Annual Report

Antimicrobial Resistance Research and Surveillance Network

January 2020 to December 2020

Division of Epidemiology and Communicable Diseases

INDIAN COUNCIL OF MEDICAL RESEARCH
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CONTENTS

List of acronyms

AMRSN	Antimicrobial Resistance Research & Surveillance Network
AMS	Antimicrobial Susceptibility
BAL	Bronchoalveolar lavage
CARD	Comprehensive Antibiotic Resistance Database
CDS	Coding sequence regions
CGPS	Center for Genomic Pathogen Surveillance
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase-negative Staphylococci
CRAB	Carbapenem-resistant*Acinetobacter baumannii*
CRE	Carbapenem resistant Enterobacterales
CSF	Cerebrospinal fluid
DI	Deep infections
DEC	Diarrheagenic *E coli*
ESBLs	Extended spectrum beta lactamases
HCWs	Health care workers
ICU	Intensive care unit
OPD	Out-patient department
LRT	Lower Respiratory tract
MBL	Metallo-beta-lactamase
MFS	Major Facilitator superfamily
MIC	Minimum inhibitory concertation
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
NFGNB	Non fermenting Gram-negative bacilli
OXA	Oxacillinases
PBP2a	Penicillin binding protein 2a
PMQR	Plasmid mediated quinolone resistance
QUAST	Quality assessment tool
RC	Regional centers
RGI	Resistance gene identifier
SCC *mec*	Staphylococcal cassette chromosome mec
SI	Superficial infections
SD	Standard deviation
SS	Sterile body fluids
ST	Sequence types
TMP-SMX	Trimethoprim sulfamethoxazole
VRE	Vancomycin-resistant enterococci
WGS	Whole-genome sequencing

Executive summary

The Indian Council of Medical Research (ICMR) has been supporting research on antimicrobial resistance through the Antimicrobial Resistance Research & Surveillance Network (AMRSN) since 2013. The data collected from the network has enabled compilation of drug resistance data on six pathogenic groups on antimicrobial resistance from the country. *(i)Enterobacterales* causing sepsis *(ii)* Gram-negative non-fermenters *(iii)* Typhoidal *Salmonella (iv)* Diarrhoeagenic bacterial organisms, (v) Gram-positives: staphylococci and enterococci, and (vi) Fungal pathogens from thirty tertiary care hospitals/laboratories across the country. Data collected from the network is used to track resistance trends and to better understand mechanisms of resistance in the key priority pathogens using genomics and whole genome sequencing (WGS).

Highlights of data:

- This report presents data from January 1st, 2020 to December 31st, 2020. Total number of culture positive isolates studied during the year 2020 was 65,561.
- *Escherichia coli* was most commonly isolated followed by the *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus*.
- Imipenem susceptibility of *E. coli* has dropped steadily from 86% in 2016 to 63% in 2019 and showed slight recovery to 72% in 2020 and that of *Klebsiella pneumoniae* dropped steadily from 65% in 2016 to 46% in 2019 and remained at 45% in 2020.
- Reduced susceptibility of 10-20% was observed against cephalosporins, carbapenems, monobactams and β-lactam-β-lactamase inhibitors in *A. baumannii*,.
- In *Pseudomonas aeruginosa*, the least susceptibility of 40% was observed for fluoroquinolones; and 60-70% to cephalosporins, carbapenems, and aminoglycosides.
- *Staphylococcus aureus* has shown increasing trends of resistance to most antibiotics over the years, no such prominent trend could be observed with MSSA isolates. Susceptibility to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. The anti MRSA antibiotics such as vancomycin and tigecycline showed excellent in vitro activity (100% against MRSA isolates). Teicoplanin and linezolid resistance was encountered in MRSA isolates albeit at very low rates of 0.5 and 1 %, respectively.
- Fungal infections among hospitalized patients are significantly increasing. Majority of the fungal infections are caused by few common fungal agents nevertheless rare species are also increasing requiring newer treatment strategies.

- *C. auris*, multidrug resistant yeast known to cause hospital outbreaks has been consistently isolated from regional centers across India. Majority of the *C. auris* isolates were resistant to fluconazole and incidences of echinocandin resistance is on the rise.

This is the fourth detailed report on AMR trends and patterns from the country, published by ICMR. Since the network collects data from tertiary care hospitals, the data presented in this report is not reflective of the community levels of AMR in the country and should not be extrapolated to community settings. In this report we also present trends of resistance of key pathogens to the critically important antimicrobials which should guide the prevention and treatment interventions for AMR in the country. Since India experienced COVID-19 pandemic in the year 2020, the report includes a chapter on AMR profile in isolates from COVID-19 patients.

Systematic collection, evaluation and analysis of resistance data of specific pathogens for last five years have highlighted that certain pathogens have become highly drug resistant and have become clinians dilemma. Aggressive action for prevention, containment and treatment are needed at the national level. Based on the laboratory evidence andthe inputs of clinicians, ID physicians and clinical microbiologists, these drug resistant difficult to pathogens can be classified into three groups (Table I):

- **Group I pathogens** include pathogens that have become resistant to last -resort antibiotics including carbapenems, the best available antibiotics for treating multi-drug resistant bacteria and pose a high risk to patients. They can cause severe and often deadly infections such as ventilator-associated or hospital-acquired pneumonia, bloodstream infections and urinary tract infections. *Candida auris*, pathogenic yeast has also been included under urgent threat that causes bloodstream and other invasive infections and is resistant to most of the antifungal drugs.
- **Group II** pathogens include multi drug resistant bacteria, conferring high risk to patients, mainly prevalent in hospital acquired infections and is associated with serious multidrug-resistant infections and ventilator associated pneumonia, complicated urinary tract infections and surgical site.
- **Group III pathogens** include drug resistant bacteria that are responsible for only a small number of infections but detection and early prevention of such infections can have significant impact on public health and need to be carefully watched in future.

Table I: Difficult to treat drug resistant pathogens in Indian hospitals

	Group I	Group II	Group III
Pathogens	• Carbapenem Resistant Enterobacterales • Carbapenem Resistant *A. baumannii* • Drug resistant *Salmonella* Typhi • *Candida auris*	• ESBL producing Enterobacterales • Multidrug resistant *P. aeruginosa* • Vancomycin-resistant enterococci, • Azole Resistant *Candida* spp	• Methicillin Resistant *Staphylococcus aureus* • Azole resistant *Aspergillus fumigatus* • Amphotericin B resistant *Aspergillus flavus* • Drug-resistant *Stenotrophomonas maltophilia* • Colistin Resistant Enterobacterales • Colistin resistant *Acinetobacter* spp.
Action required for containment	Aggressive action	Sustained action	Continuous monitoring and prevention efforts

The infections caused by the pathogens listed under Group I and II have been documented to be associated with high rates of mortality and morbidity, both in India and globally. Additionally, they increase hospital lengths of stay and result in major increase in healthcare expenditure and healthcare resource utilization. Detailed summaries on diagnosis, treatment and containment of each of the high risk pathogens have been included in this document. This list intends to flag the imminent threat of rising resistance to higher generation antimicrobials and highlight the urgent need to implement appropriate interventions to prevent development of resistance, contain the spread of drug resistant pathogens and improve treatment of drug resistant infections.

Chapter 1 Summary of surveillance data

January 2020 to December 2020

Total number of culture positive isolates studied during the year 2020 was 65,561. Of these, 13,109 from blood, 16,009 from urine, 10,557 Lower Respiratory tract (LRT), 14,843 Superficial infections, 4,055 Deep infections, 541 CSF, 1,823 Sterile spaces (SS), 331 Faeces and 4,293 others. Majority of the isolates were from Enterobacterales except *Salmonella and Shigella* (51%) followed by Non fermenting Gram-negative bacilli (NFGNB) (25%), staphylococci (12.7%), enterococci (7.3%), Typhoidal *Salmonella* (0.7%) and *fungi* (2.5%) (Table 1.1). In the distribution of major group of organisms in different specimens, member of the Enterobacterales group were the commonest isolates in urine (75.7%), sterile body fluids (SS) (60.6%), deep infections (DI) (49.2%), others (48.6%), superficial infections (SI) (44.8%), blood (40.1%), LRT (37.7%) and CSF (30.3%). Non fermenting Gram-negative bacilli (NFGNB) group were the predominant isolates in the lower respiratory tract (56.2%), CSF (43.1%), others (26.2%), superficial infections (SI) (24.9%), deep infection (DI) (22.2%), blood (21%), sterile sites (SS) (17.9%) and urine (8.7%). *Staphylococci* constituted 23.8% of the superficial infections (SI) followed by blood infection (19.9%), deep infection (DI) (18.3%) and CSF (14.6%). Enterococci group constituted 12.1% of the isolates from urine followed by sterile body fluid (11.6%), CSF (9.8%), deep infections (8.7%), blood (8%) and superficial infections (5.7%). Typhoidal *Salmonella* group constituted 2.3% of the isolates from blood. *Yeast* group were significant isolates in the blood infection (7.9%) (Table 1.1 and Figure 1.1).

The distribution of top 10 isolates from different specimens is presented in Table 1.2 and Figure 1.2. *Escherichia coli* was most commonly isolated (25.1%) followed by the *Klebsiella pneumoniae* (18%), *Pseudomonas aeruginosa* (12%), *Acinetobacter baumannii* (10.4%) and *Staphylococcus aureus* (9.6%). Among these isolates, *Escherichia coli* was the most predominant isolate from the urine (51.2%), *K. pneumoniae* from the LRT, *Acinetobacter baumannii* from LRT (27.6%), *S. aureus* from SS (21.5%), *Enterococcus faecalis* and *Enterococcus faecium* from Urine (5.7%), and (4.9%) respectively. The relative distribution of the various species isolated from patients in the out-patient department (OPD), admitted to the wards and intensive care unit (ICUs) are presented in Table 1.3 and Figures 1.3a &1.3b. Top 5 isolates in descending order in OPD specimen were *E. coli, K. pneumoniae, S. aureus , P. aeruginosa* and *Enterococcus faecalis*; in Wards *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter baumannii* and *S. aureus*; and in ICU *K. pneumoniae, Acinetobacter baumannii*, *E. coli, P. aeruginosa* and *S. aureus. Enterococcus faecium* was common isolate from the ICU (3.7%) followed by ward and OPD; whereas, *E. faecalis* was common isolate from the OPD (3.8%) followed by the wards and the ICU. (Table 1.3, Figure 1.3).

Table 1.1: Specimen wise distributions of major groups of organisms

Isolate	Culture positive																			
	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=541		SS n=1823		Faeces n=331		Others n=4293	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterobacterales except Salmonella and Shigella	33360 (50.9)	100	5255 (40.1)	15.8	12124 (75.7)	36.3	3983 (37.7)	11.9	6646 (44.8)	19.9	1994 (49.2)	6	164 (30.3)	0.5	1105 (60.6)	3.3	0 (0)	0	2089 (48.6)	6.3
Typhoidal Salmonella	458 (0.7)	100	304 (2.3)	66.4	9 (0.1)	2	2 (0)	0.4	12 (0.1)	2.6	6 (0.1)	1.3	1 (0.2)	0.2	3 (0.2)	0.7	0 (0)	0	121 (2.8)	26.4
NFGNB	16362 (25)	100	2756 (21)	16.8	1394 (8.7)	8.5	5930 (56.2)	36.2	3696 (24.9)	22.6	902 (22.2)	5.5	233 (43.1)	1.4	327 (17.9)	2	0 (0)	0	1124 (26.2)	6.9
staphylococci	8299 (12.7)	100	2611 (19.9)	31.5	305 (1.9)	3.7	472 (4.5)	5.7	3530 (23.8)	42.5	742 (18.3)	8.9	79 (14.6)	1	124 (6.8)	1.5	0 (0)	0	436 (10.2)	5.3
enterococci	4798 (7.3)	100	1048 (8)	21.8	1930 (12.1)	40.2	23 (0.2)	0.5	844 (5.7)	17.6	351 (8.7)	7.3	53 (9.8)	1.1	211 (11.6)	4.4	0 (0)	0	338 (7.9)	7
Fungi	1632 (2.5)	100	1034 (7.9)	63.4	195 (1.2)	11.9	116 (1.1)	7.1	51 (0.3)	3.1	30 (0.7)	1.8	10 (1.8)	0.6	34 (1.9)	2.1	0 (0)	0	162 (4)	9.9
Diarrheal pathogens	390 (0.6)	100	21 (0.2)	5.4	5 (0)	1.3	0 (0)	0	5 (0)	1.3	5 (0.1)	1.3	1 (0.2)	0.3	17 (0.9)	4.4	331 (100)	84.9	5 (0.1)	1.3

Note:

1. **Blood** includes: Blood-central catheter, Blood-peripheral and Peripheral catheter-blood.
2. **LRT** (Lower Respiratory Tract) includes: Bronchoalveolar lavage (BAL), Sputum, Lung aspirate, Endotracheal aspirate (ETA) and Lobectomy tissue (Lung tissue).
3. **SSI: Superficial Infection** includes SST (Skin & Soft Tissue), Pus/exudate, Wound swab, Superficial Biopsy and Superficial Tissue.
4. **Deep Infection** includes: Abscess aspirate, Pus aspirate, Deep Biopsy and Deep Tissue.
5. **SS** (Sterile sites) includes: Fluid from sterile spaces, abdominal fluid, Intracostal tube fluid, Pancreatic drain fluid, Pericardial fluid, Peritoneal fluid and Pleural fluid.

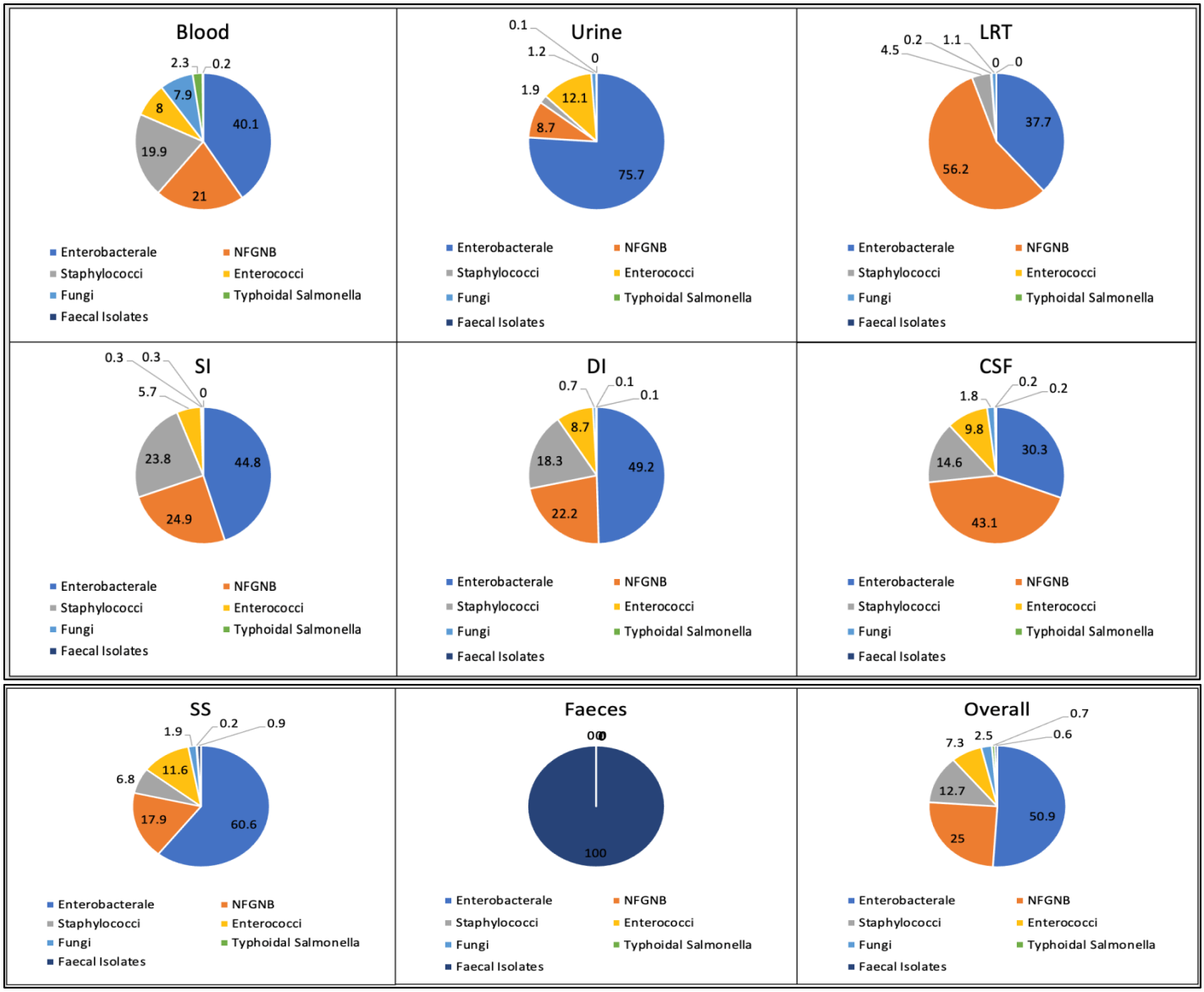


Figure 1.1: Specimen wise distribution of major groups of organisms (number shown in percentage)

Table 1.2: Isolation distribution of top 10 isolates from different specimens

Organism	Total	Blood	Urine	LRT	Superficial Infection	Deep Infection	SS	Faeces
Escherichia coli	16483/65561 (25.1)	2228/13109 (17)	8201/16009 (51.2)	703/10557 (6.7)	2971/14838 (20)	796/4055 (19.6)	578/1823 (31.7)	84/572 (14.7)
Klebsiella pneumoniae	11810/65561 (18)	2283/13109 (17.4)	2860/16009 (17.9)	2698/10557 (25.6)	2153/14838 (14.5)	629/4055 (15.5)	314/1823 (17.2)	18/572 (3.1)
Pseudomonas aeruginosa	7843/65561 (12)	788/13109 (6)	1115/16009 (7)	2335/10557 (22.1)	2182/14838 (14.7)	565/4055 (13.9)	151/1823 (8.3)	4/572 (0.7)
Acinetobacter baumannii	6851/65561 (10.4)	1474/13109 (11.2)	197/16009 (1.2)	2916/10557 (27.6)	1292/14838 (8.7)	288/4055 (7.1)	136/1823 (7.5)	2/572 (0.3)
Staphylococcus aureus	628/65561 (9.6)	1110/13109 (8.5)	272/16009 (1.7)	459/10557 (4.3)	3197/14838 (21.5)	720/4055 (17.8)	105/1823 (5.8)	1/572 (0.2)
Enterococcus faecalis	2101/65561 (3.2)	318/13109 (2.4)	912/16009 (5.7)	6/10557 (0.1)	456/14838 (3.1)	203/4055 (5)	40/1823 (2.2)	1/572 (0.2)
Enterococcus faecium	1994/65561 (3)	556/13109 (4.2)	788/16009 (4.9)	8/10557 (0.1)	287/14838 (1.9)	104/4055 (2.6)	82/1823 (4.5)	9/572 (1.6)
Proteus mirabilis	1236/65561 (1.9)	61/13109 (0.5)	281/16009 (1.8)	82/10557 (0.8)	487/14838 (3.3)	199/4055 (4.9)	31/1823 (1.7)	1/572 (0.2)
Enterobacter cloacae	1057/65561 (1.6)	232/13109 (1.8)	175/16009 (1.1)	90/10557 (0.9)	333/14838 (2.2)	115/4055 (2.8)	25/1823 (1.4)	3/572 (0.5)
Enterococcus spp.	703/65561 (1.1)	174/13109 (1.3)	230/16009 (1.4)	9/10557 (0.1)	101/14838 (0.7)	44/4055 (1.1)	89/1823 (4.9)	0/0 (-)

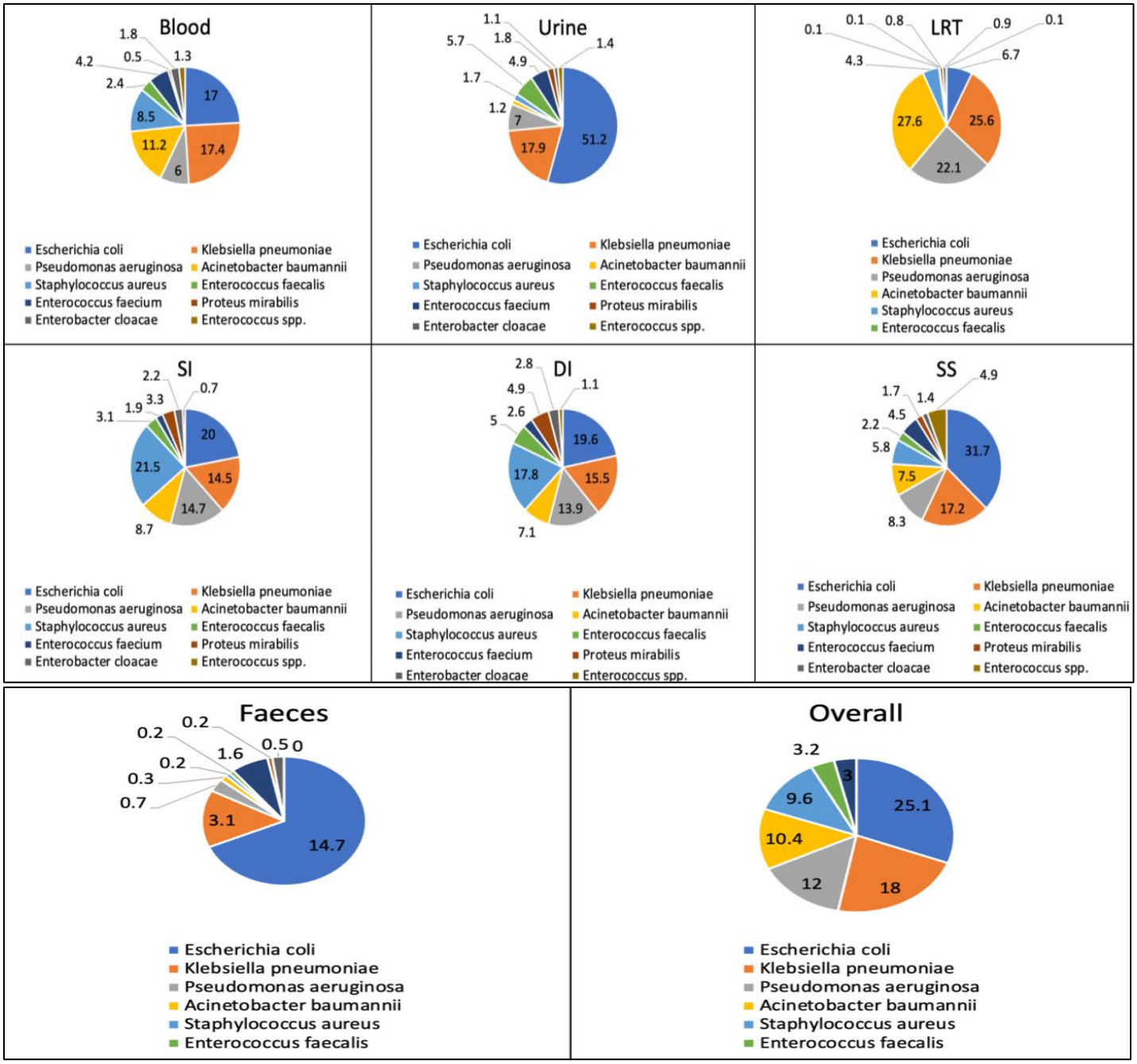


Figure 1.2: Isolation distribution of top 10 isolates from different specimens

Table 1.3: Distribution of top 10 isolates from all specimens across OPD, ward and ICU

Organism	Total (65561)	OPD (16298)	Ward (35847)	ICU (13416)
Escherichia coli	16483 (25.1)	5589 (34.3)	9139 (25.5)	1755 (13.1)
Klebsiella pneumoniae	11810 (18)	2286 (14)	6475 (18.1)	3049 (22.7)
Pseudomonas aeruginosa	7843 (12)	1941 (11.9)	4217 (11.8)	1685 (12.6)
Acinetobacter baumannii	6851 (10.4)	559 (3.4)	3484 (9.7)	2808 (20.9)
Staphylococcus aureus	6281(9.6)	2251(13.8)	3392(9.5)	638(4.8)
Enterococcus faecalis	2101 (3.2)	626 (3.8)	1183 (3.3)	292 (2.2)
Enterococcus faecium	1994 (3)	254 (1.6)	1243 (3.5)	497 (3.7)
Proteus mirabilis	1236 (1.9)	400 (2.5)	665 (1.9)	171 (1.3)
Enterobacter cloacae	1057 (1.6)	289 (1.8)	615 (1.7)	153 (1.1)
Enterococcus spp	703(1.1)	151(0.9)	431(1.2)	121(0.9)
Others	9202 (14.0)	1952 (12.0)	5003 (14.0)	2247 (16.8)

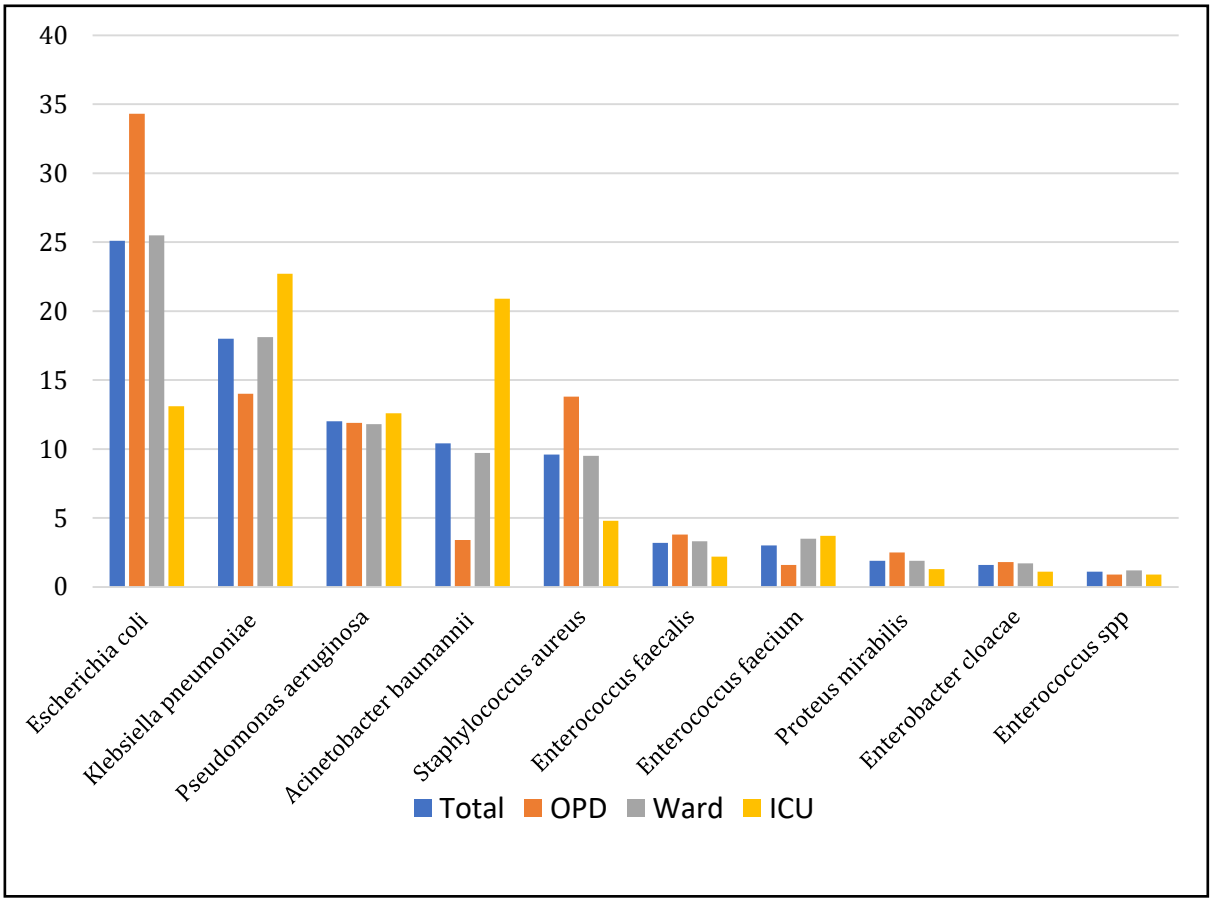


Figure 1.3a: Distribution of top 10 isolates from all specimens across OPD, ward and ICU

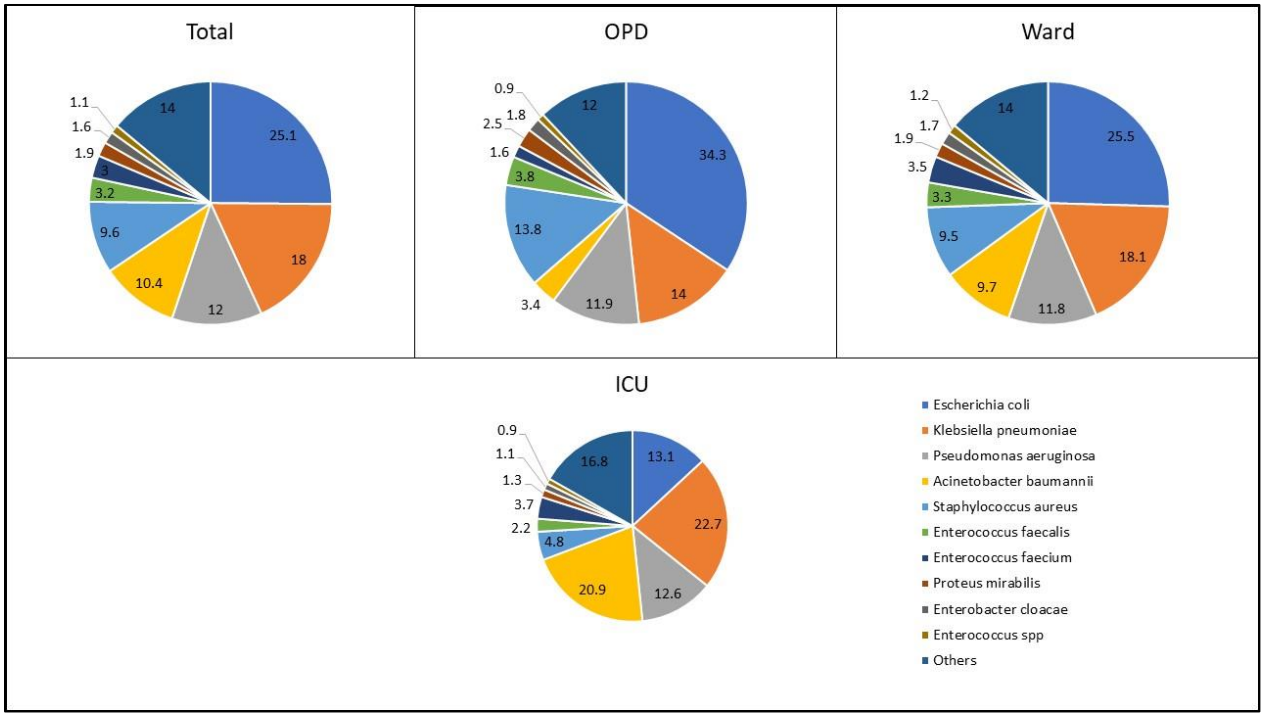


Figure 1.3b: Distribution of species of organisms in isolates of OPD, Ward and ICU

Table 1.4 Yearly isolation trends of top 10 isolates from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Escherichia coli	1398/7237 (19.3)	10413/45521 (22.9)	19317/74295 (26)	30652/108465 (28.3)	16483/65561 (25.1)
Klebsiella pneumoniae	1027/7237 (14.2)	6735/45521 (14.8)	11062/74295 (14.9)	18456/108465 (17)	11810/65561 (18)
Pseudomonas aeruginosa	1057/7237 (14.6)	5689/45521 (12.5)	8883/74295 (12)	12638/108465 (11.7)	7843/65561 (12)
Acinetobacter baumannii	396/7237 (5.5)	3361/45521 (7.4)	4550/74295 (6.1)	8533/108465 (7.9)	6851/65561 (10.4)
Staphylococcus aureus	960/7237 (13.3)	5708/45521 (12.5)	8644/74295 (11.6)	12320/108465 (11.4)	6281/65561 (9.6)
Enterococcus faecalis	126/7237 (1.7)	1034/45521 (2.3)	2014/74295 (2.7)	2895/108465 (2.7)	2101/65561 (3.2)
Enterococcus faecium	180/7237 (2.5)	937/45521 (2.1)	1476/74295 (2)	2700/108465 (2.5)	1994/65561 (3)
Proteus mirabilis	137/7237 (1.9)	882/45521 (1.9)	1285/74295 (1.7)	1958/108465 (1.8)	1236/65561 (1.9)
Enterobacter cloacae	45/7237 (0.6)	619/45521 (1.4)	1097/74295 (1.5)	1495/108465 (1.4)	1057/65561 (1.6)
Enterococcus spp.	31/7237 (0.4)	421/45521 (0.9)	711/74295 (1)	1079/108465 (1)	703/65561 (1.1)

Yearly isolation rates of top ten isolates from all samples showed a steady increase of *Klebsiella pneumoniae* from 14.2% in 2016 to 18% in 2020 (Table 1.4, Figure 1.4a) and *A. baumannii* from 6.1% in 2018 to 10.4% in 2020 without much change in the isolation rates of the other species. Yearly isolation trend of only *Klebsiella pneumoniae* from all samples in the ICU showed a steady increasing trend as compared to other isolates (Figure 1.4b).

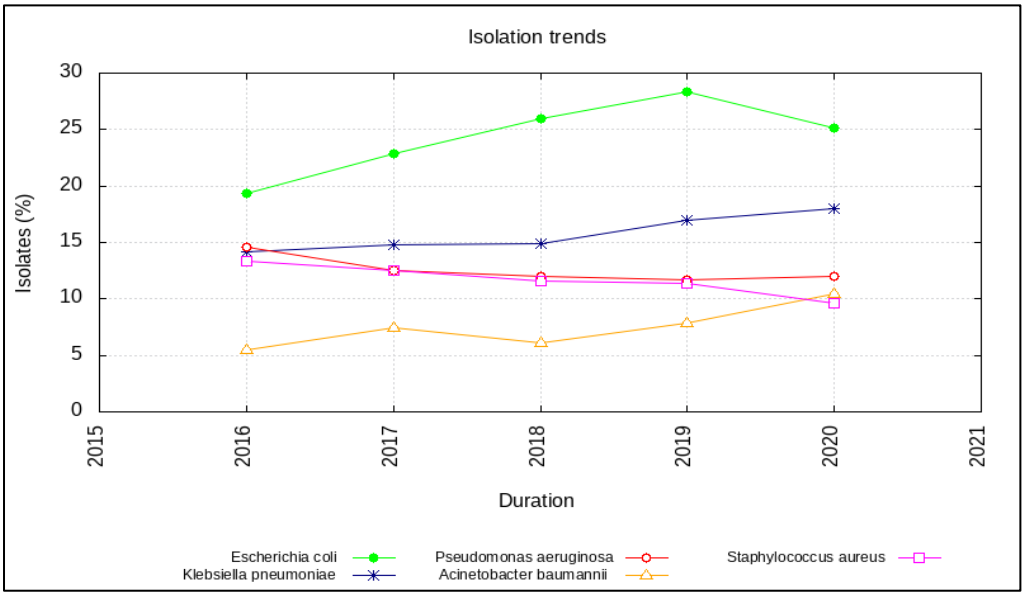


Figure 1.4a Yearly isolation trend of top 5 isolates from all samples

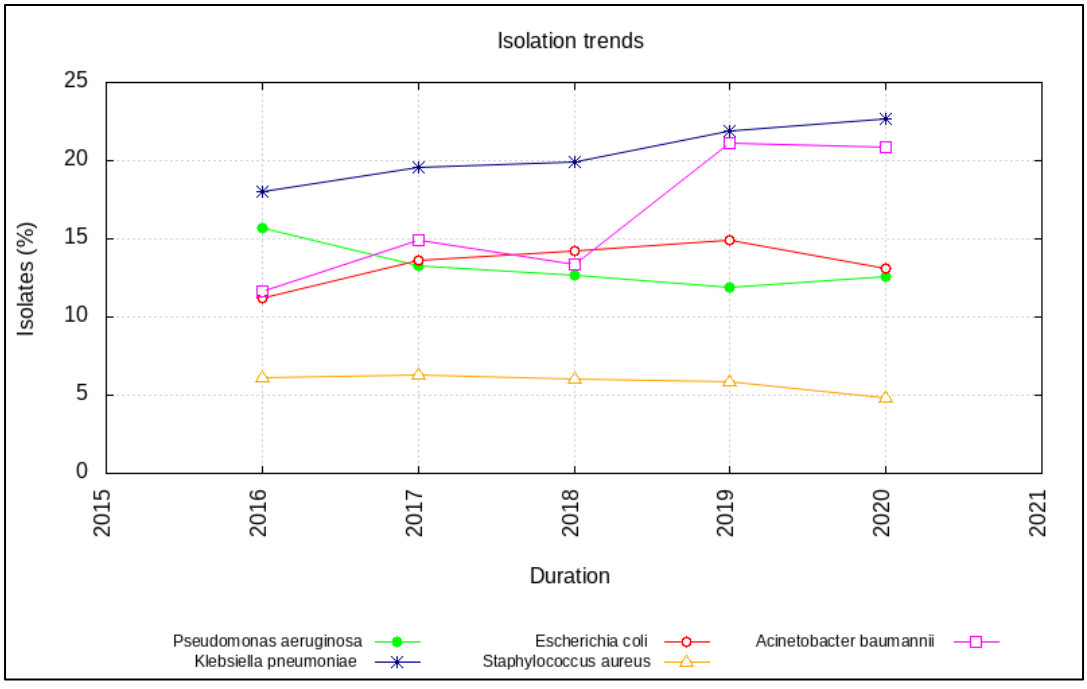


Figure 1.4b Yearly isolation trend of top 5 isolates from all samples in the ICU

Enterobacterales

Enterobacterales (except *Salmonella and Shigella*) constituted the major group (51%) of the overall isolates (Table 1.1). Out of a total of 65,561 culture positive isolates, specimen percentage wise distribution of major species within family Enterobacterales is shown in the Table 1.5 and Figures 1.5a and 1.5b. Overall, *Escherichia coli* was the commonest species (25.1%) followed by *Klebsiella pneumoniae* (18%), *Proteus mirabilis* (1.9%) and *Enterobacter cloacae* (1.6%) (Table 1.5). *Escherichia coli* was the most predominant isolate from the urine (51.2%), sterile site (31.7%), others (20.4%), superficial infection (20%), blood (17%) and CSF (10.7%). *Klebsiella pneumoniae* was the most predominant isolate in the lower respiratory tract (25.6%), urine (17.9%), blood (17.4%), sterile sites (SS) and others (17.2%), deep infection (DI) (15.5%) and CSF and superficial infection (SI)(14.6 and 14.5 & respectively). *Proteus mirabilis* was common in 5% of deep and 3.3% of superficial infections and other specimens (2%). *Enterobacter cloacae* constituted 2.8 % of deep infections and 2.2% of superficial infections. *Klebsiella species* constituted 3.8% of sterile site infections (SS). Isolates from the regional centers (RC 4) had higher percentage isolate rate of *E. coli*, *Klebsiella pneumoniae, Proteus mirabilis and Enterobacter cloacae* than the rest of RCs (Table 1.6).

Table 1.5: Specimen wise distributions of major species of family Enterobacterales

Isolate	Culture positive																			
	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=541		SS n=1823		Faeces n=*0		Others n=4624	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Escherichia coli	16483 (25.1)	100	2228 (17)	13.5	8201 (51.2)	49.8	703 (6.7)	4.3	2974 (20)	18	796 (19.6)	4.8	58 (10.7)	0.4	578 (31.7)	3.5	*0 (-)	0	945 (20.4)	5.7
Klebsiella pneumoniae	11810 (18)	100	2283 (17.4)	19.3	2860 (17.9)	24.2	2698 (25.6)	22.8	2153 (14.5)	18.2	629 (15.5)	5.3	79 (14.6)	0.7	314 (17.2)	2.7	*0 (-)	0	794 (17.2)	6.7
Proteus mirabilis	1236 (1.9)	100	61 (0.5)	4.9	281 (1.8)	22.7	82 (0.8)	6.6	487 (3.3)	39.4	199 (4.9)	16.1	1 (0.2)	0.1	31 (1.7)	2.5	*0 (-)	0	94 (2)	7.6
Enterobacter cloacae	1057 (1.6)	100	232 (1.8)	21.9	175 (1.1)	16.6	90 (0.9)	8.5	333 (2.2)	31.5	115 (2.8)	10.9	11 (2)	1	25 (1.4)	2.4	*0 (-)	0	76 (1.6)	7.2
Citrobacter koseri	445 (0.7)	100	30 (0.2)	6.7	184 (1.1)	41.3	39 (0.4)	8.8	113 (0.8)	25.4	50 (1.2)	11.2	0 (0)	0	5 (0.3)	1.1	*0 (-)	0	24 (0.5)	5.4
Klebsiella spp.	401 (0.6)	100	105 (0.8)	26.2	42 (0.3)	10.5	129 (1.2)	32.2	38 (0.3)	9.5	3 (0.1)	0.7	6 (1.1)	1.5	69 (3.8)	17.2	*0 (-)	0	9 (0.2)	2.2
Serratia marcescens	313 (0.5)	100	105 (0.8)	33.5	14 (0.1)	4.5	82 (0.8)	26.2	57 (0.4)	18.2	17 (0.4)	5.4	8 (1.5)	2.6	7 (0.4)	2.2	*0 (-)	0	23 (0.5)	7.3
Morganella morganii	333 (0.5)	100	21 (0.2)	6.3	81 (0.5)	24.3	10 (0.1)	3	122 (0.8)	36.6	60 (1.5)	18	0 (0)	0	11 (0.6)	3.3	*0 (-)	0	28 (0.6)	8.4
Providencia rettgeri	76 (0.1)	100	6 (0)	7.9	38 (0.2)	50	3 (0)	3.9	12 (0.1)	15.8	8 (0.2)	10.5	1 (0.2)	1.3	3 (0.2)	3.9	*0 (-)	0	5 (0.1)	6.6

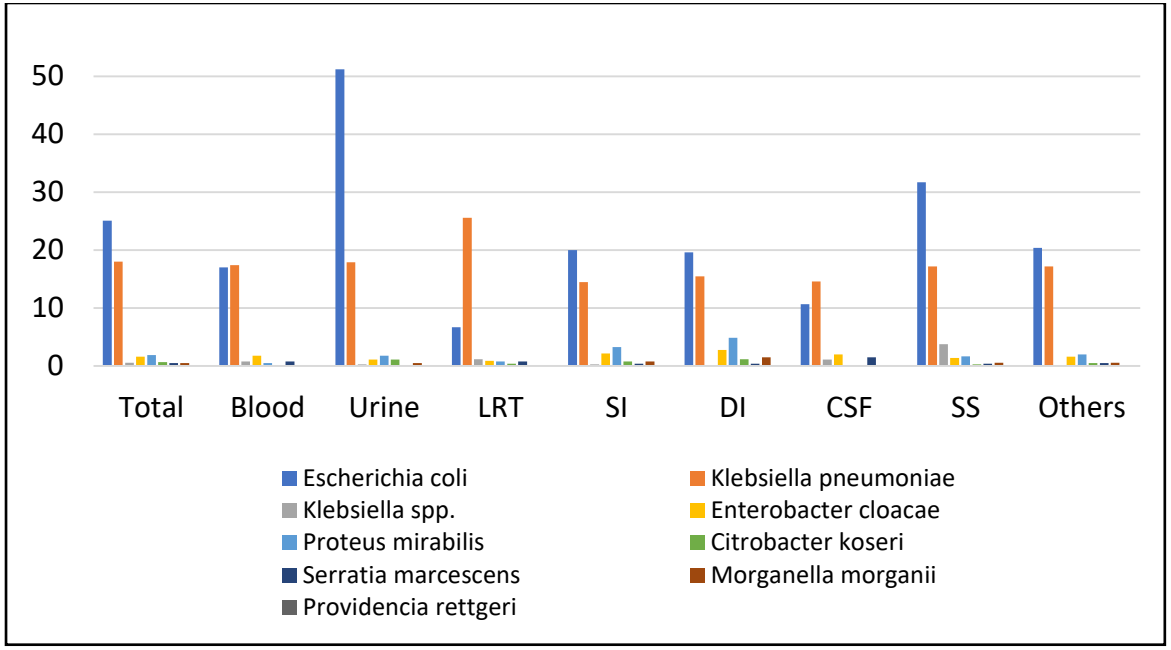


Figure 1.5a: Specimen wise distribution of major species of family Enterobacterales (Percentage calculated from total of Enterobacterales isolates)

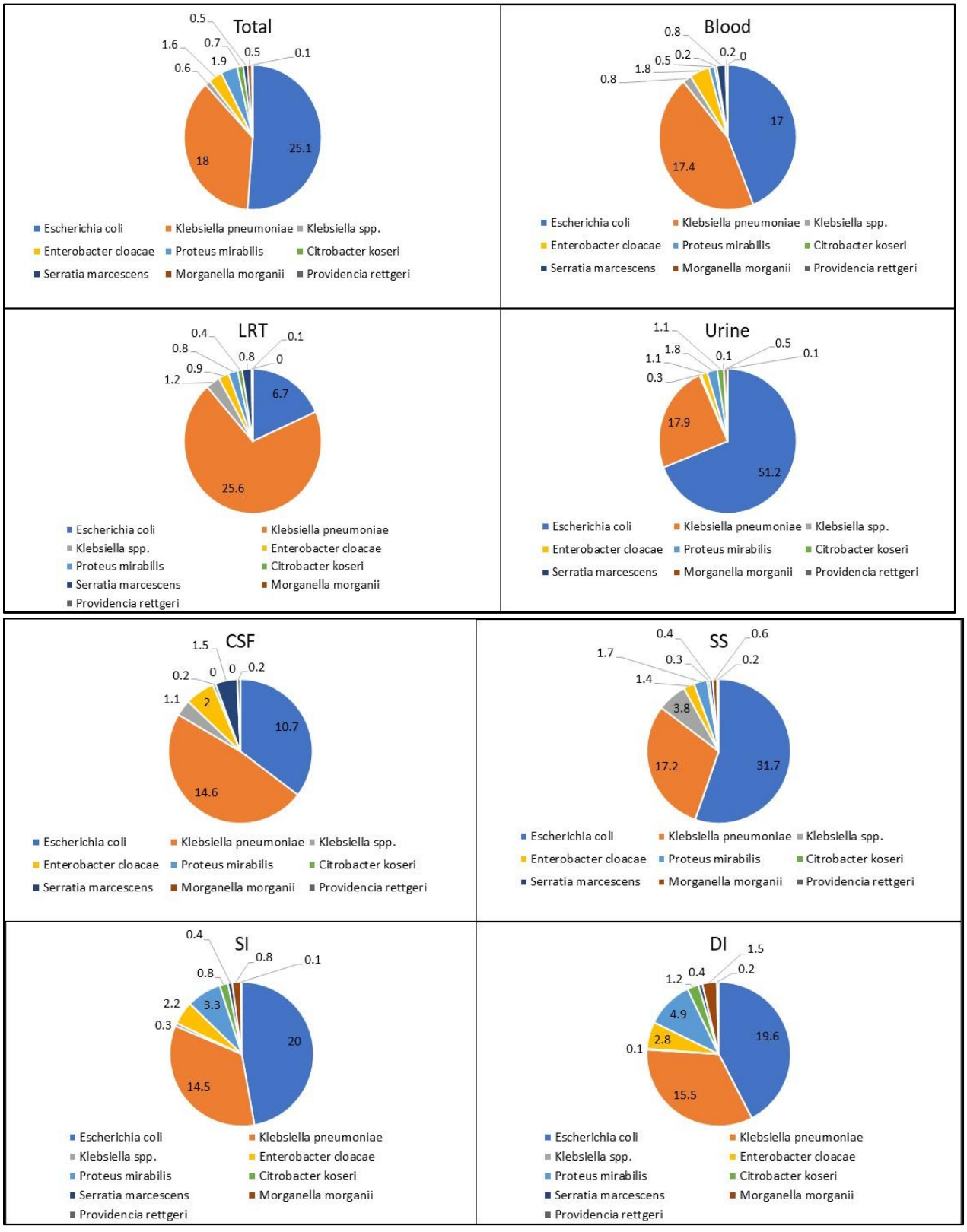


Figure 1.5b: Specimen wise distribution of major species of family Enterobacterales

Table 1.6: Regional center wise distribution of major species of family Enterobacterales except *Salmonella* in Total (except Faeces) specimen type

Regional Center	Total (n=33246)	*Escherichia coli* (n=16399)	*Klebsiella pneumoniae* (n=11792)	*Proteus mirabilis* (n=1235)	*Enterobacter cloacae* (n=1054)	*Citrobacter koseri* (n=445)	*Enterobacter spp.* (n=385)	*Citrobacter freundii* (n=180)	*Proteus vulgaris* (n=118)	*Citrobacter spp.* (n=68)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC4	4203 (12.6)	1991 (12.1)	1403 (11.9)	243 (19.7)	253 (24)	58 (13)	15 (3.9)	10 (5.6)	10 (8.5)	0 (0)
RC10	3681 (11.1)	1697 (10.3)	1277 (10.8)	199 (16.1)	158 (15)	88 (19.8)	12 (3.1)	8 (4.4)	11 (9.3)	20 (29.4)
RC6	2701 (8.1)	1125 (6.9)	1327 (11.3)	119 (9.6)	68 (6.5)	15 (3.4)	0 (0)	9 (5)	0 (0)	0 (0)
RC14	2496 (7.5)	1523 (9.3)	726 (6.2)	59 (4.8)	140 (13.3)	32 (7.2)	0 (0)	2 (1.1)	7 (5.9)	0 (0)
RC1	2441 (7.3)	1217 (7.4)	950 (8.1)	58 (4.7)	57 (5.4)	10 (2.2)	14 (3.6)	4 (2.2)	4 (3.4)	4 (5.9)
RC3	1887 (5.7)	917 (5.6)	461 (3.9)	38 (3.1)	10 (0.9)	8 (1.8)	103 (26.8)	1 (0.6)	6 (5.1)	15 (22.1)
RC7	1886 (5.7)	966 (5.9)	699 (5.9)	64 (5.2)	34 (3.2)	30 (6.7)	1 (0.3)	38 (21.1)	10 (8.5)	1 (1.5)
RC18	1820 (5.5)	757 (4.6)	674 (5.7)	35 (2.8)	111 (10.5)	137 (30.8)	0 (0)	57 (31.7)	5 (4.2)	0 (0)
RC15	1537 (4.6)	644 (3.9)	654 (5.5)	47 (3.8)	6 (0.6)	5 (1.1)	140 (36.4)	5 (2.8)	11 (9.3)	4 (5.9)
RC5	1514 (4.6)	741 (4.5)	490 (4.2)	53 (4.3)	65 (6.2)	27 (6.1)	16 (4.2)	7 (3.9)	6 (5.1)	10 (14.7)
RC17	1274 (3.8)	832 (5.1)	360 (3.1)	16 (1.3)	36 (3.4)	3 (0.7)	1 (0.3)	2 (1.1)	0 (0)	0 (0)
RC21	1115 (3.4)	454 (2.8)	521 (4.4)	42 (3.4)	0 (0)	7 (1.6)	1 (0.3)	2 (1.1)	1 (0.8)	1 (1.5)
RC9	1079 (3.2)	632 (3.9)	334 (2.8)	17 (1.4)	4 (0.4)	7 (1.6)	0 (0)	5 (2.8)	12 (10.2)	0 (0)
RC16	964 (2.9)	550 (3.4)	261 (2.2)	33 (2.7)	5 (0.5)	4 (0.9)	30 (7.8)	8 (4.4)	23 (19.5)	0 (0)
RC20	935 (2.8)	603 (3.7)	248 (2.1)	51 (4.1)	0 (0)	1 (0.2)	0 (0)	4 (2.2)	7 (5.9)	0 (0)

Regional Center	Total (n=33246)	*Escherichia coli* (n=16399)	*Klebsiella pneumoniae* (n=11792)	*Proteus mirabilis* (n=1235)	*Enterobacter cloacae* (n=1054)	*Citrobacter koseri* (n=445)	*Enterobacter spp.* (n=385)	*Citrobacter freundii* (n=180)	*Proteus vulgaris* (n=118)	*Citrobacter spp.* (n=68)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC19	908 (2.7)	434 (2.6)	392 (3.3)	30 (2.4)	4 (0.4)	1 (0.2)	3 (0.8)	3 (1.7)	1 (0.8)	0 (0)
RC12	826 (2.5)	469 (2.9)	242 (2.1)	16 (1.3)	29 (2.8)	3 (0.7)	28 (7.3)	1 (0.6)	1 (0.8)	6 (8.8)
RC2	799 (2.4)	368 (2.2)	264 (2.2)	79 (6.4)	21 (2)	0 (0)	9 (2.3)	5 (2.8)	1 (0.8)	7 (10.3)
RC11	566 (1.7)	164 (1)	283 (2.4)	24 (1.9)	43 (4.1)	0 (0)	0 (0)	6 (3.3)	1 (0.8)	0 (0)
RC8	320 (1)	164 (1)	111 (0.9)	10 (0.8)	10 (0.9)	7 (1.6)	2 (0.5)	3 (1.7)	0 (0)	0 (0)
RC13	294 (0.9)	151 (0.9)	115 (1)	2 (0.2)	0 (0)	2 (0.4)	10 (2.6)	0 (0)	1 (0.8)	0 (0)

Centre wise distribution showed that regional centre (RC) 3 had higher blood isolates than rest of RCs. This distribution showed that isolates from the RC 12 had higher percentage isolate rate (5.5%) of *Salmonella Typhi* from blood than the rest of RCs (Table 1.7). On the contrary, *Salmonella paratyphi A* isolate percentage was more in RC 13 (1.7%) as compared to other RCs. The relative distribution of Typhoidal *Salmonella* isolated from blood in the OPD, admitted to the wards and ICUs are presented in Table 1.8 and Figures 1.6. Typhoidal Salmonella was common isolate from the OPD (7.5%) followed by the wards and were least isolated from the ICU. (Table 1.8). Among Typhoidal Salmonella, *Salmonella Typhi* had higher percentage isolation rate than *Salmonella paratyphi A.* Yearly isolation trend showed that there is a decline in isolation rates of *Salmonella typhi* in 2020 from last four years from all over India. (Table 1.9 & fig1.7).

Table 1.7: Isolates percentages across regional centers of typhoidal *Salmonella* isolated from blood

Regional Centre	Total Blood isolates (n=13109)	*Salmonella Typhi*	*Salmonella Paratyphi A*
	n	n(%)	n(%)
RC3	2353	52 (2.2)	7 (0.3)
RC6	931	29 (3.1)	15 (1.6)
RC10	1031	27 (2.6)	13 (1.3)
RC1	1212	18 (1.5)	1 (0.1)
RC15	473	18 (3.8)	1 (0.2)
RC14	745	15 (2)	2 (0.3)
RC13	239	11 (4.6)	4 (1.7)
RC5	668	10 (1.5)	5 (0.7)
RC12	199	11 (5.5)	0 (0)
RC17	576	4 (0.7)	1 (0.2)
RC11	249	3 (1.2)	2 (0.8)
RC4	1501	5 (0.3)	0 (0)
RC9	419	2 (0.5)	0 (0)
RC21	351	0 (0)	1 (0.3)
RC16	70	1 (1.4)	0 (0)
RC8	182	0 (0)	0 (0)
RC20	47	0 (0)	0 (0)
RC19	741	0 (0)	0 (0)
RC18	332	0 (0)	0 (0)
RC2	548	0 (0)	0 (0)
RC7	242	0 (0)	0 (0)

Table 1.8: Location wise isolation distribution of Typhoidal Salmonella from blood

Organism	Total	OPD	Ward	ICU
Total Blood cultures(n)	13109	1646	7333	4130
Total Typhoidal Salmonella	258 (1.9)	123 (7.5)	126 (1.7)	9 (0.2)
Salmonella Typhi	206 (1.6)	93 (5.7)	107 (1.5)	6 (0.1)
Salmonella Paratyphi A	52 (0.4)	30 (1.8)	19 (0.3)	3 (0.1)

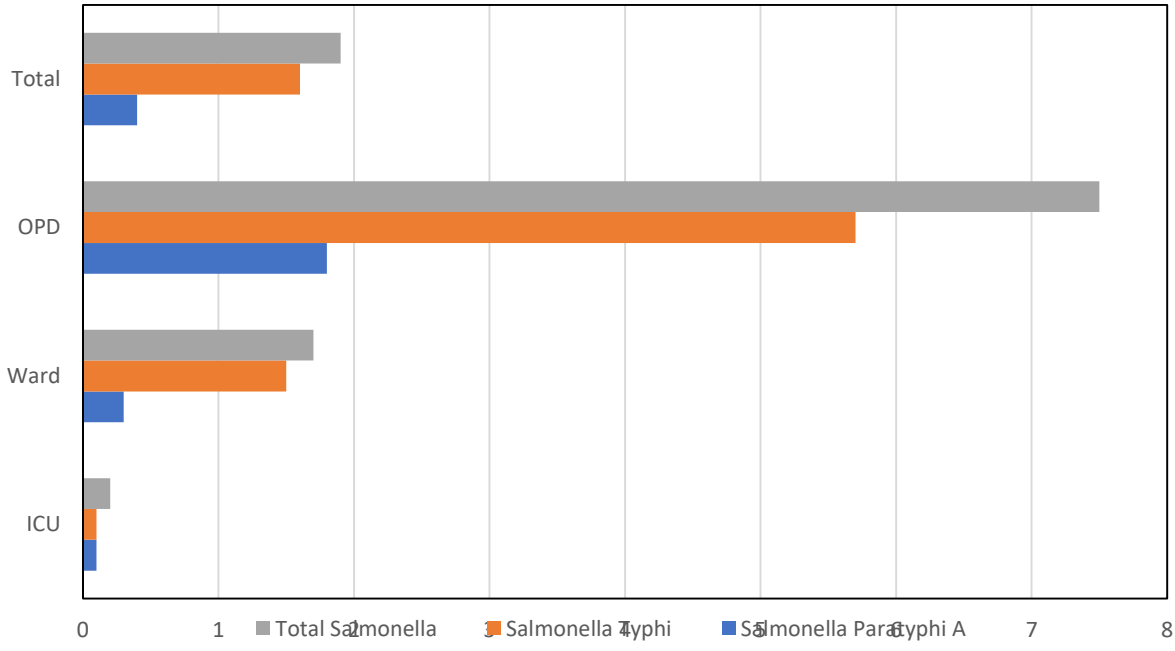


Figure 1.6: Location-wise isolation pattern of Typhoidal Salmonella isolated from blood across OPD, Ward and ICU

Table 1.9: Yearly-isolation trend of *Salmonella typhi* from Blood across different regions

Years	2016	2017	2018	2019	2020
North	12/636 (1.9%)	138/4272 (3.2%)	246/5248 (4.7%)	174/4533 (3.8%)	47/3479 (1.4%)
Central	0/0* (-)	0/0* (-)	12/110 (10.9%)	36/570 (6.3%)	14/448 (3.1%)
East	0/0* (-)	0/171* (0%)	2/712 (0.3%)	4/1443 (0.3%)	1/935 (0.1%)
West	0/0* (-)	31/648 (4.8%)	116/2011 (5.8%)	164/2761 (5.9%)	41/2041 (2%)
South	25/989 (2.5%)	176/4400 (4%)	204/6018 (3.4%)	350/8033 (4.4%)	103/6206 (1.7%)
National	37/1625 (2.3%)	345/9491 (3.6%)	580/14099 (4.1%)	728/17340 (4.2%)	206/13109 (1.6%)

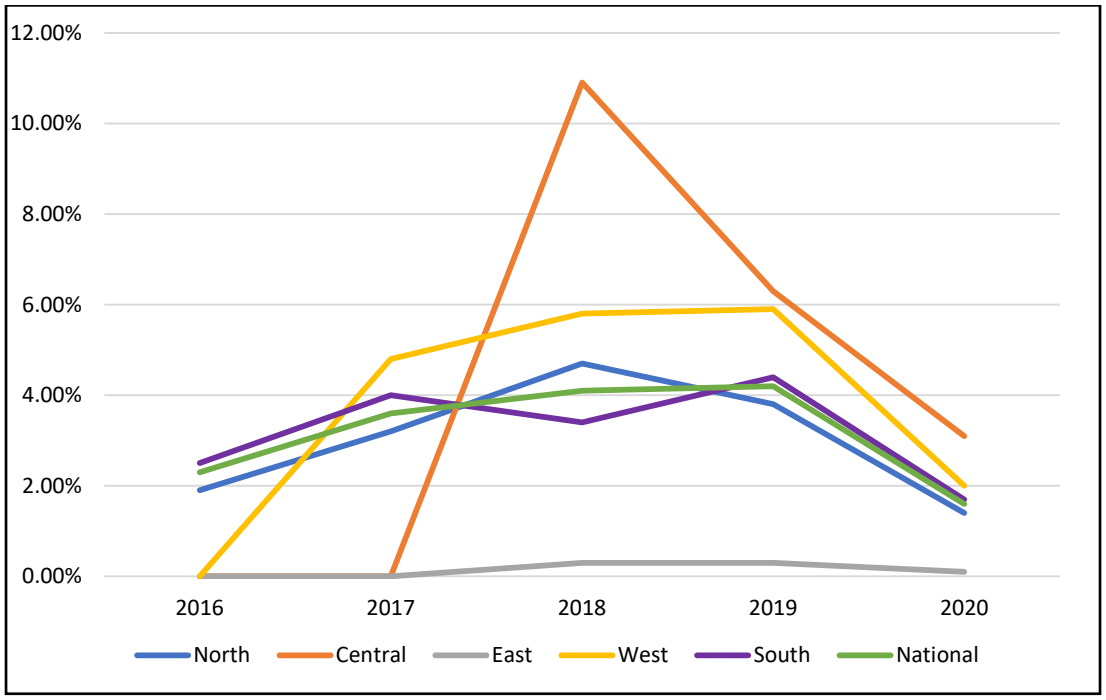


Figure1.7: Yearly-isolation trend of *Salmonella typhi* from Blood across different regions

Non-fermenting Gram negative bacteria

Non-fermenting Gram negative bacteria (NFGNB) constituted 25% of the total isolates (16,362 out of 65,561) (Table 1.10). Among the NFGNB, *Pseudomonas aeruginosa* was the commonest isolate (12%) followed by *Acinetobacter baumannii (*10.4%*). Stenotrophomonas maltophilia* and *Burkholderia cepacia* accounted for 0.5% and 0.3% of all isolates respectively. *Pseudomonas aeruginosa* was grossly predominant in LRT (22.1%) followed by others (16.1), superficial infection (14.7) and deep infections (13.9%). *Acinetobacter baumannii* was the predominant isolate from LRT (27.6%) and CSF (26.1%) followed by blood (11.2%) (Table 1.10 and Figure 1.8).

Regional center (RC) wise distribution showed that *Pseudomonas aeruginosa* was the predominant isolate among regional centers overall. RC 15 had higher percentage isolate rate of *Pseudomonas aeruginosa* and RC 11 had higher percentage isolate rate of *Acinetobacter baumannii* than the rest of RCs (Table 1.11). Among clinical settings, *P. aeruginosa* was predominantly isolated in all ICU, OPD and ward (11.9-12.6%), while *A. baumannii* was predominant in ICU (21%), followed by ward (9.8%) and OPD (3.5%) respectively (Table 1.12a and Figure 1.9).

However, trend analysis over the years 2016 – 2020 has shown a steady decline in the isolation rates of *P. aeruginosa* from 15% to 12% in 2016 to 2020, respectively (Table 1.12b). In contrast, isolation rates of *A. baumannii* increased from 5% to 10.4% between 2016 and 2020 respectively. No significant changes in the isolation rates of other pathogens such as *B. cepacia* and *S. maltophilia* have been noted (Figure 1.10).

Table 1.10: Specimen wise distribution of NFGNB

Isolate	Culture positive																			
	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=541		SS n=1823		Faeces n=572		Others n=4293	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
NFGNB	16362 (25)	100	2756 (21)	16.8	1394 (8.7)	8.5	5930 (56.2)	36.2	3696 (24.9)	22.6	902 (22.2)	5.5	233 (43.1)	1.4	327 (17.9)	2	6 (1)	0	1118 (27.6)	6.8
Pseudomonas aeruginosa	7843 (12)	100	788 (6)	10	1115 (7)	14.2	2335 (22.1)	29.8	2182 (14.7)	27.8	565 (13.9)	7.2	51 (9.4)	0.7	151 (8.3)	1.9	4 (0.7)	0.1	652 (16.1)	8.3
Acinetobacter baumannii	6851 (10.4)	100	1474 (11.2)	21.5	197 (1.2)	2.9	2916 (27.6)	42.6	1293 (8.7)	18.9	288 (7.1)	4.2	141 (26.1)	2.1	136 (7.5)	2	2 (0.3)	0	404 (10)	5.9
Stenotrophomonas maltophilia	360 (0.5)	100	90 (0.7)	25	9 (0.1)	2.5	164 (1.6)	45.6	56 (0.4)	15.6	13 (0.3)	3.6	3 (0.6)	0.8	8 (0.4)	2.2	0 (0)	0	17 (0.4)	4.7
Burkholderiacepacia	200 (0.3)	100	107 (0.8)	53.5	11 (0.1)	5.5	41 (0.4)	20.5	10 (0.1)	5	10 (0.2)	5	1 (0.2)	0.5	0 (0)	0	0 (0)	0	20 (0.5)	10
Acinetobacter baumanii-calcoaceticus complex	85 (0.1)	100	36 (0.3)	42.4	0 (0)	0	43 (0.4)	50.6	1 (0)	1.2	0 (0)	0	3 (0.6)	3.5	2 (0.1)	2.4	0 (0)	0	0 (0)	0

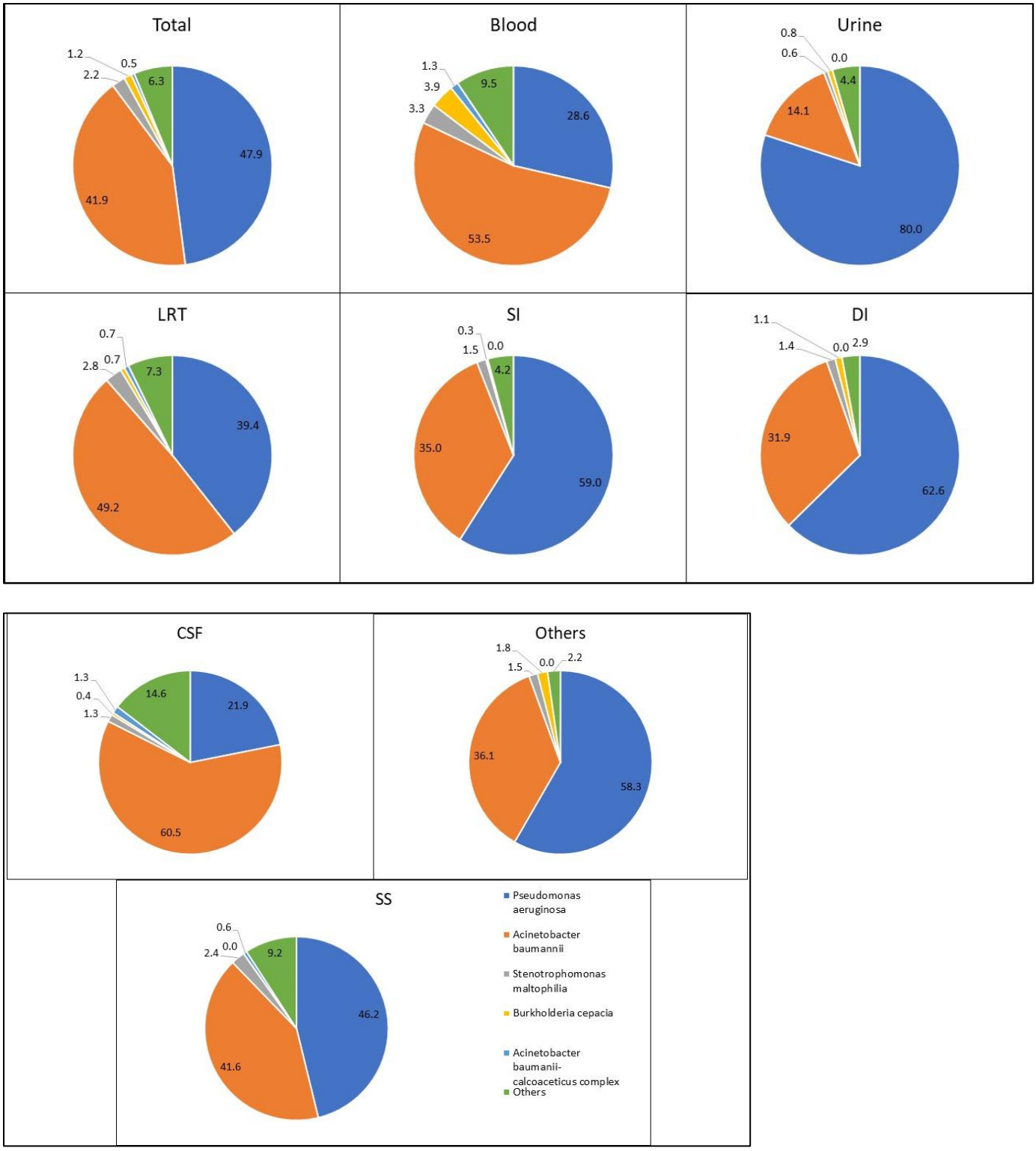


Figure 1.8: Specimen wise distribution of NFGNB (Percentage calculated from total of NFGNB isolates)

Table 1.11: Isolates percentages across Regional Centers of *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia* and from all specimens (except Faeces)

Regional Centers	Total isolates from all samples (except faeces)	*Pseudomonas aeruginosa*	*Acinetobacter baumanni*	*Stenotrophomonas maltophilia*	*Burkholderia cepacia*
	n	n(%)	n(%)	n(%)	n(%)
RC4	9277	1090 (11.7)	986 (10.6)	179 (1.9)	29 (0.3)
RC1	6092	947 (15.5)	589 (9.7)	63 (1)	27 (0.4)
RC15	3511	571 (16.3)	780 (22.2)	0 (0)	2 (0.1)
RC6	4812	775 (16.1)	536 (11.1)	31 (0.6)	20 (0.4)
RC3	4366	679 (15.6)	525 (12)	0 (0)	0 (0)
RC10	6547	749 (11.4)	381 (5.8)	16 (0.2)	69 (1.1)
RC19	2391	223 (9.3)	472 (19.7)	5 (0.2)	1 (0)
RC2	2179	220 (10.1)	365 (16.8)	0 (0)	1 (0)
RC5	2709	385 (14.2)	99 (3.7)	33 (1.2)	35 (1.3)
RC9	2202	248 (11.3)	226 (10.3)	0 (0)	0 (0)
RC14	3924	336 (8.6)	210 (5.4)	0 (0)	0 (0)
RC21	1791	158 (8.8)	321 (17.9)	2 (0.1)	0 (0)
RC20	1861	213 (11.4)	238 (12.8)	0 (0)	0 (0)
RC18	3097	155	266	9	9

		(5)	(8.6)	(0.3)	(0.3)
Regional Centers	**Total isolates from all samples (except faeces)**	***Pseudomonas aeruginosa***	***Acinetobacter baumanni***	***Stenotrophomonas maltophilia***	***Burkholderia cepacia***
	n	**n(%)**	**n(%)**	**n(%)**	**n(%)**
RC7	**2619**	**360 (13.7)**	**86 (3.3)**	**0 (0)**	**2 (0.1)**
RC11	**1161**	**148 (12.7)**	**307 (26.4)**	**8 (0.7)**	**0 (0)**
RC12	**1450**	**137 (9.4)**	**147 (10.1)**	**6 (0.4)**	**0 (0)**
RC17	**2126**	**158 (7.4)**	**142 (6.7)**	**1 (0)**	**0 (0)**
RC16	**1606**	**149 (9.3)**	**118 (7.3)**	**1 (0.1)**	**2 (0.1)**
RC13	**670**	**74 (11)**	**44 (6.6)**	**0 (0)**	**0 (0)**
RC8	**598**	**64 (10.7)**	**11 (1.8)**	**6 (1)**	**3 (0.5)**
Total National	**64989**	**7839**	**6849**	**360**	**200**

Table 1.12a: Location-wise isolates percentage of *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Stenotrophomonas maltophilia and Burkholderia cepacia* isolated from all samples except faeces across OPD, Ward and ICU

Organisms	Total	OPD	Ward	ICU
Pseudomonas aeruginosa	7839/64989 (12.1)	1941/16076 (12.1)	4213/35519 (11.9)	1685/13394 (12.6)
Acinetobacter baumannii	6849/64989 (10.5)	559/16076 (3.5)	3482/35519 (9.8)	2808/13394 (21)
Stenotrophomonas maltophilia	360/64989 (0.6)	45/16076 (0.3)	199/35519 (0.6)	116/13394 (0.9)
Burkholderia cepacia	200/64989 (0.3)	22/16076 (0.1)	104/35519 (0.3)	74/13394 (0.6)

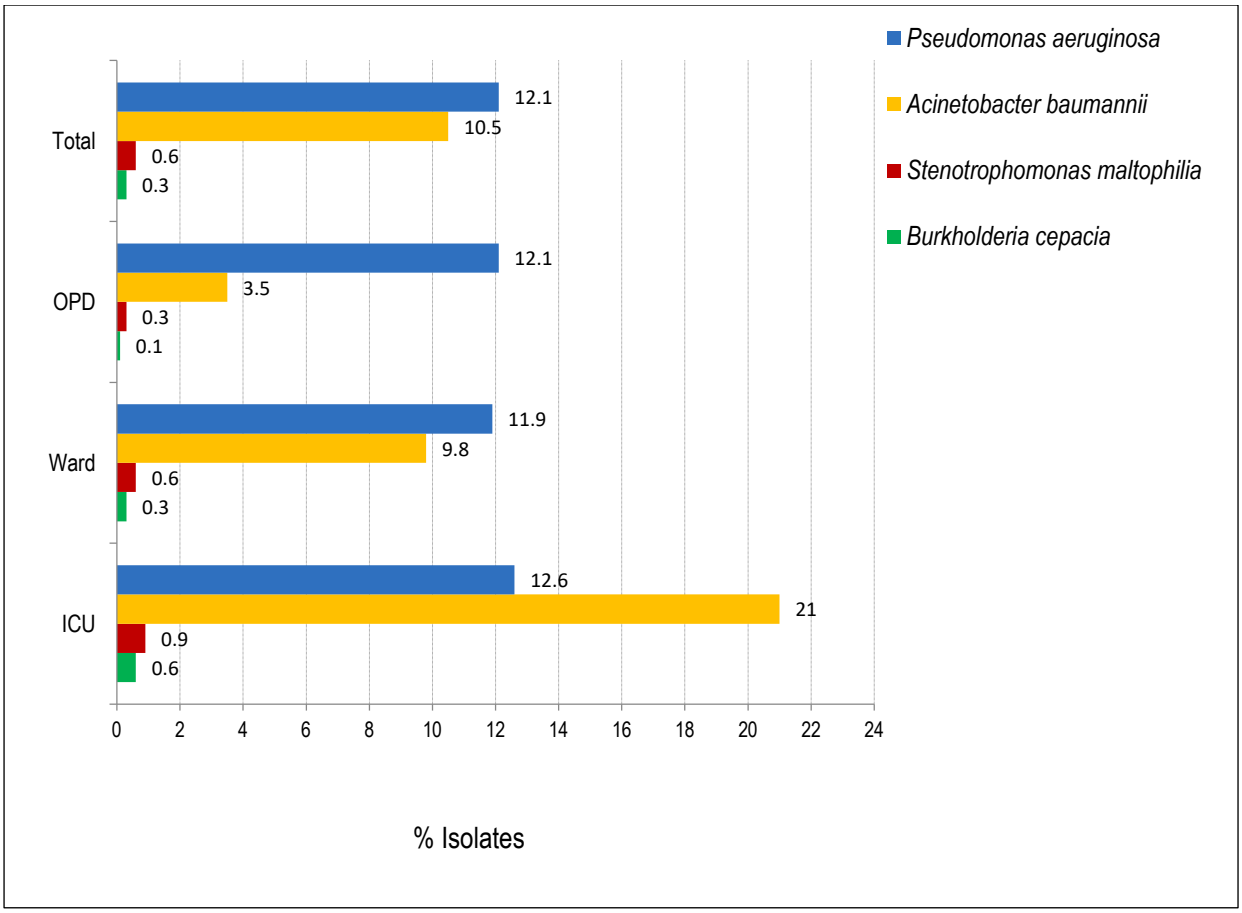


Figure 1.9: Location-wise isolation pattern of *A. baumannii, B. cepacia, P. aeruginosa,* and *S. maltophilia* isolated from all samples except faeces across OPD, Ward and ICU

Table 1.12b: Yearly Isolation trend of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Pseudomonas aeruginosa	1057/7237 (14.6)	5689/45521 (12.5)	8883/74295 (12)	12638/108465 (11.7)	7843/65561 (12)
Acinetobacter baumannii	396/7237 (5.5)	3361/45521 (7.4)	4550/74295 (6.1)	8533/108465 (7.9)	6851/65561 (10.4)
Stenotrophomonas maltophilia	23/7237 (0.3)	157/45521 (0.3)	310/74295 (0.4)	374/108465 (0.3)	360/65561 (0.5)
Burkholderia cepacia	18/7237 (0.2)	112/45521 (0.2)	197/74295 (0.3)	181/108465 (0.2)	200/65561 (0.3)

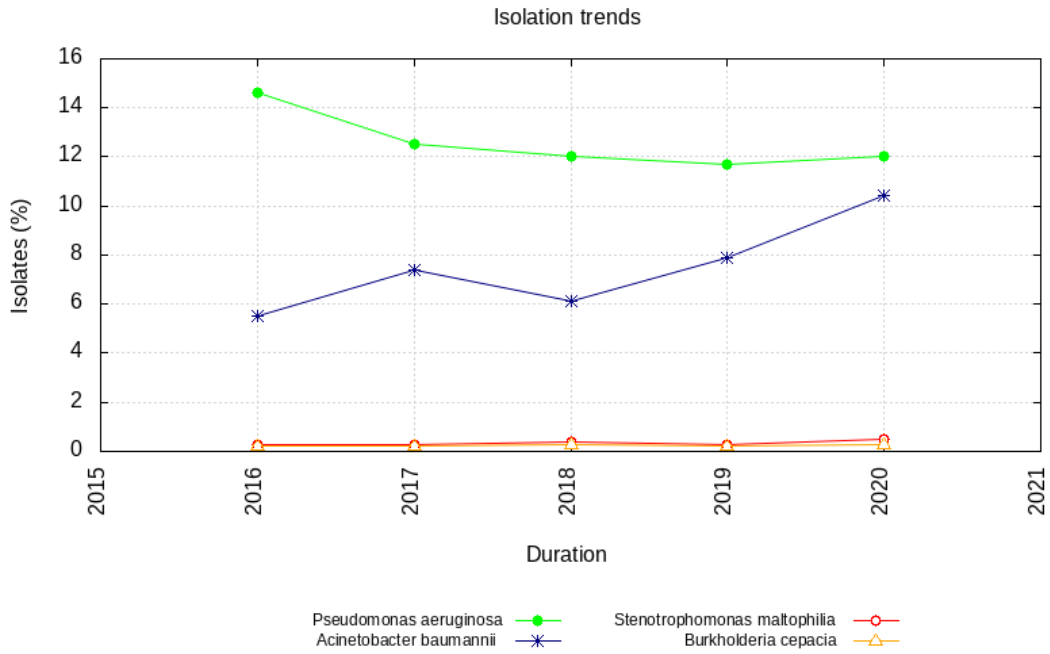


Figure 1.10: Yearly Isolation trend of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples

Staphylococci

Staphylococci constituted overall 12.7% of all the isolates (Table 1.13). *Staphylococcus aureus* was the predominant species in the superficial infections (21.5%), deep infections (17.8%), miscellaneous infections (9.5%), sterile body fluids (5.8%), blood (8.5%) and urine (1.7%) (Table 1.13). Coagulase-negative staphylococci (CoNS) were the predominant isolates in blood (11.5%) and CSF (8.5%) reflecting the high incidence of shunt infections and intra vascular device associated infections respectively. In blood and CSF, *Staphylococcus epidermidis* isolation rate was 1.8% and 1.7% respectively, reflecting the ability of the species to form biofilms and high incidence of shunt associated and dialysis associated infections. Predominant percentage isolation of Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin sensitive *Staphylococcus aureus* (MSSA) was from the superficial infections (SI) i.e., 8.8% and 12.6% respectively. This was followed by isolation from deep infection (DI), 6.9% and 10.5% and from blood, 4% and 4.6% respectively (Figure 1.11). Amongst the coagulase-negative Staphylococci (CoNS), *S. haemolyticus* (30%) were the commonest species followed by *S. epidermidis* (19.2%) and *S. hominis* (15%) (Table1.13). Regional centre wise distribution showed the predominance of isolation of *Staphylococcus aureus* in RC 14 and RC18 (16.8%) with MRSA percentage isolation (10%). The least percentage isolation of *Staphylococcus aureus* and MRSA was found among RC 6 and RC 19 i.e., 4.7% and 2.1-2.4% respectively (Table 1.14).

Among clinical settings, *Staphylococcus aureus* was predominantly isolated in OPD (13.8%), followed by ward (9.5%) and ICU (4.8%), while the coagulase-negative staphylococci (CoNS) was predominant in ICU (3.9%), followed by ward (3%) and OPD (2.6%) (Table 1.15 and Figure 1.12).

Trend analysis over the years 2016 – 2020 have shown a steady decline in the isolation rates of *Staphylococcus aureus* from 13% to 9.6% in 2016 to 2020 respectively (Table 1.16 and Figure 1.13).

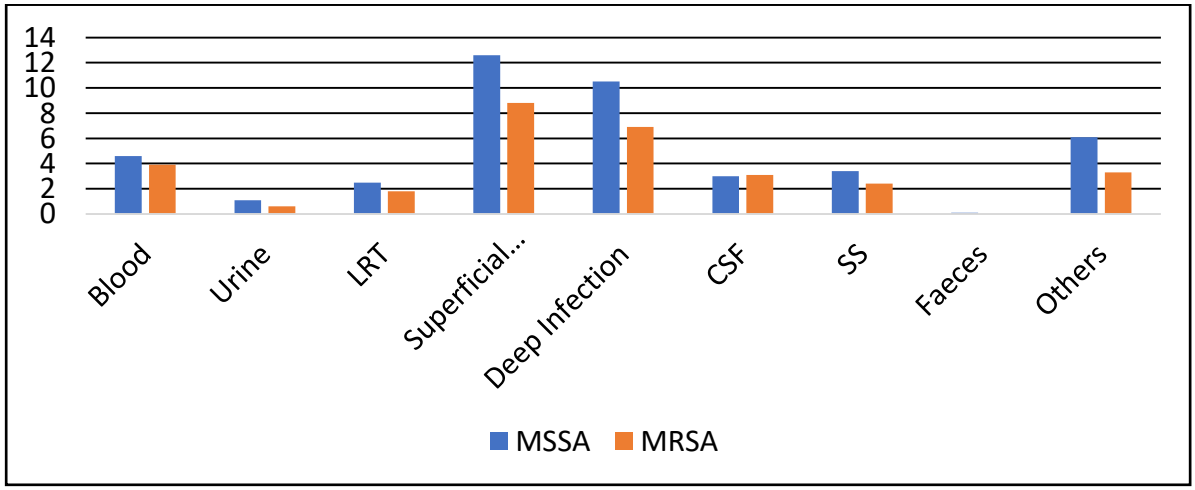


Figure1.11: Specimen wise relative distribution of MSSA and MRSA

Table 1.13: Specimen wise relative distribution of *S. aureus* and CoNS species

Isolate	Culture positive																			
	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=541		SS n=1823		Faeces n=572		Others n=4293	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Staphylococcus aureus	6281 (9.6)	100	1110 (8.5)	17.7	272 (1.7)	4.3	459 (4.3)	7.3	3197 (21.5)	50.9	720 (17.8)	11.5	33 (6.1)	0.5	105 (5.8)	1.7	1 (0.2)	0	384 (9.5)	6.1
MSSA	3655 (5.6)	100	597 (4.6)	16.3	169 (1.1)	4.6	261 (2.5)	7.1	1875 (12.6)	51.3	425 (10.5)	11.6	16 (3)	0.4	62 (3.4)	1.7	1 (0.2)	0	249 (6.1)	6.8
MRSA	2582 (3.9)	100	507 (3.9)	19.6	98 (0.6)	3.8	195 (1.8)	7.6	1308 (8.8)	50.7	281 (6.9)	10.9	17 (3.1)	0.7	43 (2.4)	1.7	0 (0)	0	133 (3.3)	5.2
CoNS	2018 (3.1)	100	1501 (11.5)	74.4	33 (0.2)	1.6	13 (0.1)	0.6	333 (2.2)	16.5	22 (0.5)	1.1	46 (8.5)	2.3	19 (1)	0.9	0 (0)	0	51 (1.3)	2.5
Staphylococcus spp.	648 (1)	100	506 (3.9)	78.1	16 (0.1)	2.5	1 (0)	0.2	77 (0.5)	11.9	7 (0.2)	1.1	15 (2.8)	2.3	10 (0.5)	1.5	0 (0)	0	16 (0.4)	2.5
Staphylococcus haemolyticus	615 (0.9)	100	449 (3.4)	73	2 (0)	0.3	7 (0.1)	1.1	123 (0.8)	20	3 (0.1)	0.5	13 (2.4)	2.1	5 (0.3)	0.8	0 (0)	0	13 (0.3)	2.1
Staphylococcus epidermidis	389 (0.6)	100	236 (1.8)	60.7	7 (0)	1.8	2 (0)	0.5	110 (0.7)	28.3	8 (0.2)	2.1	9 (1.7)	2.3	3 (0.2)	0.8	0 (0)	0	14 (0.3)	3.6
Staphylococcus hominis	301 (0.5)	100	272 (2.1)	90.4	0 (0)	0	2 (0)	0.7	12 (0.1)	4	2 (0)	0.7	7 (1.3)	2.3	1 (0.1)	0.3	0 (0)	0	5 (0.1)	1.7
staphylococci	8299 (12.7)	100	2611 (19.9)	31.5	305 (1.9)	3.7	472 (4.5)	5.7	3530 (23.8)	42.5	742 (18.3)	8.9	79 (14.6)	1	124 (6.8)	1.5	1 (0.2)	0		

Table 1.14 Isolates percentages across Regional Centers of *S. aureus*, MRSA, MSSA and CoNS isolated from all samples (Except Faeces)

Regional Center	Total Isolates	*S. aureus*	*MSSA*	*MRSA*	*Staphylococcus haemolyticus*	*S. epidermidis*	*S. lugdunensis*	*Staphylococcus hominis*	*Staphylococcus saprophyticus*	*Staphylococcus spp.*
	n	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC4	9277 (14.3)	1081 (11.7)	792 (8.5)	288 (3.1)	60 (0.6)	26 (0.3)	5 (0.1)	5 (0.1)	0 (0)	5 (0.1)
RC1	6092 (9.4)	540 (8.9)	286 (4.7)	253 (4.2)	209 (3.4)	157 (2.6)	2 (0)	60 (1)	10 (0.2)	69 (1.1)
RC3	4366 (6.7)	345 (7.9)	213 (4.9)	132 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	409 (9.4)
RC14	3924 (6)	660 (16.8)	408 (10.4)	252 (6.4)	3 (0.1)	7 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)
RC18	3097 (4.8)	519 (16.8)	314 (10.1)	205 (6.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC19	2391 (3.7)	112 (4.7)	58 (2.4)	54 (2.3)	182 (7.6)	49 (2)	1 (0)	125 (5.2)	4 (0.2)	23 (1)
RC10	6547 (10.1)	450 (6.9)	299 (4.6)	127 (1.9)	5 (0.1)	10 (0.2)	1 (0)	4 (0.1)	0 (0)	2 (0)
RC15	3511 (5.4)	412 (11.7)	218 (6.2)	194 (5.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	27 (0.8)
RC9	2202 (3.2)	204 (9.3)	104 (4.7)	99 (4.5)	56 (2.5)	22 (1)	31 (1.4)	54 (2.5)	2 (0.1)	42 (1.9)
RC5	2709 (4.2)	232 (8.6)	164 (6.1)	68 (2.5)	29 (1.1)	65 (2.4)	4 (0.1)	18 (0.7)	0 (0)	19 (0.7)

Regional Center	Total Isolates	*S. aureus*	*MSSA*	*MRSA*	*Staphylococcus haemolyticus*	*S epidermidis*	*Staphylococcus lugdunensis*	*Staphylococcus hominis*	*Staphylococcus saprophyticus*	*Staphylococcus spp.*
RC2	2179 (3.4)	218 (10)	155 (7.1)	54 (2.5)	22 (1)	15 (0.7)	0 (0)	17 (0.8)	0 (0)	2 (0.1)
RC17	2126 (3.3)	250 (11.8)	134 (6.3)	116 (5.5)	17 (0.8)	3 (0.1)	0 (0)	2 (0.1)	0 (0)	1 (0)
RC6	4812 (7.4)	227 (4.7)	100 (2.1)	127 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC16	1606 (2.5)	182 (11.3)	66 (4.1)	115 (7.2)	6 (0.4)	12 (0.7)	0 (0)	0 (0)	3 (0.2)	16 (1)
RC20	1861 (2.9)	197 (10.6)	54 (2.9)	143 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC7	2619 (4)	173 (6.6)	66 (2.5)	106 (4)	7 (0.3)	4 (0.2)	0 (0)	2 (0.1)	1 (0)	0 (0)
RC12	1450 (2.2)	116 (8)	61 (4.2)	55 (3.8)	8 (0.6)	8 (0.6)	0 (0)	4 (0.3)	1 (0.1)	1 (0.1)
RC21	1791 (2.8)	131 (7.3)	44 (2.5)	87 (4.9)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	3 (0.2)
RC13	670 (1)	100 (14.9)	42 (6.3)	56 (8.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	26 (3.9)
RC8	598 (0.9)	66 (11)	46 (7.7)	20 (3.3)	4 (0.7)	11 (1.8)	0 (0)	9 (1.5)	0 (0)	3 (0.5)
RC11	1161 (1.8)	65 (5.6)	30 (2.6)	31 (2.7)	7 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total National	64989	6280	3654	2582	615	389	44	301	21	648

Table 1.15: Location-wise isolates percentage of *S. aureus*, MSSA, MRSA and CoNS isolated from all samples across OPD, Ward and ICU

Organisms	All Specimen			
	Total	OPD	Ward	ICU
Staphylococcus aureus	6281/65561 (9.6)	2251/16298 (13.8)	3392/35847 (9.5)	638/13416 (4.8)
MSSA	3655/65561 (5.6)	1415/16298 (8.7)	1914/35847 (5.3)	326/13416 (2.4)
MRSA	2582/65561 (3.9)	819/16298 (5)	1459/35847 (4.1)	304/13416 (2.3)
CoNS	2018/65561 (3.1)	425/16298 (2.6)	1064/35847 (3)	529/13416 (3.9)

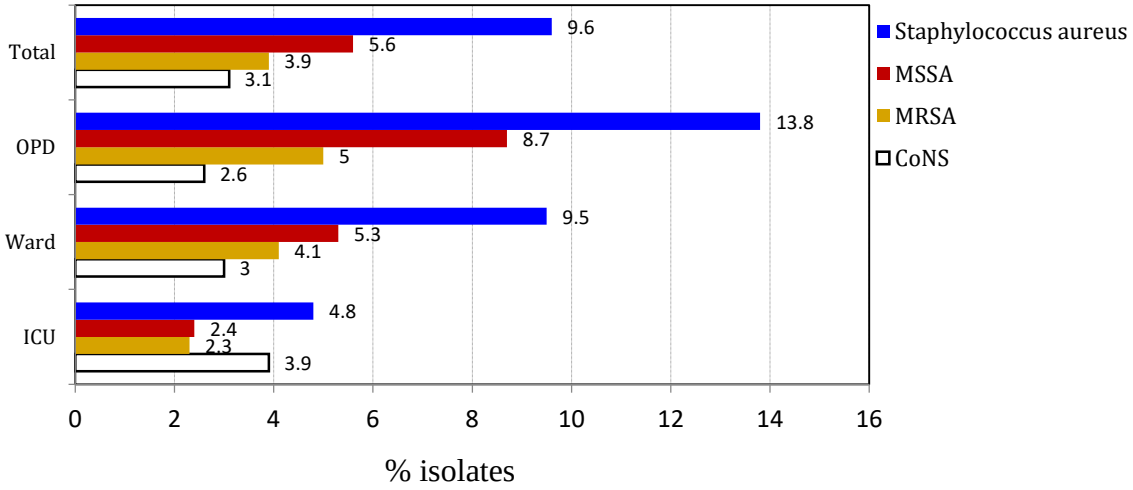


Figure 1.12: Location-wise Isolation pattern of *Staphylococcus aureus*, CoNS, MRSA, MSSA isolated from all samples across OPD, Ward and ICU

Table 1.16: Yearly isolation trend of *Staphylococcus* species

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Staphylococcus aureus	960/7237 (13.3)	5708/45521 (12.5)	8644/74295 (11.6)	12320/108465 (11.4)	6281/65561 (9.6)
Staphylococcus spp.	387/7237 (5.3)	1216/45521 (2.7)	1689/74295 (2.3)	1525/108465 (1.4)	648/65561 (1)
Staphylococcus haemolyticus	33/7237 (0.5)	628/45521 (1.4)	863/74295 (1.2)	805/108465 (0.7)	615/65561 (0.9)
Staphylococcus epidermidis	51/7237 (0.7)	575/45521 (1.3)	894/74295 (1.2)	705/108465 (0.6)	389/65561 (0.6)
Staphylococcus hominis	18/7237 (0.2)	381/45521 (0.8)	489/74295 (0.7)	432/108465 (0.4)	301/65561 (0.5)

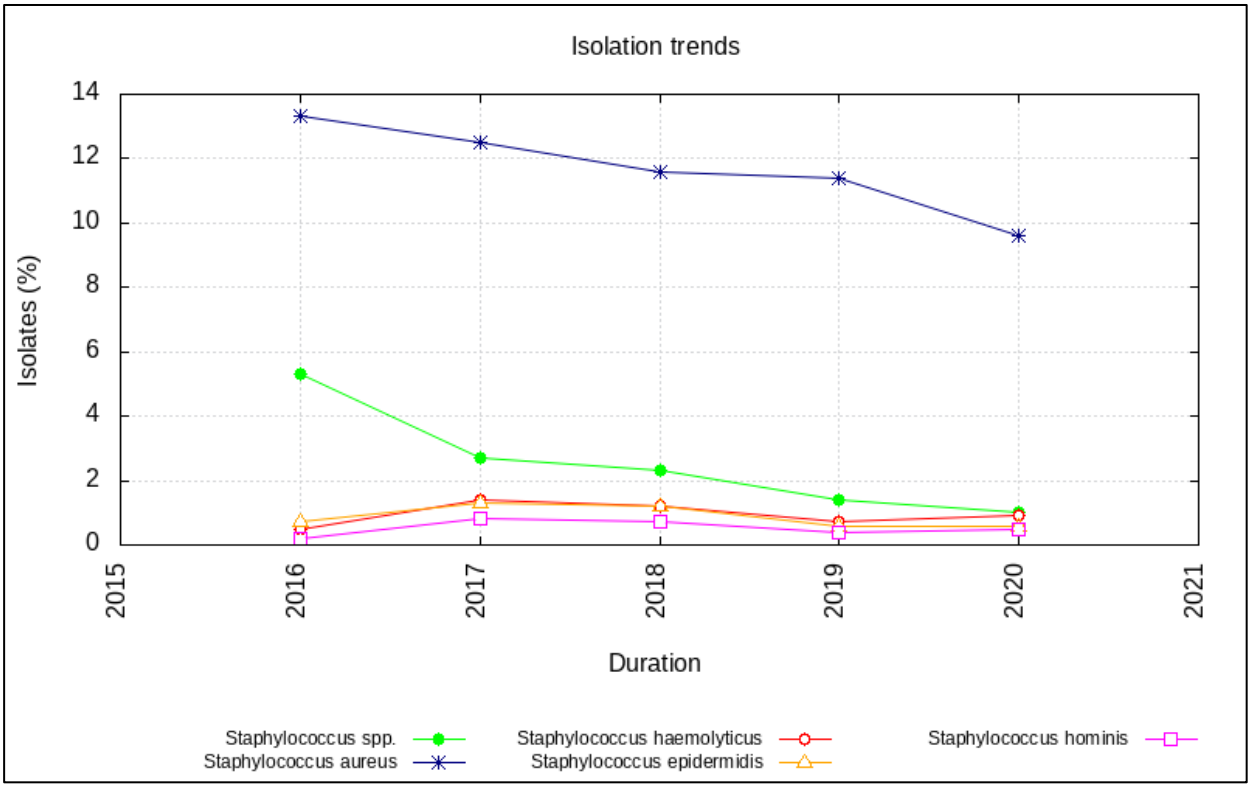


Figure 1.13 Yearly isolation trends of *Staphylococcus* species

Enterococci

Enterococci constituted overall 7.3% of all the isolates (Table 1.17). Among the *Enterococcus* species, *E. faecalis* and *E. faecium* accounted for 85% of all the total isolates, both *E. faecalis* (43.7%) and *E. faecium* (41.5%) were the predominant species. *E. faecium* was relatively more frequent in the CSF (6.1%) and urine (4.9%) while *E. faecalis* was more frequent in the urine (5.7%) and deep infections (5%) (Table 1.17 and Figure 1.14). Regional centre wise distribution showed the predominance of isolation of *E. faecalis* in RC10 (8.1%) and *E. faecium* in RC12 (5.4%) (Table 1.18).

The trend analysis over the years 2016 – 2020 have shown a steady increase in the isolation rates of *E. faecium* from 2.5% to 3% and *E. faecalis* from 1.7% to 3.2% in 2016 to 2020 respectively (Table 1.19 and Figure 1.15).

Table 1.17: Specimen wise distribution of *Enterococcus* species

Isolate	Culture positive																			
	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=541		SS n=1823		Faeces n=572		Others n=4293	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterococci	4798 (7.3)	100	1048 (8)	21.8	1930 (12.1)	40.2	23 (0.2)	0.5	844 (5.7)	17.6	351 (8.7)	7.3	53 (9.8)	1.1	211 (11.6)	4.4	10 (1.7)	0.2	328 (8.1)	**6.8**
Enterococcus faecalis	2101 (3.2)	100	318 (2.4)	15.1	912 (5.7)	43.4	6 (0.1)	0.3	456 (3.1)	21.7	203 (5)	9.7	10 (1.8)	0.5	40 (2.2)	1.9	1 (0.2)	0	155 (3.8)	**7.4**
Enterococcus faecium	1994 (3)	100	556 (4.2)	27.9	788 (4.9)	39.5	8 (0.1)	0.4	287 (1.9)	14.4	104 (2.6)	5.2	33 (6.1)	1.7	82 (4.5)	4.1	9 (1.6)	0.5	127 (3.1)	**6.4**
Enterococcus spp.	703 (1.1)	100	174 (1.3)	24.8	230 (1.4)	32.7	9 (0.1)	1.3	101 (0.7)	14.4	44 (1.1)	6.3	10 (1.8)	1.4	89 (4.9)	12.7	0 (0)	0	46 (1.1)	**6.5**

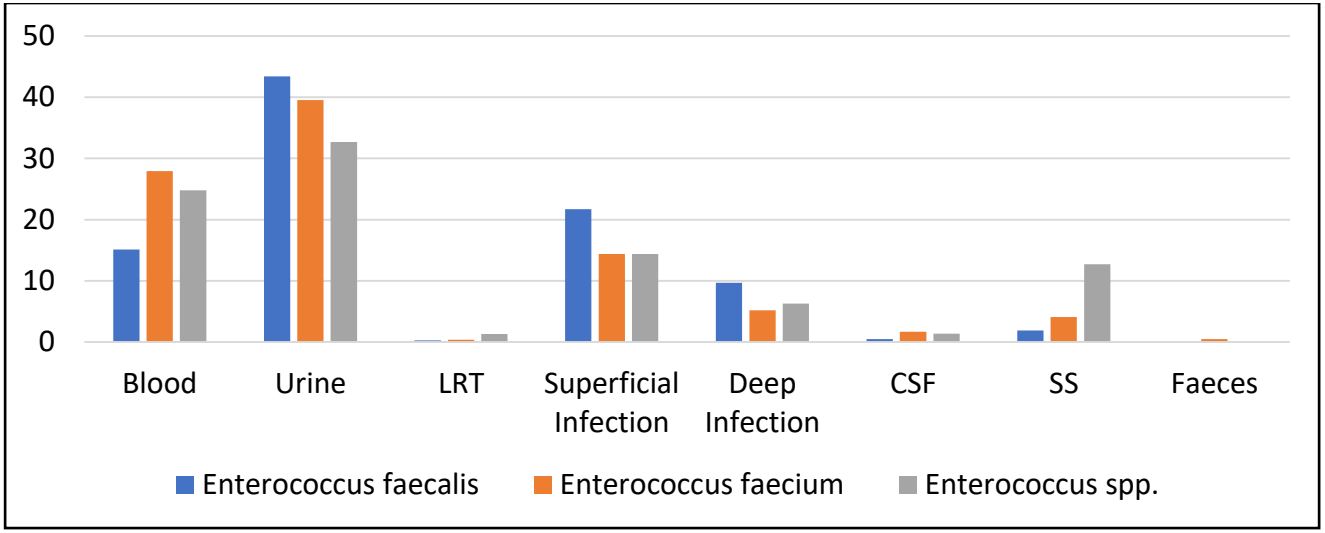


Figure 1.14: Specimen wise distribution of *Enterococcus* species

Table 1.18 Isolates percentages across Regional Centers of *Enterococcus faecalis, Enterococcus faecium, Enterococcus* spp. from All Specimen (Except Faeces)

Regional Center	Total Isolates	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus spp.*
	n	n(%)	n(%)	n(%)
RC4	9277	611 (6.6)	483 (5.2)	97 (1)
RC10	6547	533 (8.1)	277 (4.2)	62 (0.9)
RC1	6092	74 (1.2)	202 (3.3)	79 (1.3)
RC6	4812	98 (2)	204 (4.2)	0 (0)
RC3	4366	42 (1)	102 (2.3)	122 (2.8)
RC18	3097	113 (3.6)	137 (4.4)	0 (0)
RC20	1861	66 (3.5)	59 (3.2)	110 (5.9)
RC19	2391	128 (5.4)	51 (2.1)	24 (1)
RC17	2126	70 (3.3)	89 (4.2)	1 (0)
RC5	2709	63 (2.3)	72 (2.7)	14 (0.5)
RC16	1606	56 (3.5)	85 (5.3)	1 (0.1)
RC9	2202	104 (4.7)	40 (1.8)	0 (0)
RC12	1450	31 (2.1)	79 (5.4)	19 (1.3)
RC13	670	1 (0.1)	1 (0.1)	80 (11.9)
RC15	3511	3 (0.1)	14 (0.4)	53 (1.5)
RC14	3924	54 (1.4)	20 (0.5)	0 (0)
RC11	1161	7 (0.6)	41 (3.5)	0 (0)
RC21	1791	13 (0.7)	7 (0.4)	26 (1.5)
RC7	2619	22 (0.8)	7 (0.3)	7 (0.3)
RC8	598	8 (1.3)	14 (2.3)	7 (1.2)
RC2	2179	3 (0.1)	1 (0)	1 (0)
Total National	**64989**	**2100**	**1958**	**703**

Table 1.15: Yearly isolation trend of *Enterococcus* species

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Enterococcus faecium	180/7237 (2.5)	937/45521 (2.1)	1476/74295 (2)	2700/108465 (2.5)	1994/65561 (3)
Enterococcus faecalis	126/7237 (1.7)	1034/45521 (2.3)	2014/74295 (2.7)	2895/108465 (2.7)	2101/65561 (3.2)
Enterococcus spp.	31/7237 (0.4)	421/45521 (0.9)	711/74295 (1)	1079/108465 (1)	703/65561 (1.1)

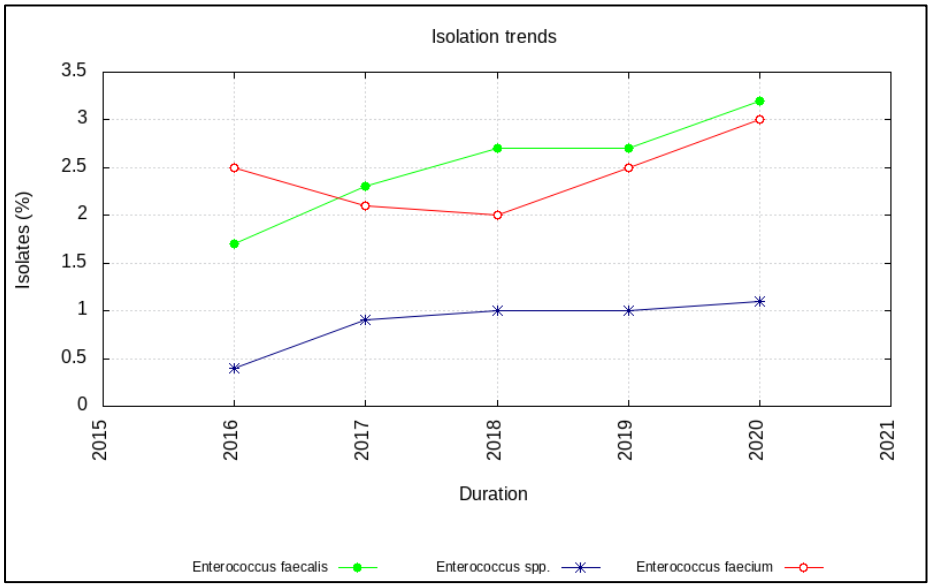


Figure 1.15 Yearly isolation trends of *Enterococcus* species

Fungal species

Total number of yeast isolates studied during the year 2020 was 1529, of those 66.4% (1016) were isolated from blood. Majority of the isolates were from *Candida tropicalis* (n=500) followed by *Candida albican*s (n=364) (Table 1.20). In the distribution of fungi species in different specimens, *C. tropicalis* was the predominant isolates in the genital (10.34%) followed by blood (2.56%), *Candida albican*s was also the predominant isolates in the genital (75.86%) followed by others (1.63) and blood (1.11%) (Table 1.20). Among

clinical settings, in ICUs, *C. tropicalis and C. albican*s were common isolates from the ICU (1.12%) and (0.75%) respectively followed by ward and OPD (Table 1.21 and Figure 1.16).

Yearly isolation trend showed that there is a steady decline in isolation of *C. tropicalis* from 1% in 2016 to 0.76% in 2020, with a slight increase from last year 0.57 in 2019 to 0.76 in 2020. Yearly isolation trend *of Candida albican*s showed a steady decline from 1% in 2016 to 0.56 in 2020. Both *C. auris and C. parapsilosis* isolates showed an increased trend from 2016 to 2020 (Table 1.22 & Figure 1.17).

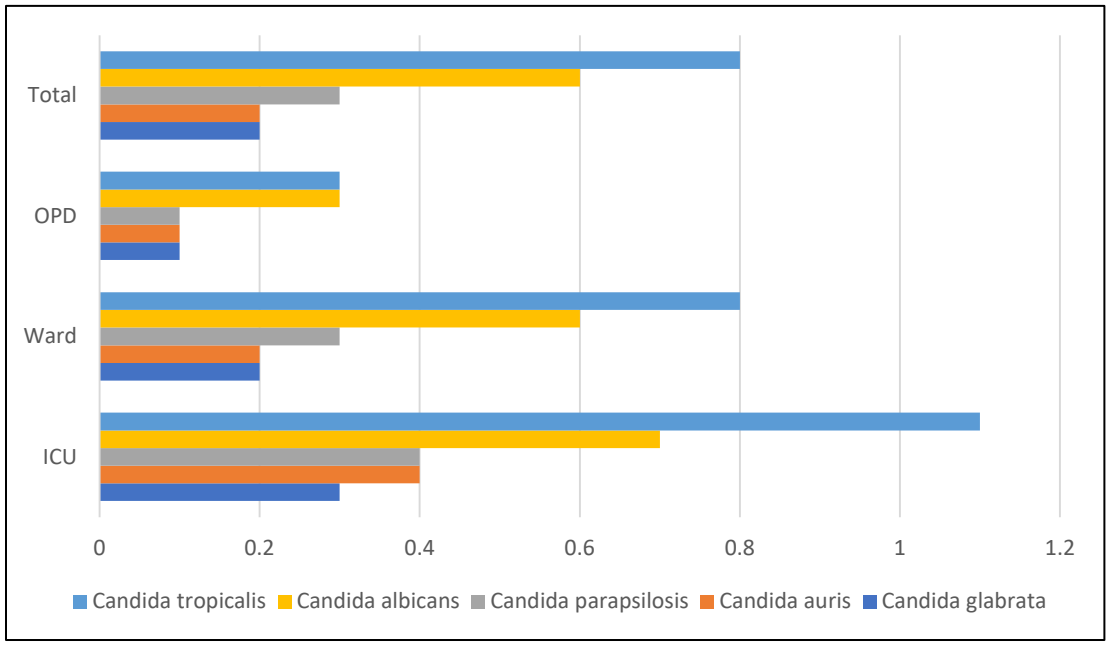


Figure 1.16: Location-wise pattern of *Candida* species isolated from all samples across OPD, Wards and ICUs.

Table 1.20 Fungi species isolated from different sample types

Isolates	Total n=65561		Blood n=13109		Urine n=16009		LRT n=10557		Superficial Infection n=14843		Deep Infection n=4055		CSF n=*0		Genital n=29		Others n=4293	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Fungal isolates	**1680 (2.56)**	**100**	**1079 (8.23)**	**64.22**	**196 (1.22)**	**11.66**	**116 (1.09)**	**6.90**	**51 (0.34)**	**3.03**	**30 (0.73)**	**1.78**	***0 (-)**	**0**	**29 (100)**	**1.72**	**143 (3.33)**	**8.51**
Candida tropicalis	500 (0.76)	100	336 (2.56)	67.20	87 (0.54)	17.40	14 (0.13)	2.80	7 (0.05)	1.40	10 (0.24)	2.00	*0 (-)	0	3 (10.34)	0.60	26 (0.61)	5.2 0
Candida albicans	364 (0.55)	100	145 (1.11)	39.83	65 (0.41)	17.85	33 (0.31)	9.07	15 (0.10)	4.12	8 (0.19)	2.20	*0 (-)	0	22 (75.86)	6.04	70 (1.63)	19.23
Candida parapsilosis	189 (0.28)	100	169 (1.28)	89.42	6 (0.04)	3.17	0 (0)	0	7 (0.05)	3.70	2 (0.04)	1.06	*0 (-)	0	0 (0)	0	2 (0)	1.06
Candida auris	121 (0.18)	100	96 (0.73)	79.34	12 (0.07)	9.92	4 (0.03)	3.31	5 (0.03)	4.13	3 (0.07)	2.48	*0 (-)	0	0 (0)	0	1 (0.02)	0.83
Candida glabrata	113 (0.17)	100	47 (0.36)	41.59	19 (0.12)	16.81	10 (0..09)	8.85	8 (0.05)	7.08	5 (0.12)	4.42	*0 (-)	0	4 (13.79)	3.54	18 (0.42)	15.93
Candida utilis	112 (0.17)	100	112 (0.85)	100	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	*0 (-)	0	0 (0)	0	0 (0)	0
Candida krusei	79 (0.12)	100	70 (0.53)	88.61	3 (0.02)	3.80	1 (0)	1.27	0 (0)	0	1 (0)	1.27	*0 (-)	0	0 (0)	0	3 (0.07)	3.80
Candida pelliculosa	11 (0.02)	-	11 (0.08)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	*0 (-)	-	0 (0)	-	0 (0)	-
Candida kefyr	8 (0.01)	-	4 (0.03)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	*0 (-)	-	0 (0)	-	3 (0.07)	-
Candida lusitaniae	8 (0.01)	-	7 (0.05)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	*0 (-)	-	0 (0)	-	1 (0.02)	-
Candida	**1529 (2.33)**	**100**	**1016 (7.75)**	**66.45**	**194 (1.21)**	**12.69**	**63 (0.59)**	**4.12**	**42 (0.28)**	**2.75**	**30 (0.74)**	**1.96**	***0 (-)**	**0**	**29 (100)**	**1.90**	**124 (2.88)**	**8.11**

1. Percentages are out of paricular specimen (column).
2. Percentages in rows below Culture positive are out of Culture positive in respective columns.
3. **Blood** includes: Blood-central catheter, Blood-peripheral and Peripheral catheter-blood.
4. **LRT** (Lower Respiratory Tract) includes: BAL, Sputum, Lung aspirate, Endotracheal aspirate (ETA) and Lobectomy tissue (Lung tissue).
5. **Superficial Infection** includes: SST (Skin & Soft Tissue), Pus/exudate, Wound swab, Superficial Biopsy and Superficial Tissue.
6. **Deep Infection** includes: Abscess aspirate, Pus aspirate, Deep Biopsy and Deep Tissue.
7. **SS** (Sterile sites) includes: Fluid from sterile spaces, Abdominal fluid, Intracostal tube fluid, Pancreatic drain fluid, Pericardial fluid, Peritoneal fluid and Pleural fluid.

Table 1.21: *Candida* species isolated from all samples across OPD, Ward and ICUs

	All Specimens			
	Total	**OPD**	**Ward**	**ICU**
Total Fungal Isolates	1680/65561 (2.56)	163/16298 (1.00)	1044/35847 (2.91)	473/13416 (3.53)
Candida tropicalis	500/65561 (0.76)	46/16298 (0.28)	304/35847 (0.85)	150/13416 (1.12)
Candida albicans	364/65561 (0.56)	50/16298 (0.31)	214/35847 (0.60)	100/13416 (0.75)
Candida parapsilosis	189/65561 (0.29)	21/16298 (0.13)	119/35847 (0.33)	49/13416 (0.37)
Candida auris	121/65561 (0.18)	11/16298 (0.07)	63/35847 (0.18)	47/13416 (0.36)
Candida glabrata	113/65561 (0.17)	16/16298 (0.10)	62/35847 (0.18)	35/13416 (0.26)
Candida utilis	112/65561 (0.17)	0/0 (-)	85/35847 (0.24)	27/13416 (0.20)
Candida krusei	79/65561 (0.12)	3/16298 (0.02)	62/35847 (0.17)	14/13416 (0.10)
Candida pelliculosa	11/65561 (0.02)	0/0 (-)	2/35847 (0.01)	9/13416 (0.07)
Candida lusitaniae	8/65561 (0.01)	0/0 (-)	7/35847 (0.02)	1/13416 (0.01)
Candida kefyr	8/65561 (0.01)	1/16298 (0.01)	4/35847 (0.01)	3/13416 (0.02)

Table 1.22 Yearly trends for isolation of *Candida* species isolated from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Candida tropicalis	78/7237 (1.08)	628/45521 (1.38)	494/74295 (0.66)	621/108465 (0.57)	500/65561 (0.76)
Candida albicans	74/7237 (1.02)	452/45521 (0.99)	560/74295 (0.75)	652/108465 (0.60)	364/65561 (0.56)
Candida parapsilosis	7/7237 (0.09)	105/45521 (0.23)	134/74295 (0.18)	232/108465 (0.21)	189/65561 (0.29)
Candida auris	0/7237 (0)	17/45521 (0.04)	55/74295 (0.07)	117/108465 (0.11)	121/65561 (0.18)
Candida glabrata	22/7237 (0.30)	136/45521 (0.30)	179/74295 (0.24)	185/108465 (0.17)	113/65561 (0.17)

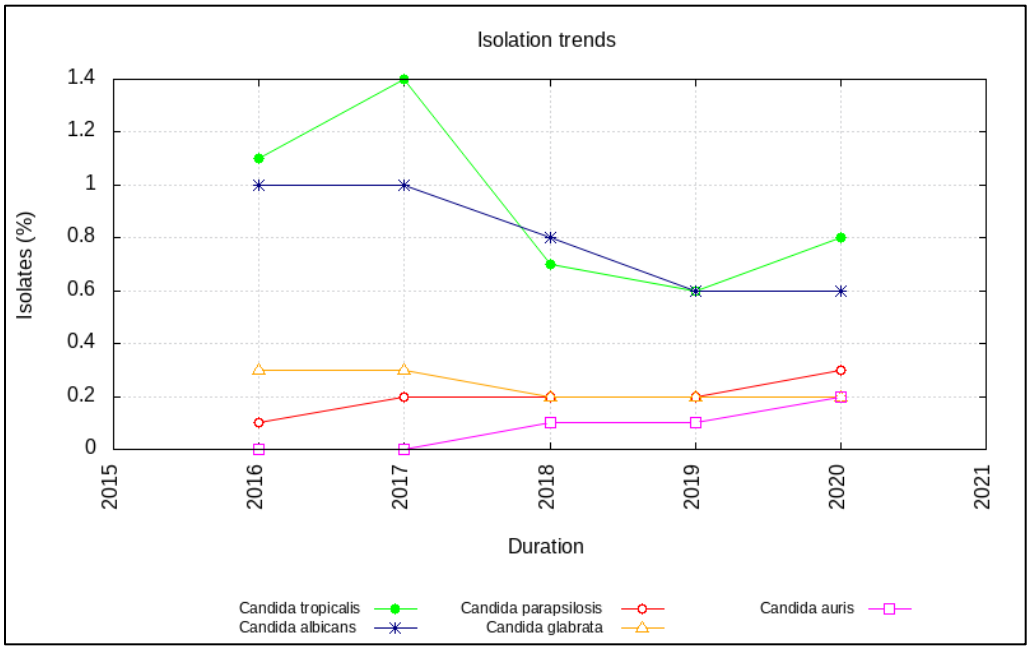


Figure 1.17: Yearly trends for isolation of *Candida* species isolated from all samples.

Table 1.23 Isolation patterns of *Aspergillus* species from all specimens

Organism	Total
Aspergillus flavus	48/65561 (0.1)
Aspergillus fumigatus	15/65561 (0)
Aspergillus terreus	5/65561 (0)
Aspergillus niger	2/65561 (0)
Aspergillus versicolor	1/65561 (0)

Diarrheal pathogens

A total of 390 diarrheal pathogen isolates were studied during the year 2020 which constituted 0.6% of total isolates (Table 1.1). The predominant species among diarrheal pathogens isolated from faeces sample identified was *Escherichia coli Diarrheagenic* (30.8%) followed by *Shigella spp* (25.4%) and *Aeromonas spp* (23.3%). *Vibrio spp* and *Salmonella spp* was isolated in 11.8% and 8.7% respectively (Table 1.24). From non-faecal specimens, *Aeromonas spp* was isolated (n=50) and constituted 0.1% of total cultures (Table 1.25).

Table 1.24: Isolation rates of faecal isolates from faeces sample isolated in 2020

Isolates	n	% Isolation from faecal isolates (n= 331)	% Isolation from total positive cultures (n=65561)
Aeromonas spp.	**77**	**23.3**	**0.1**
Escherichia coli Diarrheagenic	**102**	**30.8**	**0.2**
Shigella	**84**	**25.4**	**0.1**
Shigella flexneri	55	16.6	0.1
Shigella sonnei	14	3.6	0
Shigella spp	12	3.6	0
Shigella boydii	2	0.6	0
Shigella dysenteriae	1	0.3	0
Vibrio	**39**	**11.8**	**0.1**
Vibrio cholerae	31	9.3	0
Vibrio spp.	8	2.4	0
Salmonella	**29**	**8.7**	**0**
Salmonella spp. Faecal	24	7.2	0
Salmonella Typhimurium Faecal	3	0.9	0
Salmonella Enteritidis	2	0.6	0

Table 1.25: Isolation rates of Diarrheagenic pathogens from non-faecal specimen isolated in 2020

Isolates	n	% Isolation from total positive cultures except faeces (n=64989)
Aeromonas spp.	50	0.1
Escherichia coli Diarrhoeagenic	0	0
Shigella	1	0
Vibrio	1	0
Salmonella	7	0

Diarrheagenic pathogens were predominantly isolated from patients in OPD and wards (Table 1.26). *Escherichia coli* Diarrheagenic was mainly isolated in OPD (34.7%) followed by ward (7.6%), while the *Aeromonas spp* was predominant in OPD (15.3%), followed by ICU (13.6%) and ward (12.2%) (Table 1.26 and Figure 1.18). *Shigella flexneri* was predominant in OPD, *Vibrio cholerae* in ward and *Salmonella* spp. faecal in (13.6%) ICU. The isolation trend over the period of four years (2016– 2020) showed decreasing trend in the isolation of *Aeromonas spp*. whereas, the isolation trend of *Shigella spp* and *Vibrio spp* showed an increasing trend from last year (Table 1.27 and Figure 1.19).

Table 1.26: Location-wise Isolation pattern of top 5 faecal isolates isolated from Faeces across OPD, Ward and ICU.

Organism	Total	OPD	Ward	ICU
Escherichia coli Diarrheagenic	102/572 (17.8)	77/222 (34.7)	25/328 (7.6)	0/0 (-)
Aeromonas spp.	77/572 (13.5)	34/222 (15.3)	40/328 (12.2)	3/22 (13.6)
Shigella flexneri	55/572 (9.6)	26/222 (11.7)	28/328 (8.5)	1/22 (4.5)
Vibrio cholerae	31/572 (5.4)	2/222 (0.9)	28/328 (8.5)	1/22 (4.5)
Salmonella spp. Faecal	24/572 (4.2)	7/222 (3.2)	14/328 (4.3)	3/22 (13.6)

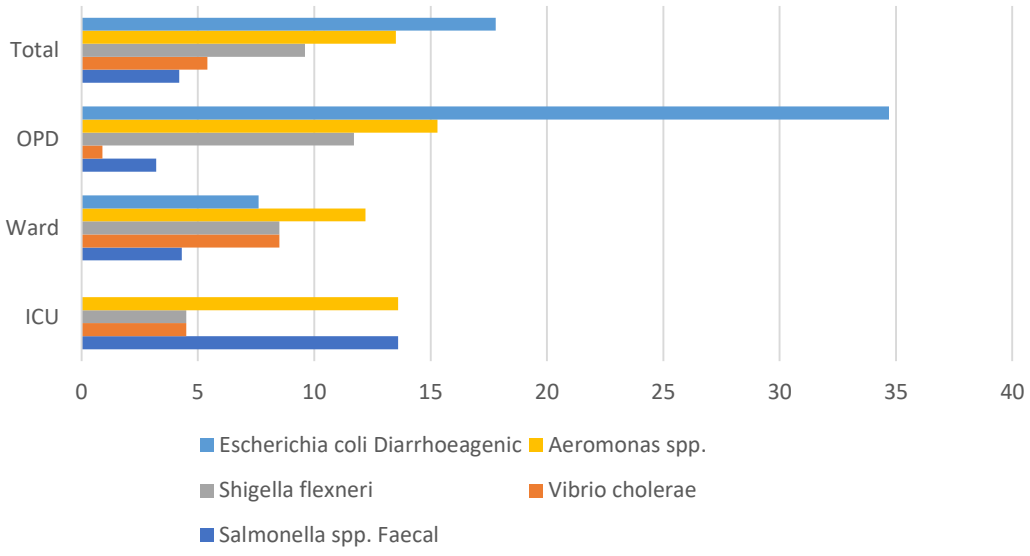


Figure 1.18: Location-wise Isolation pattern of top 5 faecal isolates isolated from Faeces across OPD, Ward and ICU

Table 1.27 Yearly Isolation trends of top 5 faecal isolates isolated from faeces.

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)
Escherichia coli Diarrhoeagenic	0/55 (0)	0/501 (0)	0/621 (0)	134/1063 (12.6)	102/572 (17.8)
Aeromonas spp.	21/55 (38.2)	131/501 (26.1)	114/621 (18.4)	170/1063 (16)	77/572 (13.5)
Shigella flexneri	7/55 (12.7)	89/501 (17.8)	47/621 (7.6)	95/1063 (8.9)	55/572 (9.6)
Vibrio cholerae	1/55 (1.8)	24/501 (4.8)	25/621 (4)	39/1063 (3.7)	31/572 (5.4)
Salmonella spp. Faecal	0/55 (0)	20/501 (4)	39/621 (6.3)	60/1063 (5.6)	24/572 (4.2)

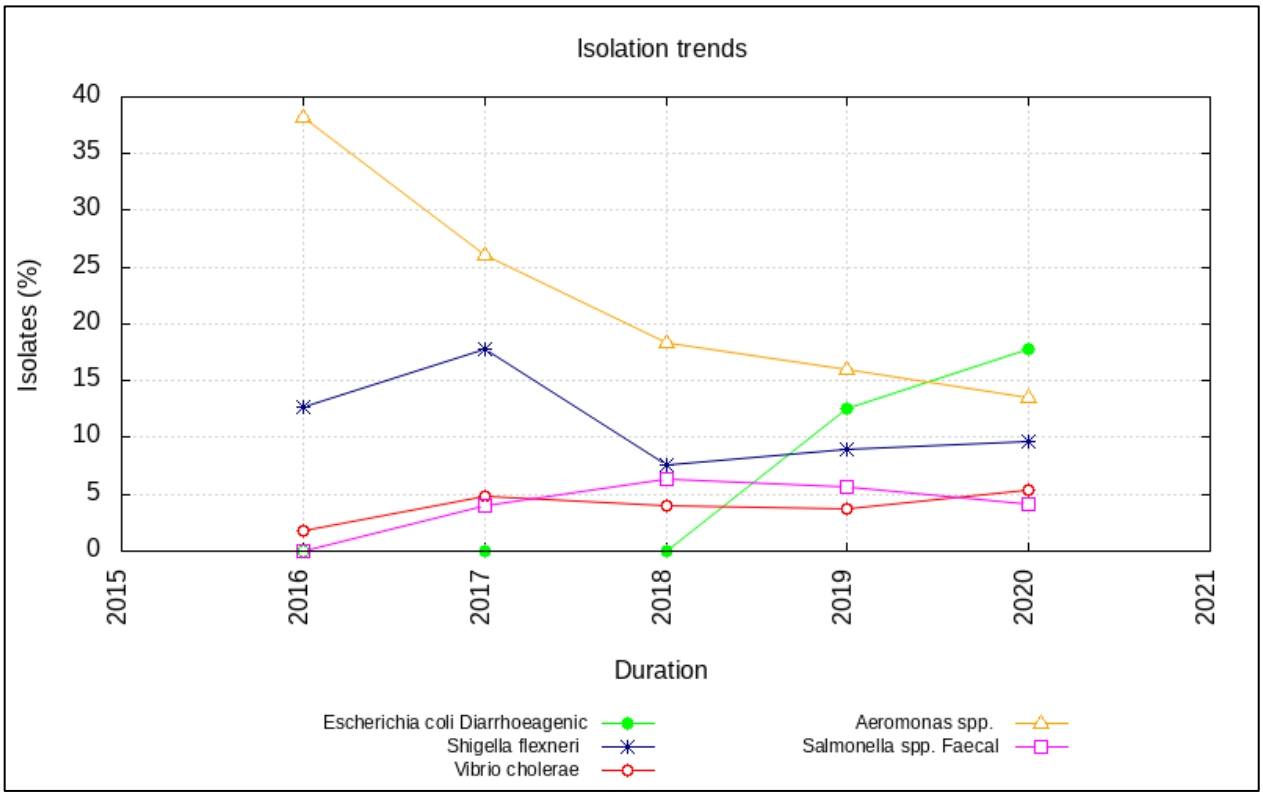


Figure 1.19: Yearly Isolation trends of top 5 faecal isolates isolated from Faeces

Chapter 2 Fungal pathogens

Antifungal susceptibility profile of *Candida* species isolated from all specimens revealed 98.2% fluconazole susceptibility in *C. utilis*, 95.0% in *C. tropicalis*, and 92.6% in *C. albicans* but only 4.2% in *C. auris:* voriconazole susceptibility was 96.9% in *C. albicans,* 96.7% in *C. tropicalis,* 91.1% in *C. glabrata*, and 34.6% in *C. auris*. More than 95% of *C. albicans* and *C. tropicalis* were susceptible to echinocandins. However, *C. auris* showed high resistance to echinocandins (caspofungin-85.5%, anidulafungin- 88.9% and micafungin - 94.7%) (Table 2.1). *C. parapsilosis*is often reported as less susceptible to echinocandins. However, *C. parapsilosis* in our study exhibited comparable susceptibility to echinocandins (Table 2.1). *C. utilis*, anemerging species, was found susceptible to all major classes of antifungals (Table 2.1). Although two most common species, *C. albicans* and *C. tropicalis* exhibited azole susceptibility in >90%, increasing resistance percentage over the years among these species is a major concern (Table 2.1-2.3). However, increased susceptibility to fluconazole and voriconazole among *C. tropicalis* was witnessed in this year compared to previous year. *C. tropicalis* isolated from blood was more susceptible to different antifungals compared to isolates obtained from urine (Table 2.2 and 2.3). *C. albicans* was predominantly isolated from genital samples (Table 2.4). Decrease in susceptibility to majority of the antifungals among *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata* needs to be cautiously monitored (Figure 2.1 – 2.6). *Aspergillus flavus* was the most frequently isolated mold followed by *A. fumigatus. A. flavus* was less susceptible to amphotericin B and caspofungin compared to *A. fumigatus* (Table 2.5). Azole resistant *Aspergillus* causing concerns in western world is not noted in our strains.

Invasive infections cases due *C. auris* are increasing across the country from the past 5 years. Isolation of *C. auris* from various regional centers during the current reporting year is provided in Figure 2.7. We witnessed eight cases of *C. auris* infection among patients hospitalized in the nodal centre within two months (November-January). Active surveillance was conducted across various wards and ICUs of the hospital. Many patients were colonized by *C. auris*. Strict infection control measures were adopted to halt the further transmission and infections. To rapidly isolate *C. auris* from the clinical as well as environmental samples, we developed a novel selective medium. This selective medium is useful in diagnostic setups where costly molecular tests are not available. Due to its high sensitivity and specificity, this medium can be usedin routine screening of suspected *C. auris* isolates. It could be particularly useful in regions with a preponderance of clades I, III, and IV of *C. auris*. For those regions with a predominance of clade II, medium slightly modified form (lower salt concentration and longer incubation at ≤42°C)

A MALDI-TOF MS-based identification protocol was developed and standardized for rapid identification of yeasts directly from blood culture bottles. Mean time for Direct-ID using

this protocol was 75 min per sample which is almost 24 h earlier than conventional identification methods. Additionally, antifungal susceptibility results were also available within 24 h compared to routine culture-AFST (2–3 days).

Molecular mechanisms of resistance in *C. tropicalis, C. parapsilosis* (azole resistance) and *C. auris* (azole and echinocandin resistance) were evaluated. Previously, we demonstrated that over-expression of multi-drug transporters genes; mutations in ergosterol pathway genes and transcription factors involved in regulating the expression of azole –target gene *ERG*11 and multidrug efflux transporters played a role in resistance in *C. tropicalis*. We also developed a MALDI TOF MS-based stable isotope method for the rapid detection of fluconazole resistance in *C. tropicalis*. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 92.31%, 100%, 100%, 90.48% and 95.56%, respectively. Three different methods for the detection of mutation in the *ERG11* gene of fluconazole-resistant *C. tropicalis* were also developed, such as Tetra-primer Amplification Refractory Mutation System (T-ARMS), Restriction Site mutation (RMS) and High-Resolution Melting curve (HRM) analysis. *C. parapsilosis* isolates exhibiting azole resistance had missense mutations, G1193T and A395T leading to the substitution in the amino acid R398I and Y132F respectively. Similarly, azole-resistant *C. auris* harbored mutation at 395th position (A to T) leading to substitution of tyrosine to phenylalanine at 132nd position in ERG11p.

Laboratory support was extended to 16 regional centers which are part of this project. Apart from them, we also received request from other Institutes for molecular analysis. In this regard, we received 12 environmental isolates from Agharkar Research Institute, Pune. MALDI TOF MS was not able to identify them with satisfactory scores. Therefore, sequencing of ITS1 and ITS4 and BLAST scores showed them close matches of *C.blankii*. Accordingly, we updated the MALDI database for this species for subsequent identification. Antifungal susceptibility testing revealed that none of the isolates were resistant to any antifungal tested. Ten *C. auris* outbreak isolates were received from JSS Medical College, Mysuru, Karnataka. These isolates were confirmed bysequencing and typed by amplified fragment length polymorphism for their clonality. Additionally, we also determined the mechanism of resistance in these isolates.

***Clinical relevance*:** Fungal infections in hospitalized patients are increasing significantly. Majority of the fungal infections are caused by few common fungal agents nevertheless isolation rates of other species are also increasing requiring newer treatment strategies. Antibiotic susceptibility testing of these fungal isolates provides insight to the resistance pattern and aids in selecting appropriate agent for management of fungal infections. *C. auris*, multidrug-resistant yeast known to cause hospital outbreaks has been consistently isolated from regional centers across India. Majority of the *C. auris* isolates were resistant to fluconazole and incidences of echinocandin resistance are on the rise. Therefore, accurate identification, susceptibility testing, and infection control strategies are necessary for reducing *C. auris burden*. Reduced susceptibility to commonly used antifungals among

most frequently isolated fungal species such as *C. tropicalis, C. albicans* and *C. parapsilosis* limits treatment options. Previous studies indicated presence of significant changes at molecular level leading to adaptation to currently used antifungals. It is known that the mutations in the *ERG*11 gene responsible to produce lanosterol-14-α-demethylase enzyme impart resistance to fluconazole. In this study period, we also developed rapid methods for identifying these mutations which eventually would help in choosing appropriate antifungal drugs for optimal therapy. Development of rapid method for the detection of fungal pathogens and antifungal susceptibility testing directly from the blood culture bottle in case of candidemia improves the turn-around time for optimal management. The protocol would also help centers with limited resources. An outbreak of *C. auris* was detected in the nodal center and effective disinfection measures helped to restrict spread of the infection. *C. auris* was also isolated from an outbreak situation for the first time in a surgical ICU of a tertiary care hospital in southern India. *C. blankii* that has occasionally been associated with outbreaks was sent to the nodal center for identification. On performing MALDI TOF MS analysis, we noticed that the spectra for this species were not available in the database. We updated the database using these strains which in turn may help in identifying this species from clinical samples using MALDI-TOF.

Table 2.1: Susceptibility pattern of *Candida* species isolated from all samples

AMA	*Candida tropicalis* n=500	*Candida albicans* n=364	*Candida parapsilosis* n=189	*Candida auris* n=121	*Candida glabrata* n=113	*Candida utilis* n=112	*Candida krusei* n=79
Anidulafungin	146/149 (98)	94/94 (100)	48/48 (100)	24/27 (88.9)	45/57 (78.9)	104/105 (99)	53/53 (100)
Caspofungin	451/470 (96)	309/325 (95.1)	172/177 (97.2)	100/117 (85.5)	44/105 (41.9)	96/108 (88.9)	26/78 (33.3)
Fluconazole	473/498 (95)	337/364 (92.6)	136/188 (72.3)	5/119 (4.2)	70/82 (85.4)	109/111 (98.2)	3/76 (3.9)
Micafungin	344/351 (98)	252/259 (97.3)	110/112 (98.2)	89/94 (94.7)	73/74 (98.6)	38/38 (100)	20/24 (83.3)
Voriconazole	473/489 (96.7)	343/354 (96.9)	161/164 (98.2)	27/78 (34.6)	82/90 (91.1)	112/112 (100)	78/79 (98.7)

Table 2.2: Susceptibility pattern of *Candida* species isolated from blood

AMA	*Candida tropicalis* n=336	*Candida parapsilosis* n=169	*Candida albicans* n=145	*Candida utilis* n=112	*Candida auris* n=96	*Candida krusei* n=70	*Candida glabrata* n=47
Anidulafungin	102/104 (98.1)	44/44 (100)	49/49 (100)	104/105 (99)	21/23 (91.3)	52/52 (100)	17/22 (77.3)
Caspofungin	303/313 (96.8)	153/157 (97.5)	123/134 (91.8)	96/108 (88.9)	80/92 (87)	19/69 (27.5)	20/43 (46.5)
Fluconazole	319/335 (95.2)	118/168 (70.2)	133/145 (91.7)	109/111 (98.2)	5/94 (5.3)	3/68 (4.4)	25/30 (83.3)
Micafungin	211/216 (97.7)	93/95 (97.9)	92/97 (94.8)	38/38 (100)	70/74 (94.6)	*14/18 (-)	33/33 (100)
Voriconazole	319/327 (97.6)	143/146 (97.9)	137/139 (98.6)	112/112 (100)	22/57 (38.6)	70/70 (100)	34/36 (94.4)

* Less than 20 samples

Table 2.3: Susceptibility pattern of *Candida* species isolated from urine

AMA	*Candida tropicalis* n=87	*Candida albicans* n=65	*Candida glabrata* n=*19	*Candida auris* n=*12
Anidulafungin	*14/15 (-)	*7/7 (-)	*9/10 (-)	*1/1 (-)
Caspofungin	78/83 (94)	56/56 (100)	*11/19 (-)	*11/12 (-)
Fluconazole	80/87 (92)	58/65 (89.2)	*14/17 (-)	*0/12 (-)
Micafungin	82/84 (97.6)	54/54 (100)	*18/19 (-)	*10/11 (-)
Voriconazole	79/85 (92.9)	59/64 (92.2)	*10/13 (-)	*1/10 (-)

* Less than 20 samples

Table 2.4: Susceptibility pattern of *Candida* species isolated from genital samples

	Candida albicans n=22
Anidulafungin	*0/0 (-)
Caspofungin	*4/5 (-)
Fluconazole	20/22 (90.9)
Micafungin	*4/5 (-)
Voriconazole	21/22 (95.5)

* Less than 20 samples

Table 2.5: Susceptibility pattern of *Aspergillus* species isolated from all samples across different locations

AMA	*Aspergillus flavus*			*Aspergillus fumigatus*	
	Total n=48	Ward n=30	ICU n=*11	Total n=*15	Ward n=*14
	(S %)	(S %)	(S %)	(S %)	(S %)
Amphotericin B	34/48 (70.8)	20/30 (66.7)	*9/11	*14/15	*13/14
Caspofungin	22/48 (45.8)	10/30 (33.3)	*8/11	*2/15	*2/14
Itraconazole	44/48 (91.7)	28/30 (93.3)	*10/11	*13/15	*12/14
Posaconazole	40/48 (83.3)	26/30 (86.7)	*10/11	*13/15	*12/14
Voriconazole	46/48 (95.8)	29/30 (96.7)	*10/11	*15/15	*14/14

* Less than 20 samples

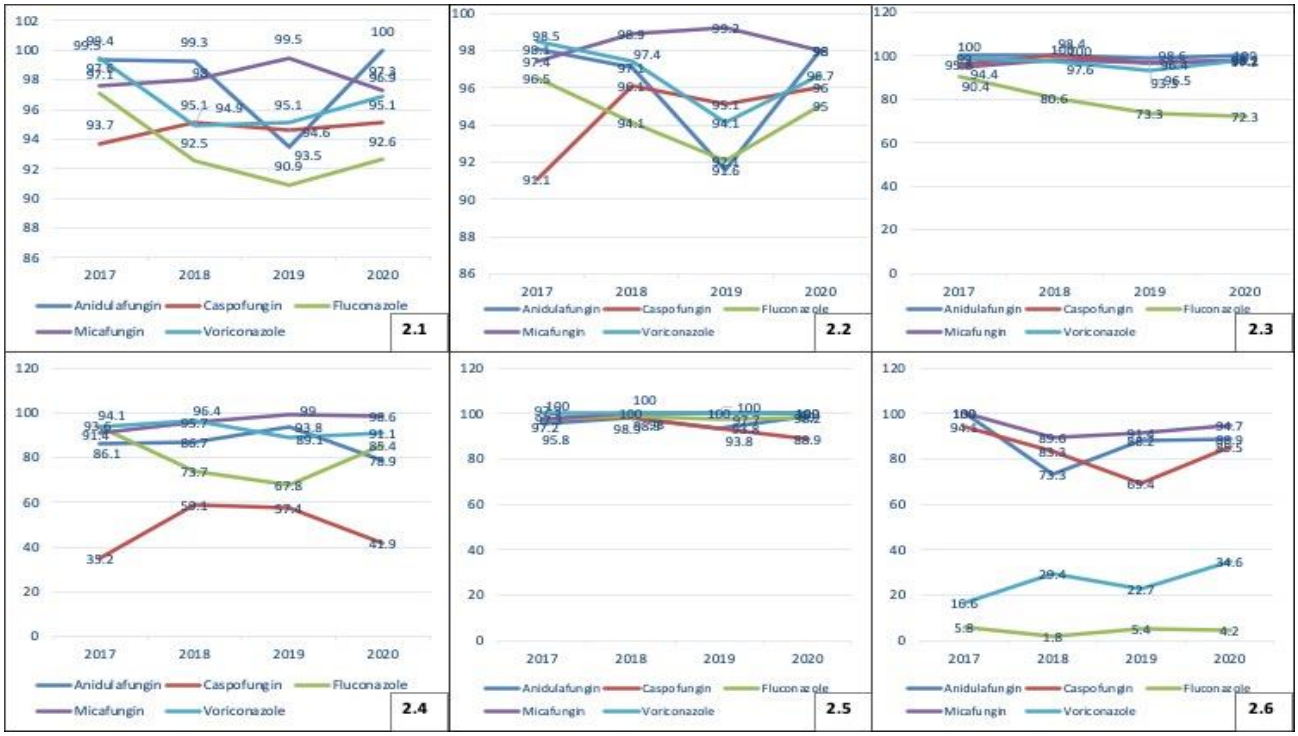


Figure- 2.1-2.6: Antifungal susceptibility trend in *C. albicans* (2.1), *C. tropicalis* (2.2), *C. parapsilosis* (2.3), *C. glabrata* (2.4), *C. utilis* (2.5), and *C. auris* (2.6) from all samples

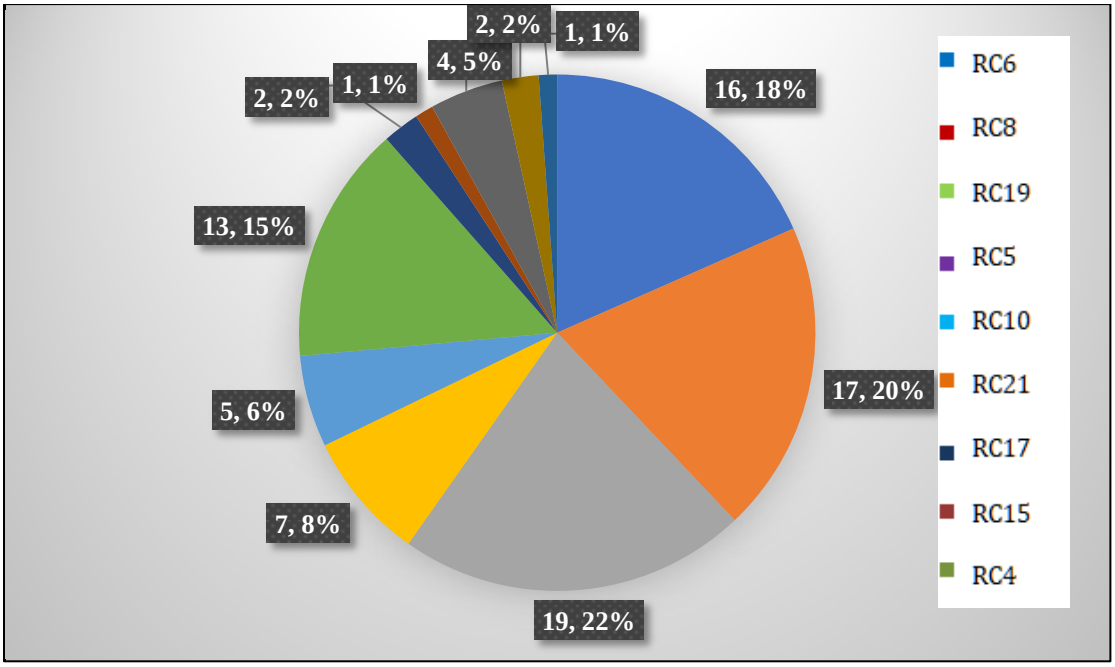


Figure 2.7: Distributionof *C. auris* among different regional centers recorded in 2020 and until 31/01/2021

A selective medium for isolation and detection of *Candida auris*

A novel selective medium was designed to isolate *C. auris* from clinical as well as environmental samples. Eighteen *C. auris* and 30 non- *C. auris* yeasts were used for the standardization of the selective medium. Sodium chloride (10% to13% concentration) and ferrous sulfate (8mM to 15mM) were added to yeast extractpeptone-dextrose (YPD) agar in various combinations followed by incubation at 37°C,40°C, or 42°C for 2 to 3 days. Representative isolates from other phylogeographic clades (clade II, III, IV) of *C. auris* were also evaluated for growth on this medium.

For validation, 579 yeast isolates and 40 signal-positive Bactec blood culture (BC) broths were used. YPD agar comprising 12.5% NaCl and 9mM ferrous sulfate incubated at 42°C for 48 h, named Selective Auris Medium (SAM), allowed selective growth of *C. auris*. A total of 95% (127/133) of C. auris isolates tested grew on the standardized media within 48 h, and the remaining 6 isolates grew after 72 h, whereas the growth of 446 non-*C. auris* yeast isolates was completely inhibited. The specificity and sensitivity of the test medium were both 100% after 72 h of incubation. The positive and negative predictive values were also noted to be 100% after 72 hr of incubation. Representative images of the findings during the development of this selective medium are depicted in figures 2.8 to 2.11.

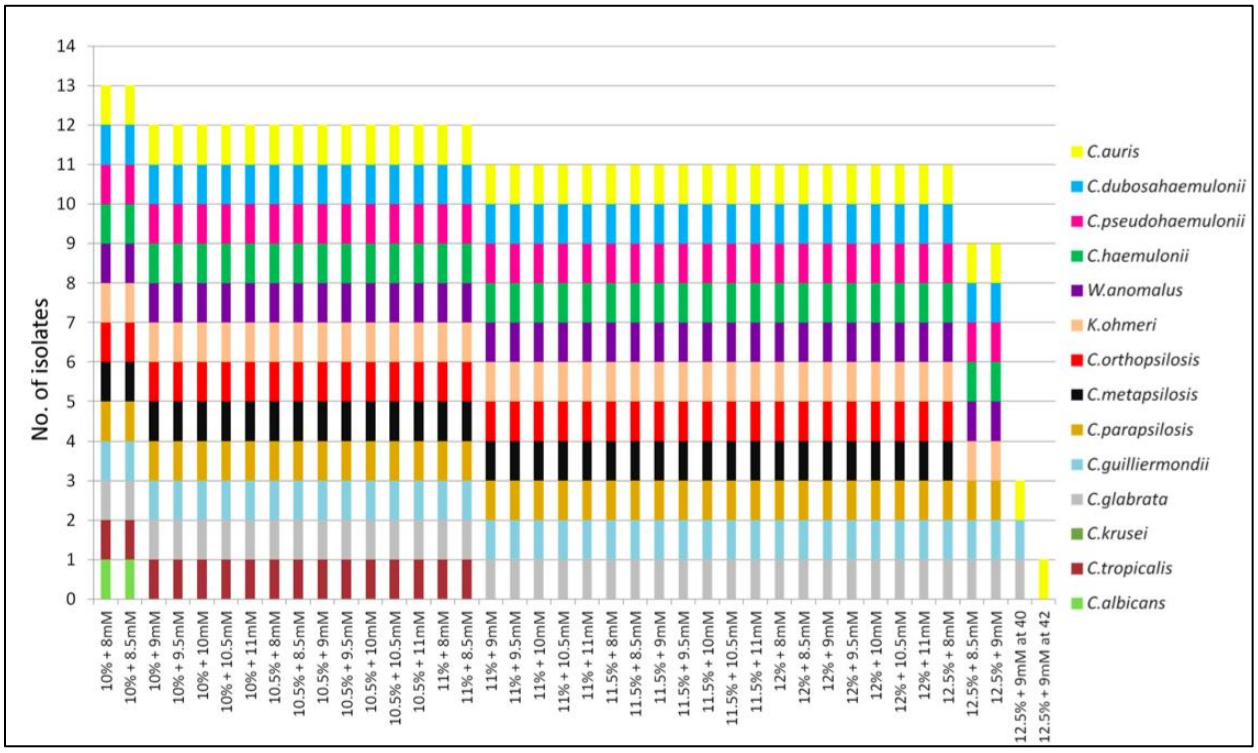


Figure 2.8: Results of dual-stress induction in *C. auris* and 13 common non- *C. auris* yeast isolates

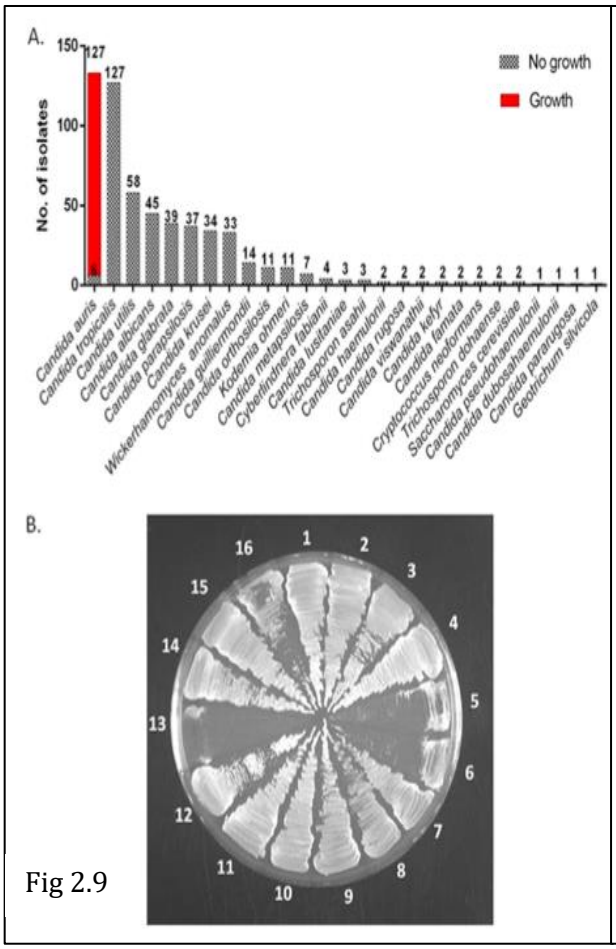


Fig 2.9

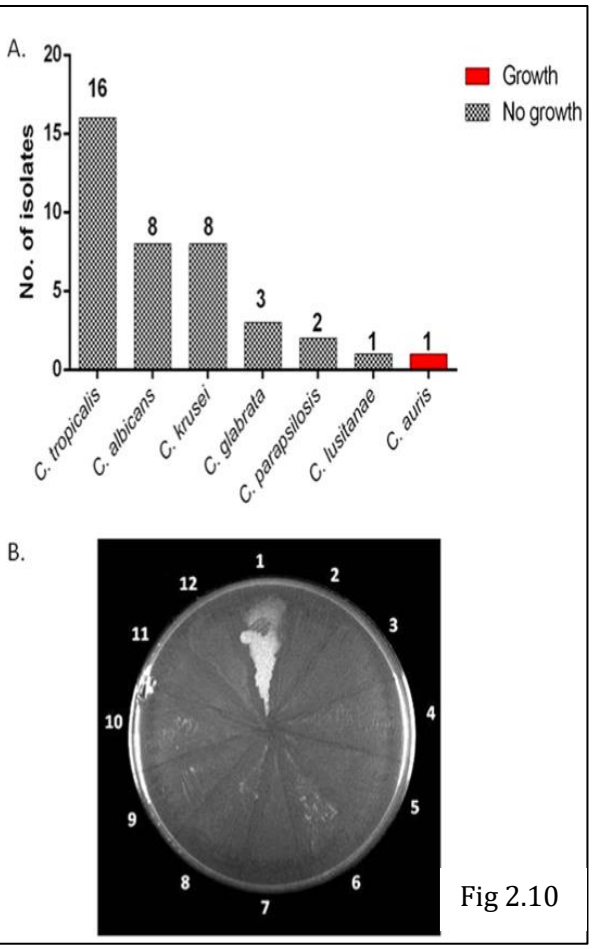


Fig 2.10

Figure 2.9: (A) Validation of results of the use of selective medium with *C. auris* and non-*C. auris* yeast species by growth at 48 h of incubation. (B) Directly streaked *C. auris* clinical isolates at positions 1 to 12 and positions 14 to 16, showing heavy confluent growth on the selective medium after 3 days of incubation. Position 13 represents non-*C. auris* yeast (*C. parapsilosis*) showing no growth.

Figure 2.10: (A) Validation of results of the use of selective medium for isolation of *C. auris* directly from positive automated blood culture vials. (B) Confluent growth of *C. auris* obtained from direct inoculation from one *C. auris*-positive blood culture vial (position 1) after 48 h of incubation.

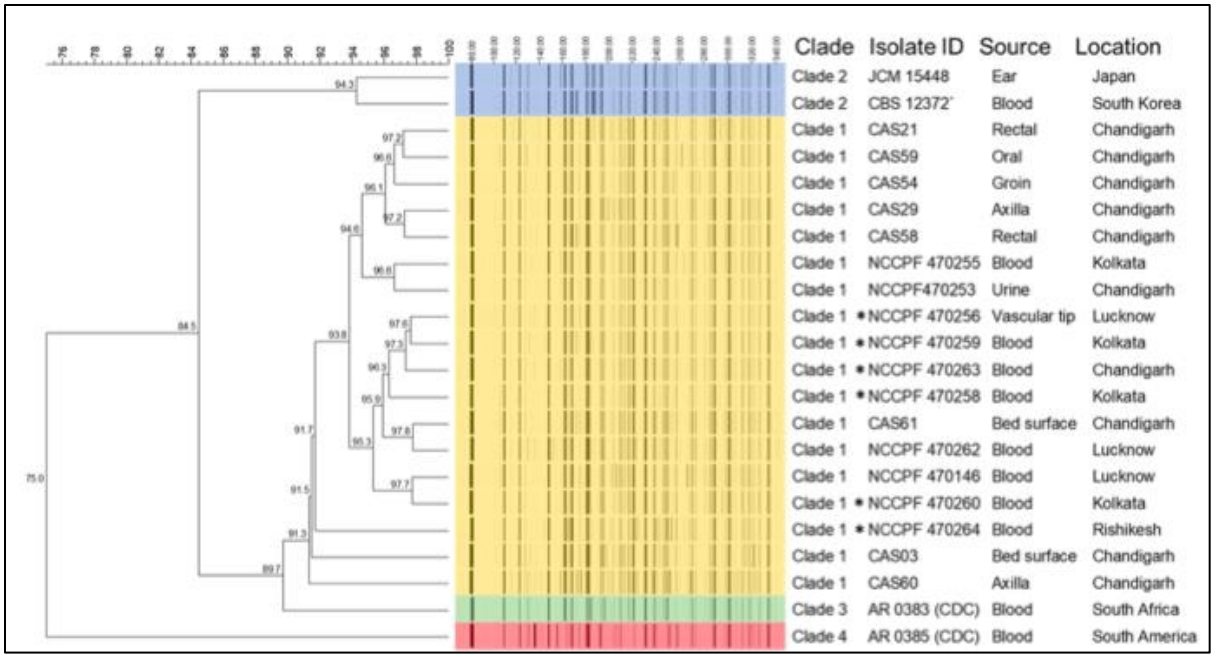


Figure 2.11: Fluorescent amplified fragment length polymorphism (FAFLP) analysis of 22 isolates of *C. auris* (9 clinical isolates [NCCPF], 6 colonizing isolates [*Candia auris* Screening isolates {CAS} no. 21, 29, and 54, aggregative; CAS no. 58, 59, and 60, non-aggregative], 2 environmental isolates [CAS 03, aggregative; CAS 61, non-aggregative], 1 *C. auris* clade I isolate [NCCPF 470146], 2 *C. auris* clade II isolates [JCM 15448, Japan; CBS 12372, South Korea], 1 *C. auris* clade III isolate [AR 0383, CDC], and 1 *C. auris* clade IV isolate [AR 0385, CDC]). *, six isolates which grew after prolonged incubation (72h) on standardized medium.

Molecular mechanism of fluconazole resistance in *C. tropicalis*

Stable isotope labelling approach for MALDI-TOF MS-based rapid detection of fluconazole resistance in *Candida tropicalis*

In total, 45 isolates were used in this study. As per the CLSI's breakpoints, 26 isolates were resistant to fluconazole, with MICs ranging from 16 to 256 mg/L, and 19 were susceptible to fluconazole, with MICs ranging from 0.5 to 1mg/L. For voriconazole, itraconazole and posaconazole, resistance was reported in 12 (1–4 mg/L), 4 (2 mg/L) and 4 (1–2mg/L) isolates, respectively (Figure 2.12). Isolates resistant to voriconazole, itraconazole, and posaconazole also exhibited resistance to fluconazole. Isolates were grown in media containing normal lysine (NL), heavy-isotope-labeled lysine (HL) and fluconazole (FLC) with labeled-lysine (HL+FLC). MALDI-TOF MS was performed, acquired spectra were visually compared and composite correlation index (CCI) values were calculated. In the case of resistant isolates, the newly dividing cells incorporated the HL into their proteins as fluconazole did not affect growth. As a result, the proteins of dividing cells were labeled with heavy isotopes of lysine and the spectra from the third setup containing fluconazole were similar to the second setup containing HL (without fluconazole) and entirely different

from the first setup containing NL. In the case of susceptible isolates, the growth was suppressed in the presence of fluconazole; since it is a fungistatic drug this inhibition was not immediate. Thus, much lower number of heavy isotopes was incorporated into the dividing cells and the resulting spectra exhibited similarity to both the spectra from isolates grown in media with NL and HL (Figure 2.13). The CCI cut-off values for susceptible and resistant isolates were significant ($P < 0.05$) (Figure 2.14). The CCI matrix, virtual gel and PCA dendrogram confirmed the results. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of this method for detection of fluconazole resistance were 92.31%, 100%, 100%, 90.48% and 95.56%, respectively.

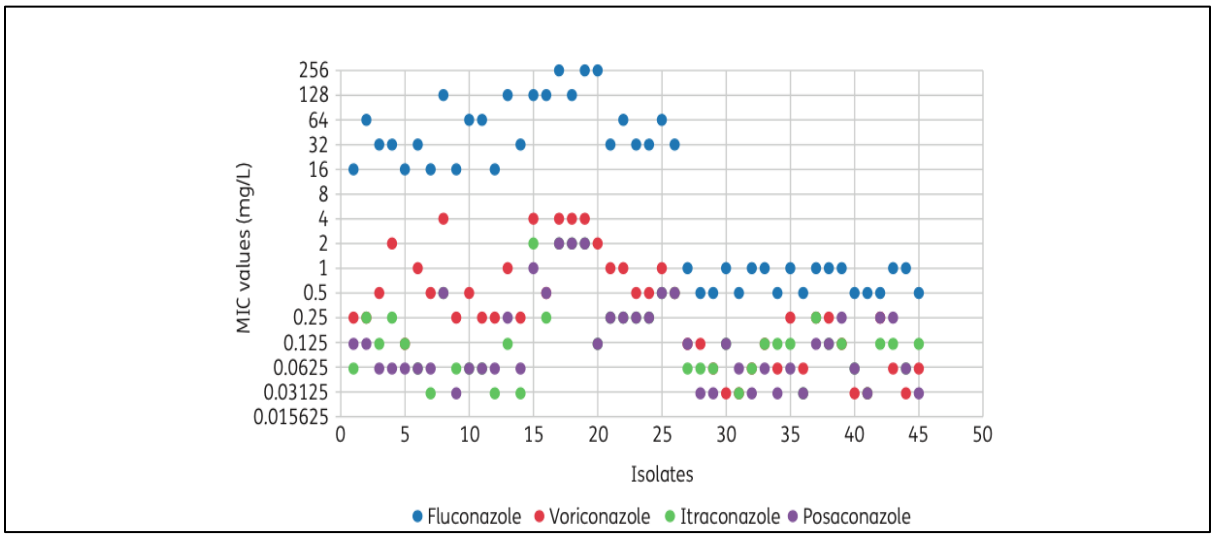


Figure 2.12: Susceptibility results for 45 *C. tropicalis* isolates for fluconazole, voriconazole, itraconazole and posaconazole. Green itraconazole dots are obscured by purple posaconazole dots for isolates 17, 18 and 19

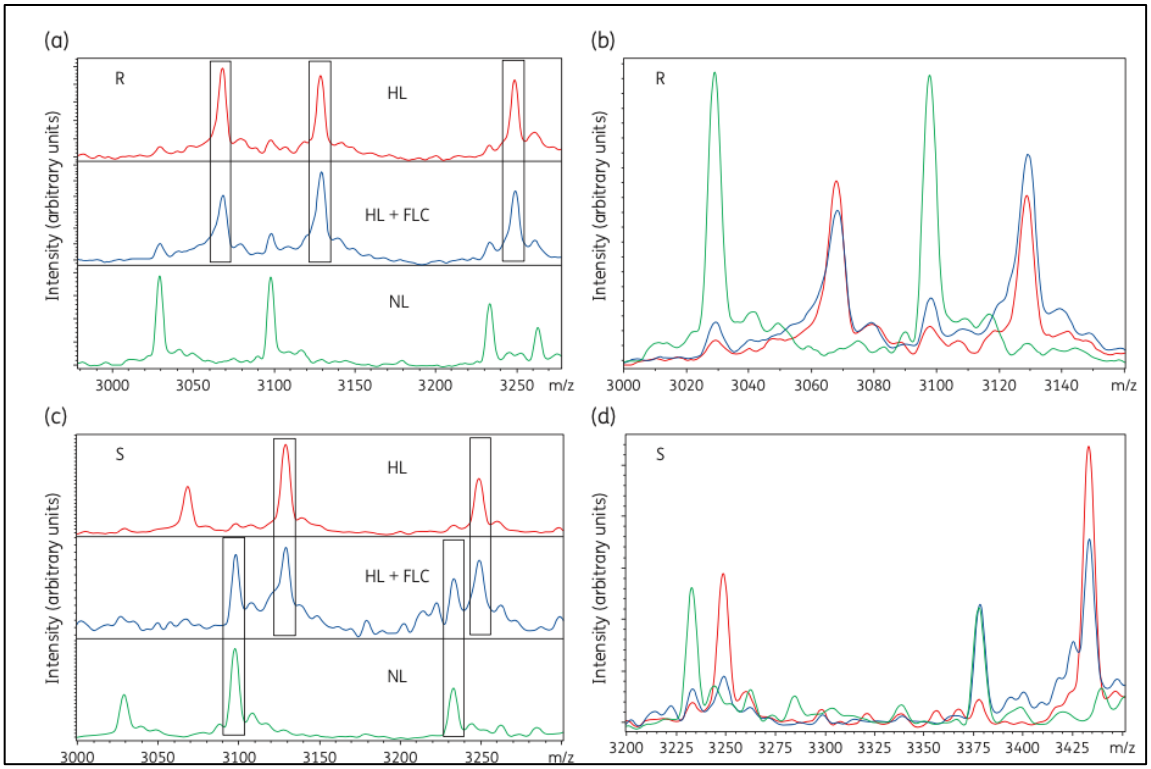


Figure 2.13: Comparison of spectra captured from susceptible and resistant isolates of *C. tropicalis* using three different setups (NL, HL and HL + FLC). (a) In resistant isolates, the HL + FLC spectrum was similar to the HL spectrum and different from the NL spectrum. (b) The overlapping view of spectra shows higher similarity between HL + FLC and HL in a resistant isolate. (c) In susceptible isolates, the HL+ FLC spectrum had similarity with both NL and HL spectra and the NL spectrum was completely different from the HL spectrum. (d) The overlapping view of spectra shows similarity of HL + FLC with both NL and HL in a susceptible isolate. R, resistant isolate; S, susceptible isolate; FLC, fluconazole.

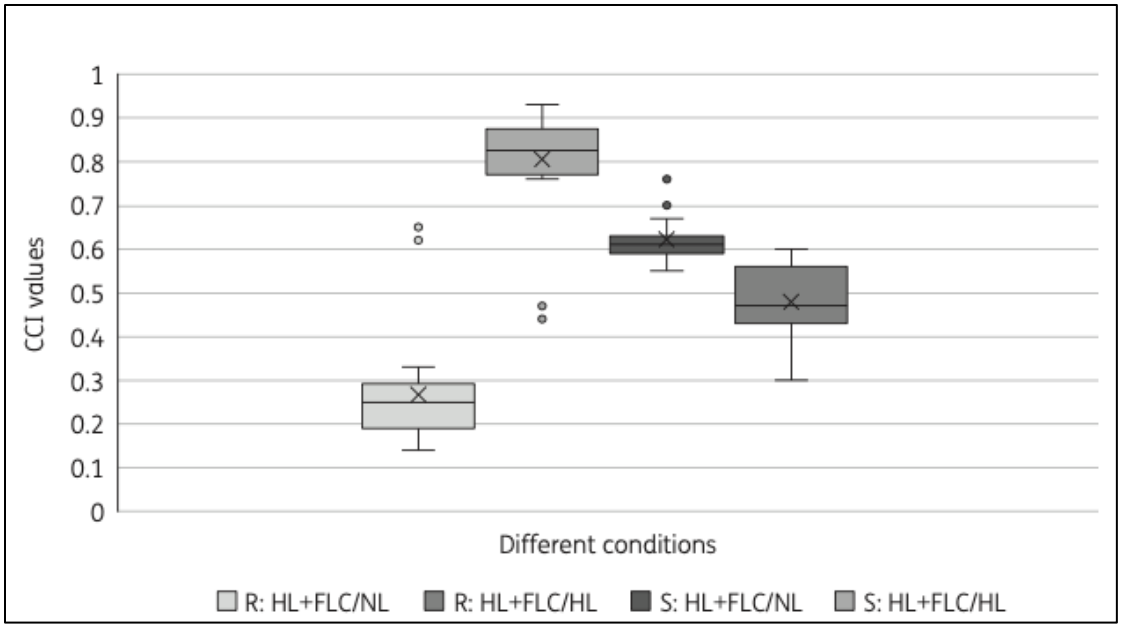


Figure 2.14: Determination of the cut-off values to differentiate resistant and susceptible isolates using the CCI values for HL + FLC/NL and HL + FLC/HL obtained for each isolate. R, resistant isolate; S, susceptible isolate; FLC, fluconazole.

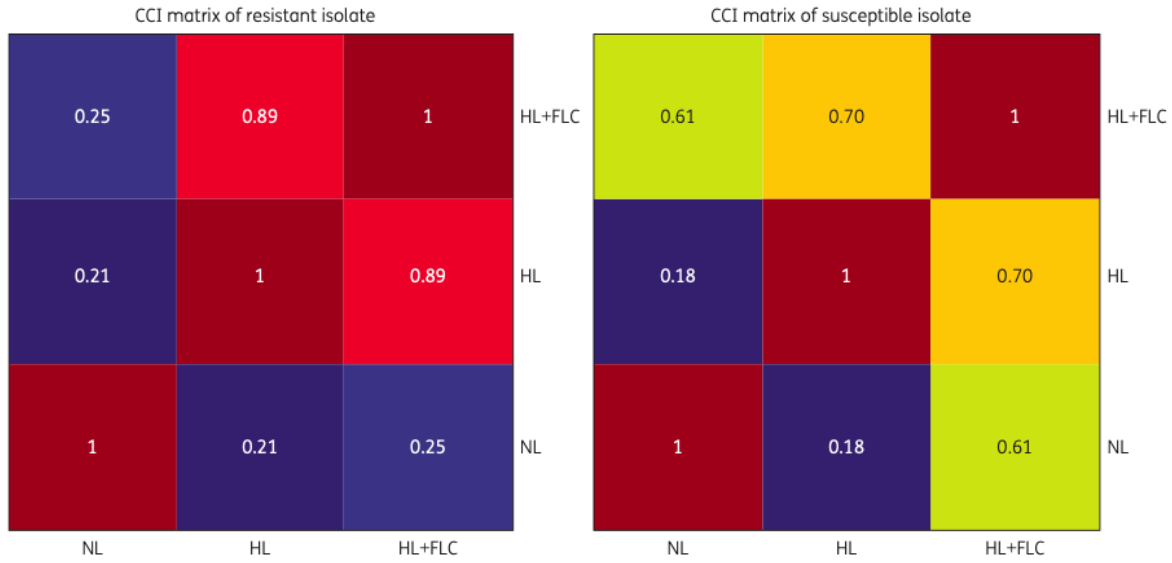


Figure 2.15: FCCI matrix of representative resistant and susceptible isolates of *C. tropicalis*. Highly similar spectra are represented by colors between yellow and deep red, whereas less similar spectra are represented by colors between green and deep blue. In resistant isolates, the HL + FLC/HL spectra are highly similar, whereas the HL + FLC/NL and HL/NL spectra are less similar. In susceptible isolates, the HL + FLC/HL and HL + FLC/NL spectra have similarity, whereas the HL/NL spectrum is less similar. FLC, fluconazole.

Molecular mechanism of fluconazole resistance in *C. parapsilosis* and *C. auris* : Sequencing of *ERG 11* geneof *C. parapsilosis*: A total of 124 *C. parapsilosis* were isolated from clinical samples. Of these, 99 (79.8%) were from blood, followed by pus samples (21, 16.9%), vitreous fluid (3, 2.4%) and gastric fluid (1, 0.8%). Identity of the isolates was confirmed by sequencing ITS region of ribosomal DNA. Among 124 *C. parapsilosis* isolates, 8 (6.4%) isolates exhibited higher MIC (≥8µg/ml) to fluconazole. Of these 8 isolates, 4 isolates were subjected to sequencing of *ERG 11*gene (till date). All these 4 isolates exhibited a silent mutation (T to C) at 591st position. A missense mutation (G1193T) (Isolate ID: 26000) was observed in one isolate, leading to the substitution in the amino acid (R398I). Whereas another isolate (Isolate ID- 34103) had a missense mutation (A395T) leading to the substitution of the amino acid (Y132F). None of the isolates were resistant to voriconazole (MIC ≤0.5µg/ml) or itraconazole (MIC ≤0.5µg/ml) or echinocandins.

We received ten *C. auris* isolates from JSS Medical College, Mysuru, Karnataka, isolated from clinical samples. *C. auris*was isolated from urine (n=4), blood (n=3) and ear discharge (n=1) and two environmental isolates from bed railings. Antifungal susceptibility testing indicated that all the isolates had an MIC of >32µg/ml to fluconazole. Lanosterol 14-alpha demethylase (*ERG11*) gene sequence showed nucleotide variation at positions A150G, A395T, T561C, C864T and T1428C. However, the only missense mutation noted at 395th position led to substitution of tyrosine to phenylalanine at 132nd position (Y132F) in the amino acid sequence of all the eight isolates whereas the other nucleotide variations were synonymous in nature.

Rapid detection of *ERG11* polymorphism associated azole resistance in *Candida tropicalis:* We evaluated the tetra primer-amplification refractory mutation system- PCR (T-ARMS-PCR), restriction-site mutation (RSM), and high-resolution melt (HRM) analysis methods for rapid resistance detection based on *ERG11* polymorphism in *C. tropicalis*. Twelve azole-resistant and 19 susceptible isolates of *C. tropicalis* were included. DNA sequencing of the isolates was performed to check the *ERG11* polymorphism status among resistant and susceptible isolates. Three approaches T-ARMS-PCR, RSM, and HRM were evaluated and validated for the rapid detection of *ERG11* mutation. The fluconazole MICs for the 12 resistant and 19 susceptible isolates were 32–256 mg/L and 0.5–1 mg/L, respectively. The resistant isolates showed A339T and C461T mutations in the *ERG11* gene. TheT-ARMS-PCR and RSM approaches discriminated all the resistant and susceptible isolates, whereas HRM analysis differentiated all except one susceptible isolate. The sensitivity, specificity, analytical sensitivity, time, and cost of analysis suggest that these three methods can be utilized for the rapid detection of *ERG11* mutations in *C. tropicalis*. Additionally, an excellent concordance with DNA sequencing was noted for all three methods. The rapid, sensitive, and inexpensive T-ARMS-PCR, RSM, and HRM approaches

are suitable for the detection of azole resistance based on *ERG11* polymorphism in *C. tropicalis* and can be implemented in clinical setups for better patient management. The details of the isolates used in the study are provided in table 2.6 and the results of these three rapid detection methods are summarized in figures 2.16 -2.20 and table 2.7.

Table 2.6: Clinical details, MIC distribution, and mutation status of the azole resistant isolates

NCCPF ID	GenBank accession number	Source of isolates	Flu MIC (mg/L)	Vori MIC (mg/L)	Itra MIC (mg/L)	Posa MICs (mg/L)	*ERG11* mutations	Amino acid alterations
420189	MW015956	Blood	128	4	0.5	0.5	A395T & C461T	Y132F & S154F
420227	MW015957	Pus	128	0.5	0.25	0.5	A395T & C461T	Y132F & S154F
420232	MW015958	Blood	32	0.5	0.5	0.5	A395T & C461T	Y132F & S154F
420233	MW015959	Blood	32	1	0.25	0.25	A395T & C461T	Y132F & S154F
420234	MW015960	Blood	64	1	0.25	0.25	A395T & C461T	Y132F & S154F
420235	MW015961	Blood	32	0.5	0.25	0.25	A395T & C461T	Y132F & S154F
420236	MW015962	Blood	32	0.5	0.25	0.25	A395T & C461T	Y132F & S154F
420237	MW015963	Blood	64	1	0.5	0.5	A395T & C461T	Y132F & S154F
420238	MW015964	Ascitic fluid	256	16	16	2	A395T & C461T	Y132F & S154F
420239	MW015965	Blood	256	16	16	0.5	A395T & C461T	Y132F & S154F
420245	MW015966	Blood	128	4	1	0.5	A395T & C461T	Y132F & S154F
420247	MW015967	Wound slough	128	4	2	0.25	A395T & C461T	Y132F & S154F

Flu: Fluconazole; Vori: Voriconazole; Itra: Itraconazole; Posa: Posaconazole; Y: Tyrosine; F: Phenylalanine; S: Serine

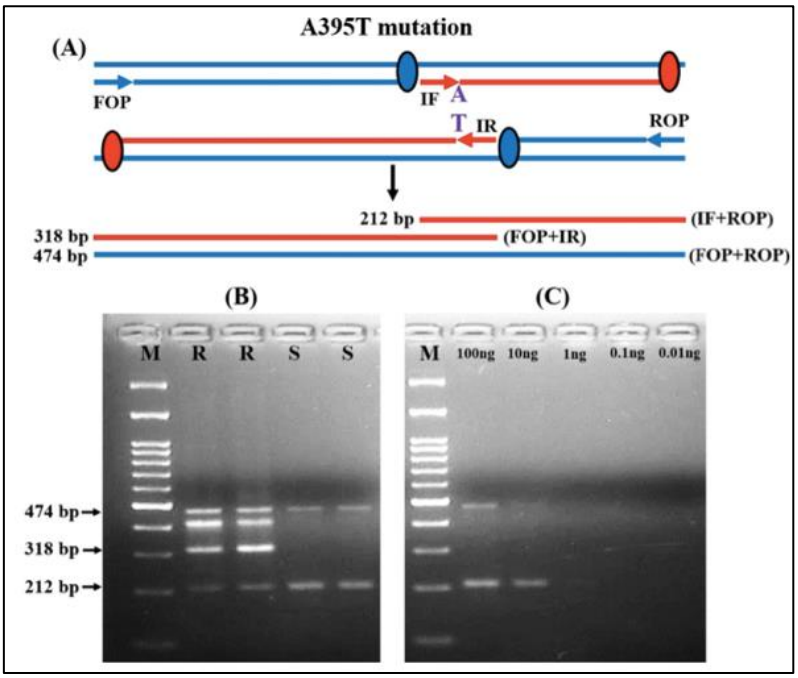


Figure 2.16: T-ARMS-PCR analysis of ERG11 gene mutation among resistant (R) and susceptible (S) isolates. (A) Schematic representation of T-ARMS-PCR assay for A395T alteration. (B) Representative agarose gel electrophoresis of the T-ARMS-PCR assay amplicons for both R and S isolates with and without ERG11 mutations. (C) Analytical sensitivity of T-ARMS-PCR examined by diluting the DNA. M: 100 bp molecular weight markers.

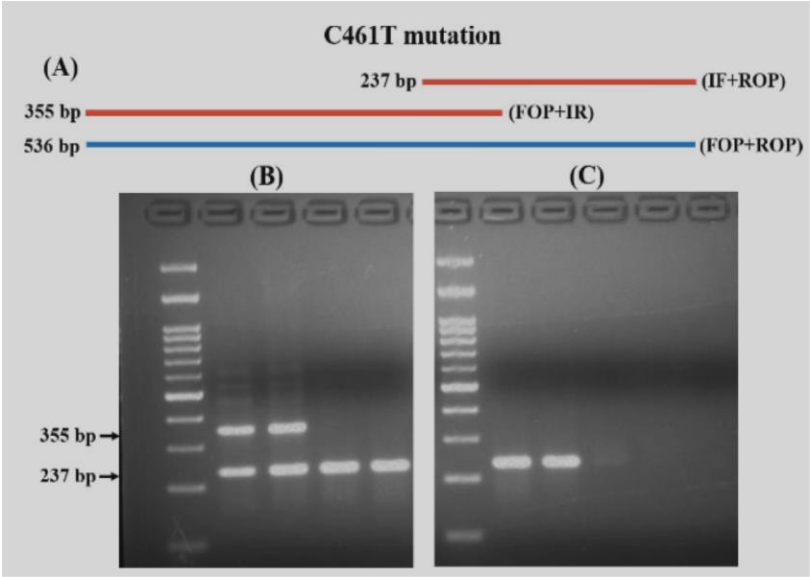


Figure 2.17: T-ARMS-PCR analysis of C461T mutation in ERG11(A) Schematic diagram of T-ARMS-PCR for C461T alteration. (B) Representative gel image of the fragment produced in R and S isolates. (C) Analytical sensitivity of T-ARMS-PCR examined by diluting the DNA. M: 100 bp molecular weight markers

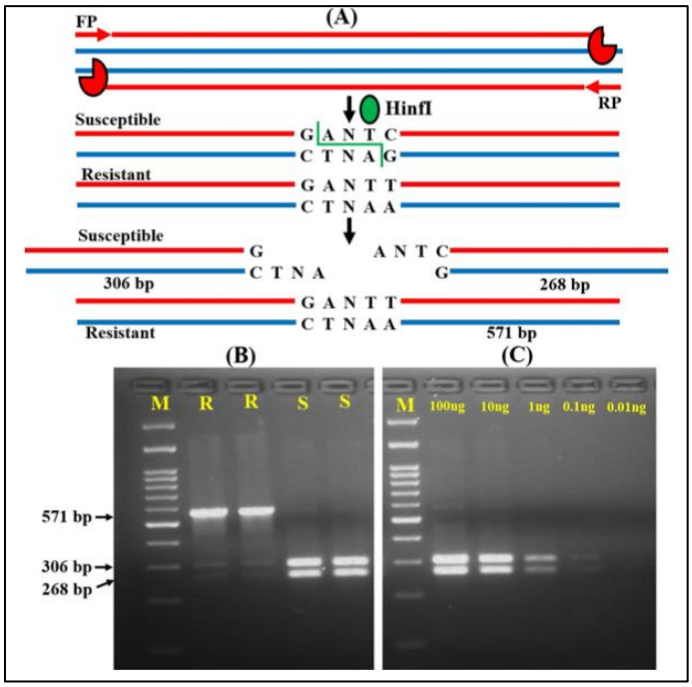


Figure 2.18: RSM analysis for *ERG11* mutation screening.(A) Schematic representation of RSM assay for the C461T mutation screening among resistant (R) and susceptible (S) isolates. (B) Agarose gel image of the fragments specific for R and S isolates (C) Gel image of gradually diluted DNA samples to confirm the analytical sensitivity of the RSM assay. M: 100 bp molecular weight markers.

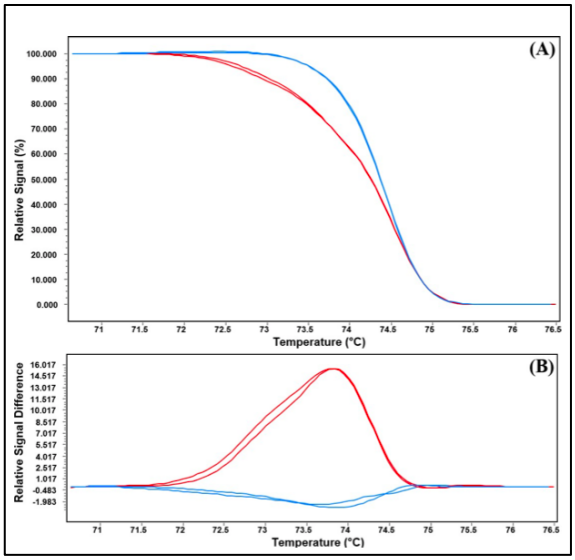


Figure 2.19: HRM analysis of the *ERG11* gene of *C. tropicalis*.(A) Normalized melting curve and (B) Difference plot presenting two variants of the *ERG11* gene fragment among the resistant and susceptible isolates. Red curves resistant variant and blue curves susceptible variant.

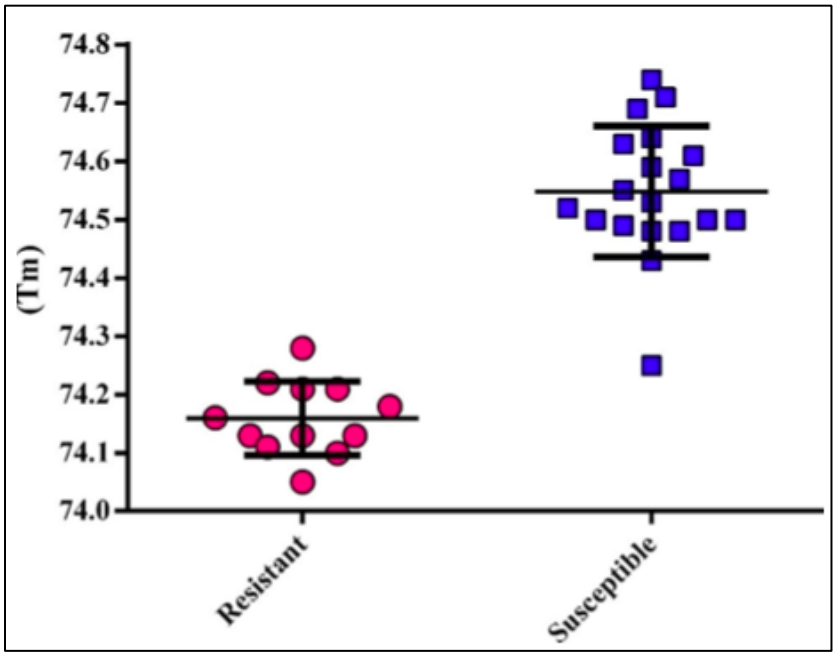


Figure 2.20: The scatter dot plot representing the Tm distributions among resistant and susceptible isolates.

Table 2.7: Comparison between DNA sequencing, T-ARMS-PCR, RSM, and HRM approaches.

	DNA sequencing	T-ARMS-PCR	RSM*	HRM
Sensitivity	100%	100%	100%	100%
Specificity	100%	100%	100%	94.74%
Detection time	~24 hours	~4 hours	~5 hours	~3 hours
Cost/reaction	~15 US dollars	<1 US dollars	~2 US dollars	<1 US dollars
Detection limit	5 ng	10 ng	1 ng	0.1 ng

* Only for C461T mutation

Identification, antifungal susceptibility and updating of MALDI TOF MS Database for *C. blankii*: A total of 12 isolates in duplicate were received from Agharkar Research Institute, Pune. Post receiving, we tried to identify those using MALDI TOF MS. But none of the isolates had satisfactory MALDI score (<1.2). Therefore, we amplified and sequenced ITS region of rDNA. All the isolates were identified as *Candida blankii* with percent identity of >99% in both NCBI and ISHAM ITS database. We further updated the MALDI database and validated. The MALDI score of the *C. blankii* isolates after database updation were >1.9 MIC distributions of *C. blankii* isolates is given in Table 2.8.

Table 2.8: Susceptibility results of *C. blankii* against different antifungal agents

LAB ID	Amphotericin B	Fluconazole	Voriconazole	Itraconazole	Posaconazole	Caspofungin	Anidulafungin	Micafungin
CSVC-7.2	0.5	0.12	0.03	0.5	0.25	1	0.06	0.06
CSVC-7.2	0.5	0.25	0.03	0.06	0.25	0.25	0.5	0.06
CSV-3.3	0.5	0.25	0.03	1	0.25	0.5	0.06	0.06
CSV-3.3	0.5	0.12	0.03	1	0.25	0.5	0.06	0.06
CSKA-8.1	0.5	0.25	0.03	1	0.5	0.5	0.06	0.06
CSKA-8.1	0.5	0.25	0.03	0.06	0.25	0.06	0.5	0.06
CSK-2.1	0.5	2	0.06	2	0.25	1	0.25	0.06
CSK-2.1	0.5	1	0.06	0.25	0.5	0.5	0.5	0.06
CSVK-8.2	0.5	0.25	0.03	1	0.25	0.5	0.06	0.06
CSVK-8.2	0.5	0.12	0.03	2	0.12	1	0.06	0.06
CSC-4.3	0.5	0.25	0.03	1	0.06	0.5	0.06	0.06
CSC-4.3	0.5	0.12	0.03	0.03	0.25	0.06	1	0.06
PMS-1.3	0.5	0.25	0.03	1	0.25	0.5	0.03	0.06
PMS-1.3	0.5	0.25	0.03	0.06	0.25	0.06	1	0.06

CSVC-7.1	0.5	0.25	0.03	1	0.25	0.5	0.03	0.06
CSV-7.1	0.5	0.25	0.03	0.06	0.25	0.06	1	0.06
CSA-1.2	0.5	1	0.06	2	0.25	0.5	0.25	0.06
CSA-1.2	0.5	0.5	0.03	0.06	0.12	0.5	1	0.06
CSV-3.2	0.5	0.25	0.06	2	0.5	1	0.25	0.06
CSV-3.2	0.5	1	0.03	0.12	0.5	0.5	1	0.06
CSV-1.7	0.5	0.25	0.06	2	0.25	0.5	0.25	0.06
CSA-1.7	0.5	0.25	0.03	0.12	0.5	0.5	1	0.06
CSA-1.1	0.5	0.25	0.06	2	0.5	1	0.12	0.06
CSA-1.1	0.5	4	0.03	0.25	0.25	1	2	0.06

Identification and broth-microdilution antifungal susceptibility testing of yeast directly from automated blood cultures: To facilitate the regional centre to rapidly identify yeasts from blood samples we performed direct identification and antifungal susceptibility testing directly from the broth cultures in the nodal centre. The graphical abstract of the protocol is summarized in Figure 2.21.

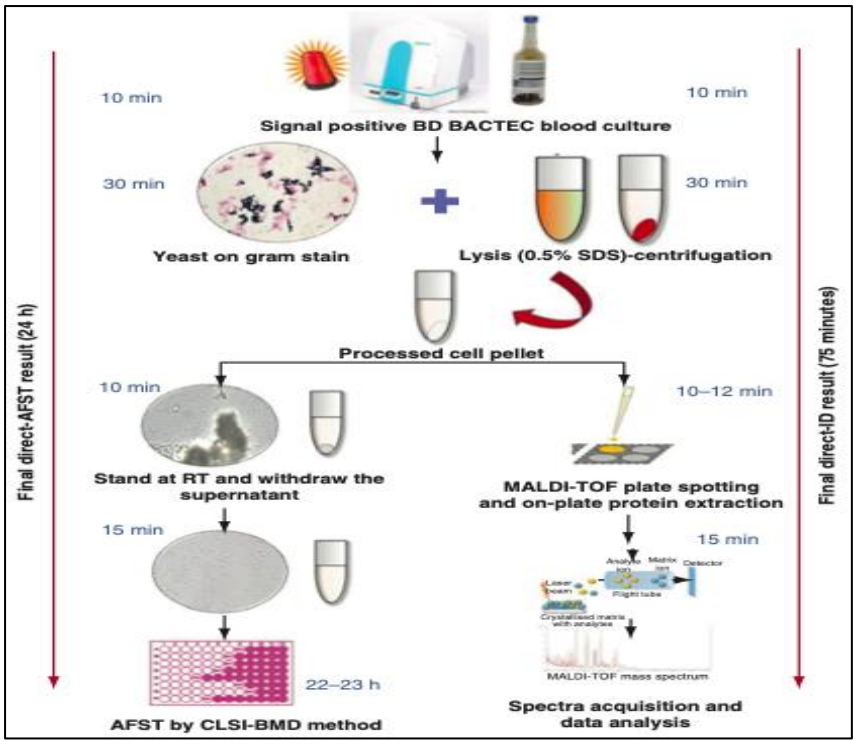


Figure 2.21 Graphical abstract of direct identification and susceptibility testing from automated blood cultures

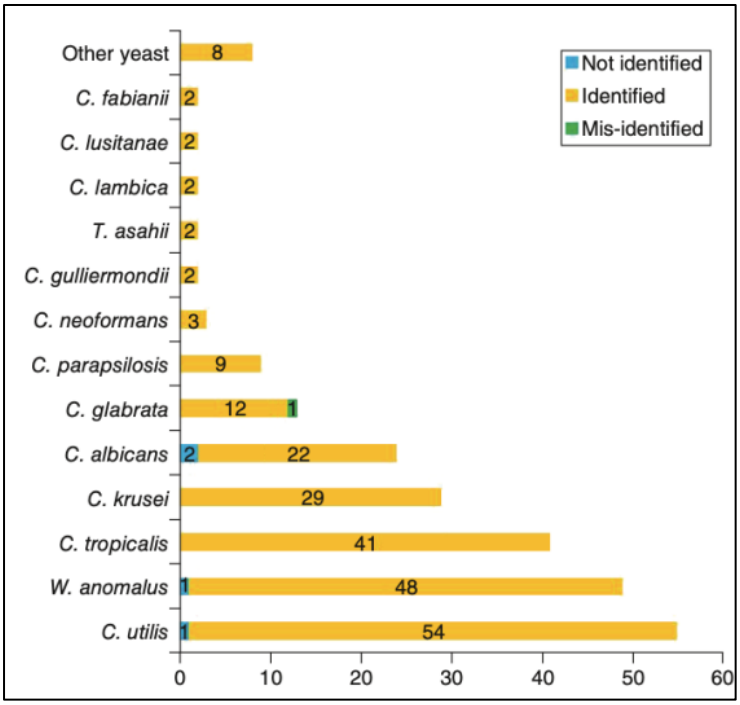


Figure 2.22: Species wise direct ID results from processed blood cultures using MALDI-TOF based protocol

Table 2.9: Mixed infection cases screened by direct MALDI-TOF protocol

S. no.	ID from culture	ID from direct MALDI TOF	Direct MALDI TOF score	Final identification
1	*C. auris* + *C. albicans*	*C. auris* + *C. albicans*	1.790/1.416	Identified
2	*C. guilliermondii* + *P. occidentalis*	*C. guilliermondii only*	1.545/–	Incompletely identified
3	*T. asahii/W. anomalus*	*T. asahii/W. anomalus*	2.008/1.743	Identified
4	*T. asahii/W. anomalus*	*T. asahii/W. anomalus*	2.229/1.632	Identified
5	*T. asahii/L. elongisporus*	*T. asahii* only	1.736/–	Incompletely identified
6	*K. ohmeri/C. krusei*	*S. epidermidis*	1.838	Not identified
7	*C. krusei/S. epidermidis*	*C. krusei/S. epidermidis*	1.586/2.07	Identified
8	*C. krusei/E. faecium*	*C. krusei/E. faecium*	1.408/1.994	Identified
9	*C. krusei/S. aureus*	*C. krusei/S. aureus*	1.681/2.27	Identified

a. *AFST testing:* AFST standardization (n = 28) results are shown inFigure 2.23. Validation using 70 BC vials revealed a 100% CA between Direct-AFST and Culture-AFST for posaconazole, amphotericin B and anidulafungin, followed by voriconazole (97%, n = 68), fluconazole (91.4%, n = 64), caspofungin (80%, n = 56) and itraconazole (70%, n = 49).

Median MIC g/l (range) for all the antifungals by direct-AFST and culture-AFST were as follows, fluconazole: 1 (0.12–64) in both, voriconazole: 0.045 (0.03–1) in Direct-AFST and 0.12 (0.03–2) in culture-AFST, itraconazole: 0.12 (0.03–1.00) in both, posaconazole: 0.06 (0.03–0.5) in Direct-AFST and 0.06 (0.03–1.00) on culture-AFST, amphotericin B: 0.5 (0.06–

2.00) in both, caspofungin: 0.25 (0.12–2.00) in both and anidulafungin 0.03 (0.03– 2.00) in both.

Among all 70 isolates, one very major error was observed in amphotericin B AFST of *C. parapsilosis* and two major errors were observed in caspofungin AFST in one *C. krusei* and one *C. glabrata* isolate. The species-wide direct-AFST results, percentage essential agreement, percentage categorical agreement, very major errors and major errors are shown in the Table 2.10.

Table 2.10: Result of direct-antifungal susceptibility and culture-antifungal susceptibility in different yeast species

Table 4. Result of direct-antifungal susceptibility and culture-antifungal susceptibility in different yeast species.

		Total (n = 70)	*W. anomala* (n = 14)	*C. utilis* (n = 14)	*C. krusei* (n = 13)	*C. tropicalis* (n = 10)	*C. albicans* (n = 9)	*C. parapsilosis* (n = 6)	Other species (n = 4)
Essential agreement (%)	Fluconazole	97.1 (68/70)	92.8	92.8	100	100	100	100	100
	Voriconazole	92.8 (65/70)	85.7	100	84.6	100	100	100	75
	Itraconazole	91.4 (64/70)	100	92.8	69.2	100	100	100	50
	Posaconazole	85.7 (60/70)	78.5	92.8	53.8	100	100	100	100
	Amphotericin B	94.2 (66/70)	100	92.8	84.6	100	100	100	100
	Caspofungin	95.7 (67/70)	92.8	100	92.3	100	100	100	75
	Anidulafungin	95.7 (67/70)	92.8	100	84.6	100	100	83.3	100
Categorical agreement (%)†	Fluconazole	91.4 (64/70)	71.4	100	92.3	100	100	100	75
	Voriconazole	97.1 (68/70)	78.5	100	100	100	100	100	75
	Itraconazole	70 (49/70)	50	78.5	38.4	90	90	90	50
	Posaconazole	100 (70/70)	100	100	100	100	100	100	100
	Amphotericin B	98.5 (69/70)	100	100	100	100	100	83.3	100
	Caspofungin	80.0 (56/70)	57.1	85.7	76.9	100	88.8	100	50
	Anidulafungin	100 (70/70)	100	100	100	100	100	100	100
Very major errors (n)	Fluconazole	0	0	0	0	-	-	0	-
	Voriconazole	0	-	-	-	-	-	0	-
	Itraconazole	0	-	-	-	-	-	-	0
	Posaconazole	-	-	-	-	-	-	-	-
	Amphotericin B	1	-	-	-	-	-	1	0
	Caspofungin	0	0	0	0	-	-	-	-
	Anidulafungin	-	-	-	-	-	-	-	-
Major errors (n)	Fluconazole	0	0	0	0	0	0	0	0
	Voriconazole	0	0	0	0	0	0	0	0
	Itraconazole	0	0	0	0	0	0	0	-
	Posaconazole	0	0	0	0	0	0	0	0
	Amphotericin B	0	0	0	0	0	0	0	0
	Caspofungin	2	0	0	1	0	0	0	1
	Anidulafungin	0	0	0	0	0	0	0	0

Others: *C. glabrata* (n = 2), *Trichosporon asahii* (n = 1) and *C. lambica* (n = 1).
†For species without available breakpoints as per CLSI guidelines, institutional breakpoints were used for % CA calculation.

Prevention of *C. auris* outbreak by active surveillance: During the study period, we witnessed 8 *C. auris* candidemia cases at our center. As soon as we identified the organism in our laboratory, we screened index patients as well as other patients in the wards. We

also carried out environmental surveillance to determine source of the yeast. We conducted surveillance of the wards housing *C. auris* candidemia patients. Result of environmental surveillance is provided in Table 2.11.

Table 2.11: Number of *C. auris* colonized patients and environmental samples in different wards

	Adult Medical ICU	**Trauma ICU and Neurosurgery ward**	**Adult gastroenterology ward**	**Adult Surgical ICU**	**COVID-19 ICU**	**Emergency ward**
No of colonisation samples	38	26	5	2	-	-
***C. auris* colonized patients**	13	4	2	1	-	-
No. of Environmental Samples	157	65	24	23	33	60
***C. auris* positive samples**	1	2	0	0	0	0

Chapter 3 Enterobacterales

In the year 2020, a total of 33360 significant clinical isolates belonging to various genera and species of family *Enterobacterales* except *Salmonella* and *Shigella* from 20 participating centers were included in the analysis. The isolates belonged to various specimens including blood (5255), sterile body fluids including cerebrospinal fluid (164), pus, wound swabs and aspirates (1105) and respiratory tract specimens (3983).

Significant clinical isolates from all specimens (except urine and feces) were tested for susceptibility to 10 antibiotics including aminoglycoside (amikacin), cephalosporins (cefotaxime and ceftazidime), fluoroquinolones (ciprofloxacin and levofloxacin), beta-lactam and beta-lactamase inhibitor combination (piperacillin-tazobactam), carbapenems (imipenem, meropenem and ertapenem) and polymyxin (colistin). Susceptibility was tested following CLSI guidelines using disc diffusion or automated systems except colistin where micro-broth dilution test was used.

Susceptibilities of different species to the antibiotics are presented in table 3.1, figure 3.1 and figure 3.2. Colistin susceptibility overall was 96% (no change from previous 4 years); *Klebsiella aerogenes* (*E. aerogenes*) and *Klebsiella oxytoca* showed 100% susceptibility followed by *Escherichia coli, Enterobacter* species, *Citrobacter* spp. and *Klebsiella pneumoniae* showing 93-100% susceptibility.

Table 3.1. Species wise susceptibility of Enterobacterales isolated from all specimens except urine and feces.

	Pip-taz		Cefotax		Ceftazid		Ertapen		Imipen		Meropen		Colistin		Amikacin		Ciproflox		Levoflox	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
C. freundii	129	41	102	22	97	27	91	49	131	53	124	56	29	97	132	58	120	47	89	58
C. koseri	251	65	244	42	176	40	197	70	252	67	250	74	21	95	257	74	244	67	133	50
Citrobacter spp	47	74	42	48	22	41	46	89	38	84	53	85	4	100	49	92	46	80	14	93
K. oxytoca	133	37	116	22	104	20	109	48	124	46	124	55	22	100	132	55	133	33	85	22
K. pneumoniae	8669	37	7658	19	5334	22	6255	41	8392	45	7771	47	2061	94	8828	47	7218	34	4913	28
Klebsiella spp	359	51	277	35	267	37	346	54	97	44	354	55	14	93	286	53	258	44	175	41
Enterobacter cloacae	839	63	739	40	489	37	559	75	863	72	814	75	91	97	872	78	812	67	311	66
Enterobacter spp	312	69	283	26	271	32	162	72	216	74	321	81	56	98	303	77	203	64	186	61
K. (E.) aerogenes	74	53	72	31	63	21	62	42	69	61	76	59	8	100	75	45	65	42	57	26
P. mirabilis	922	90	766	50	597	44	510	81	881	61	939	85			927	62	813	41	436	37
P. rettgeri	38	74	27	52	25	44	21	81	34	74	38	76			37	70	34	56	16	50
P. stuartii	117	56	105	30	94	34	38	76	118	56	121	67			117	45	118	38	31	29
E. coli	7890	53	6835	16	5072	19	5729	71	7191	72	7499	76	1065	99	7935	81	7092	22	3762	19
M. morganii	7890	53	6835	16	5072	19	5729	71	7191	72	7499	76			7935	81	7092	22	3762	19
S. marcescens	219	83	245	59	170	49	212	92	192	89	293	90			292	83	271	76	131	67
Overall	27889	**50**	24346	**20**	17853	**22**	20066	**62**	25789	**62**	26276	**67**	3371	**96**	28177	**69**	24519	**30**	14101	**26**

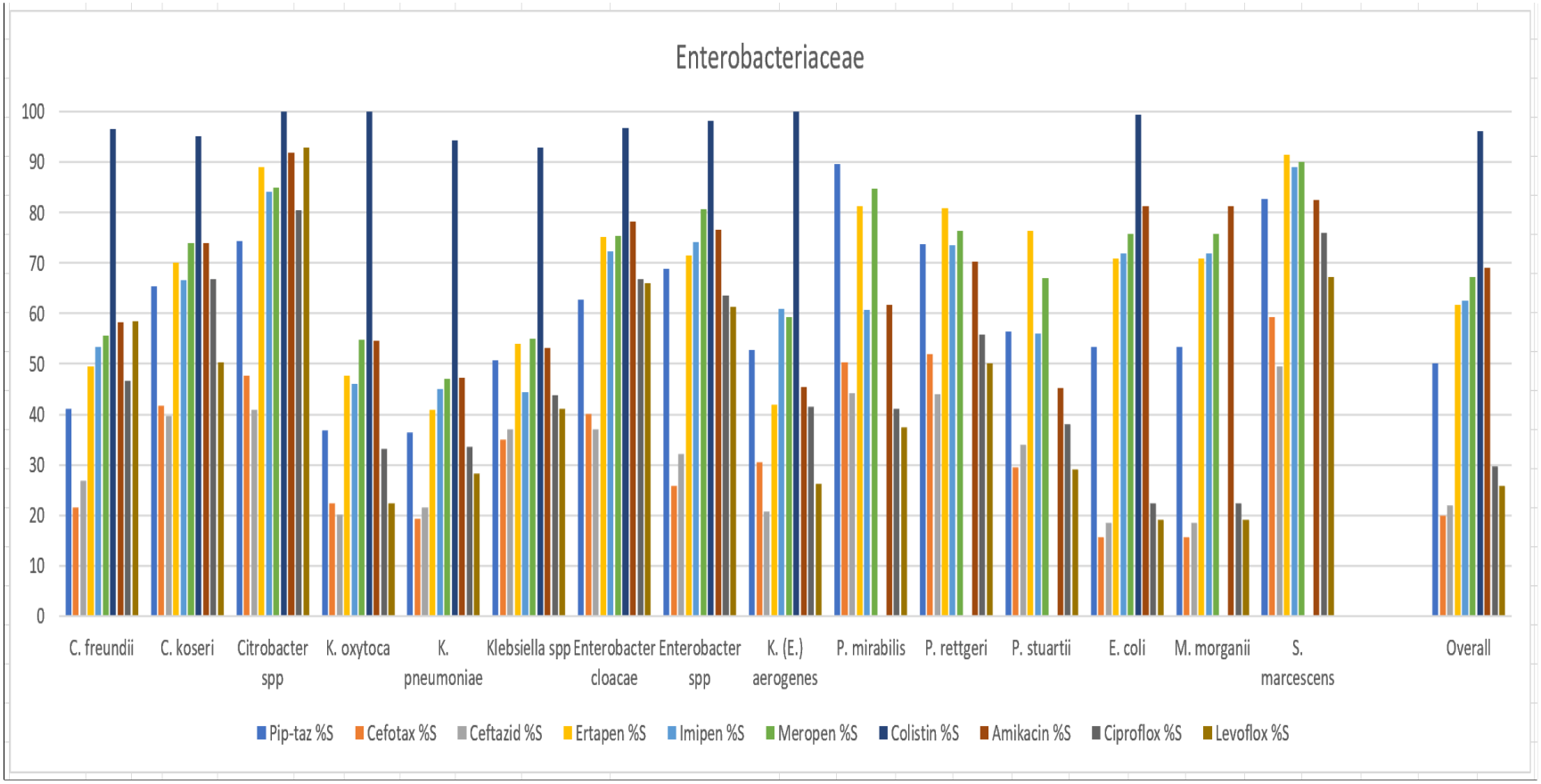


Figure 3.1 Species wise susceptibility of Enterobacterales isolated from of all specimens except urine and feces.

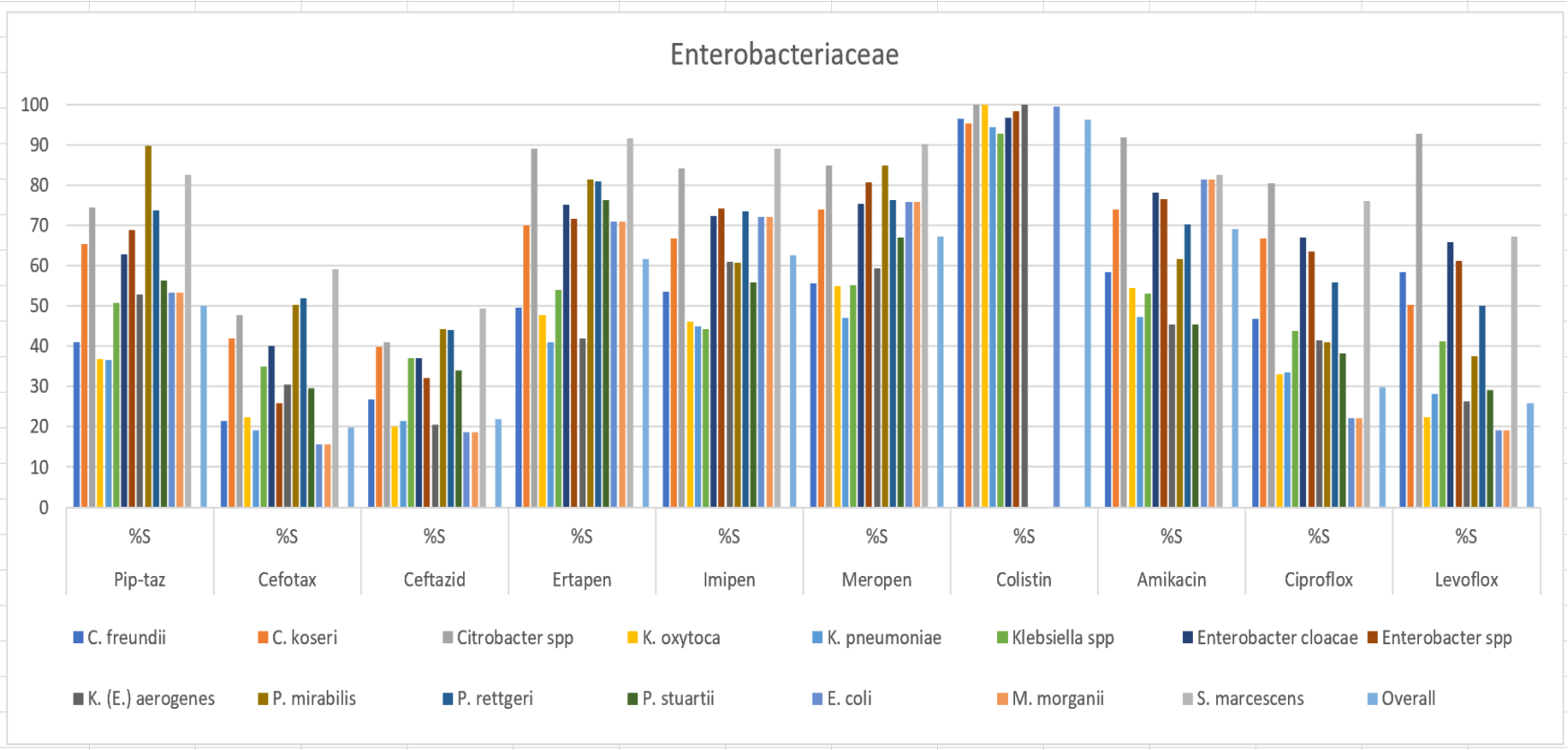


Figure 3.2. Antibiotic wise susceptibility of species of Enterobacterales isolated from of all specimens except urine and feces.

Out of the carbapenems, overall, meropenem showed 67% susceptibility followed by imipenem and ertapenem showing 62% susceptibility each. *Serratia marcescens* (90%) and *Proteus mirabilis* (85%), showed highest susceptibility to meropenem followed by *P. rettgeri* (76%), *E. coli* (76%), *Morganella morganii* (76%), *Enterobacter cloacae* (75%) and *C. koseri* (74%). *P. stuartii*, *K. aerogenes* and *C. freundii* showed lower susceptibility (56-67%) with *K. pneumoniae* showing the lowest (47%).

Piperacillin-tazobactam susceptibility was overall 50%. Maximum susceptibility was found in *Proteusmirabilis* (90%), *Serratia marcescens* (83%), and *P. Rettgeri* (74%). *C. koseri, E. cloacae*, *P. stuartii*, E. coli, *Morganellamorganii* and *K. aerogenes* showed susceptibilities from 53% to 65% with *C. freundii* (41%) and *K. pneumoniae* (37%) showing the least. Overall, less than one third (26-30%) of isolates showed fluoroquinolone susceptibility. *Serratia marcescens* (76%) and *E. cloacae* (67%) showed maximum susceptibility to ciprofloxacin. *E. coli* showed the lowest susceptibility to ciprofloxacin (22%). Ciprofloxacin and levofloxacin showed similar patterns of resistance for all species tested. Third-generation cephalosporins, cefotaxime and ceftazidime showed comparable susceptibility in 20% and 22% of isolates overall. *Serratia marcescens* (59%) *P. rettgeri* (52%) and *P. mirabilis* (50%) showed susceptibility in half of the isolates or more. Overall, two thirds (69%) of the isolates were susceptible to amikacin. *S. marcescens* (83%) followed by *E. coli* (81%), *M. morganii* (81%), *E. cloacae* (78%), *C. koseri* (74%) and *P. rettgeri* (70%) showed better susceptibility than other species.

Comparison of susceptibility of isolates from OPD, ward and ICU: Overall, for all drugs tested, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Enterobacter cloacae* isolated from out-patients were more susceptible than those from in-patients and among in-patients, isolates from wards were more susceptible than those from ICU (tables 3.2 to 3.5, figures 3.3 to 3.6). The differences were more marked for *E. coli*, *K. pneumoniae* and *Enterobacter cloacae*, and least for *Citrobacter koseri*.

Table 3.2. Comparison of susceptibility of *Escherichia coli* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	1411	87	5311	81	1213	77	7935	81
Cefotaxime	1209	20	4555	15	1071	13	6835	16
Ceftazidime	859	26	3591	17	622	17	5072	19
Ciprofloxacin	1247	26	4838	22	1007	20	7092	22
Colistin	157	100	679	99	229	100	1065	99
Ertapenem	1054	80	3644	70	1031	63	5729	71
Imipenem	1279	79	4808	71	1104	68	7191	72
Levofloxacin	695	20	2424	19	643	17	3762	19
Meropenem	1267	85	5154	75	1078	70	7499	76
Pip-taz	1355	63	5325	52	1210	48	7890	53

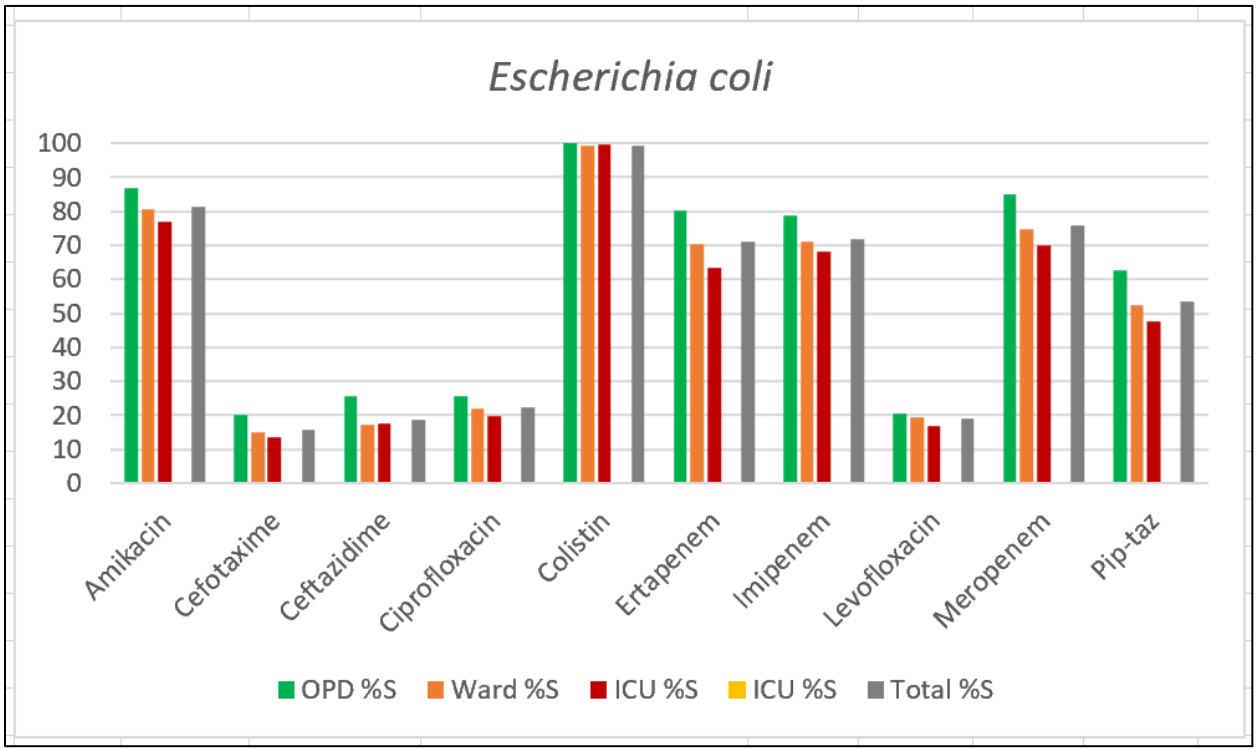


Figure 3.3: Comparison of susceptibility of *Escherichia coli* isolated from OPD, ward and ICU.

Table 3.3. Comparison of susceptibility of *Klebsiella pneumoniae* isolated from OPD, ward and ICU.

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	1184	66	4946	50	2698	33	8828	47
Cefotaxime	1033	35	4324	19	2301	12	7658	19
Ceftazidime	740	42	3276	21	1318	12	5334	22
Ciprofloxacin	1055	53	4294	34	1869	21	7218	34
Colistin	156	97	1088	95	817	93	2061	94
Ertapenem	853	60	3223	45	2179	27	6255	41
Imipenem	1137	62	4724	48	2531	31	8392	45
Levofloxacin	623	45	2530	31	1760	19	4913	28
Meropenem	1064	67	4605	49	2102	32	7771	47
Pip-taz	1151	55	4864	39	2654	24	8669	37

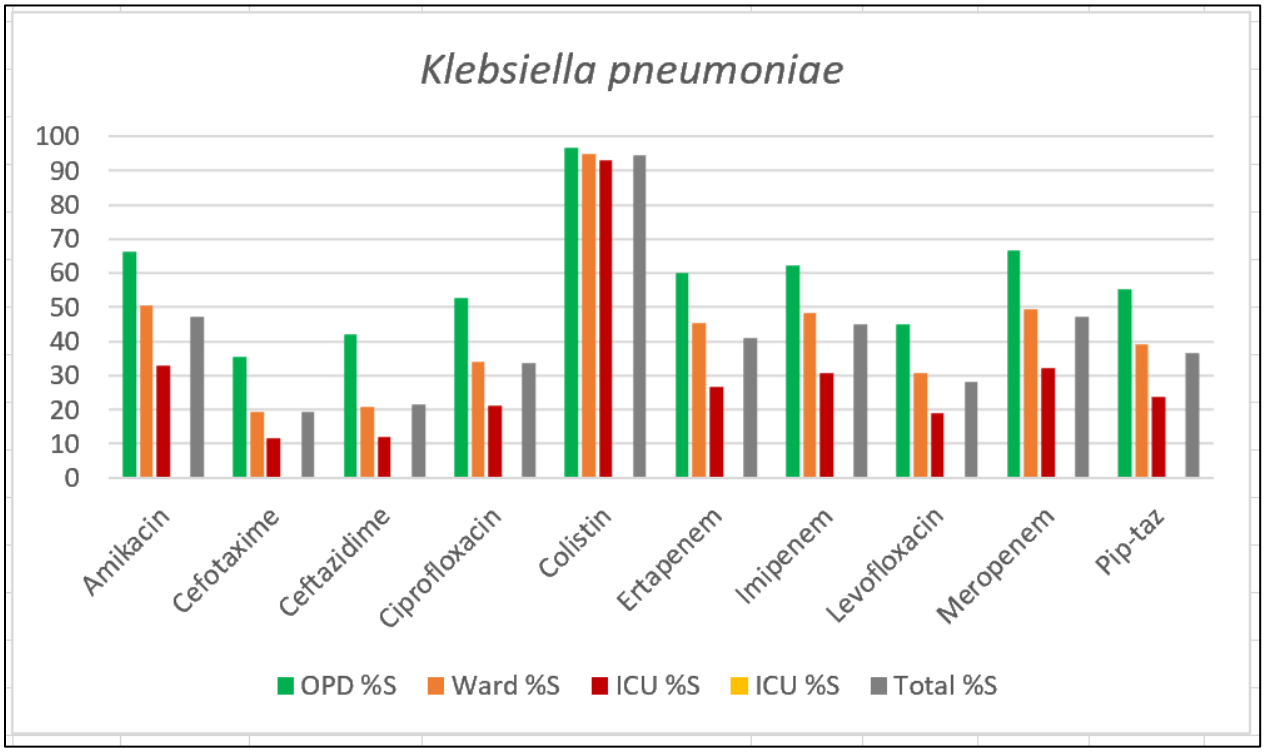


Figure 3.4. Comparison of susceptibility of *Klebsiella pneumoniae* isolated from OPD, ward and ICU.

Table 3.4. Comparison of susceptibility of *Citrobacter koseri* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	67	93	159	66	31	74	257	74
Cefotaxime	62	71	154	31			244	42
Ceftazidime	37	73	123	29			176	40
Ciprofloxacin	63	87	157	59			244	67
Ertapenem	47	94	122	60			197	70
Imipenem	67	96	157	55			252	67
Levofloxacin			96	47			133	50
Meropenem	63	98	158	65			250	74
Pip-taz	66	95	155	54	30	57	251	65

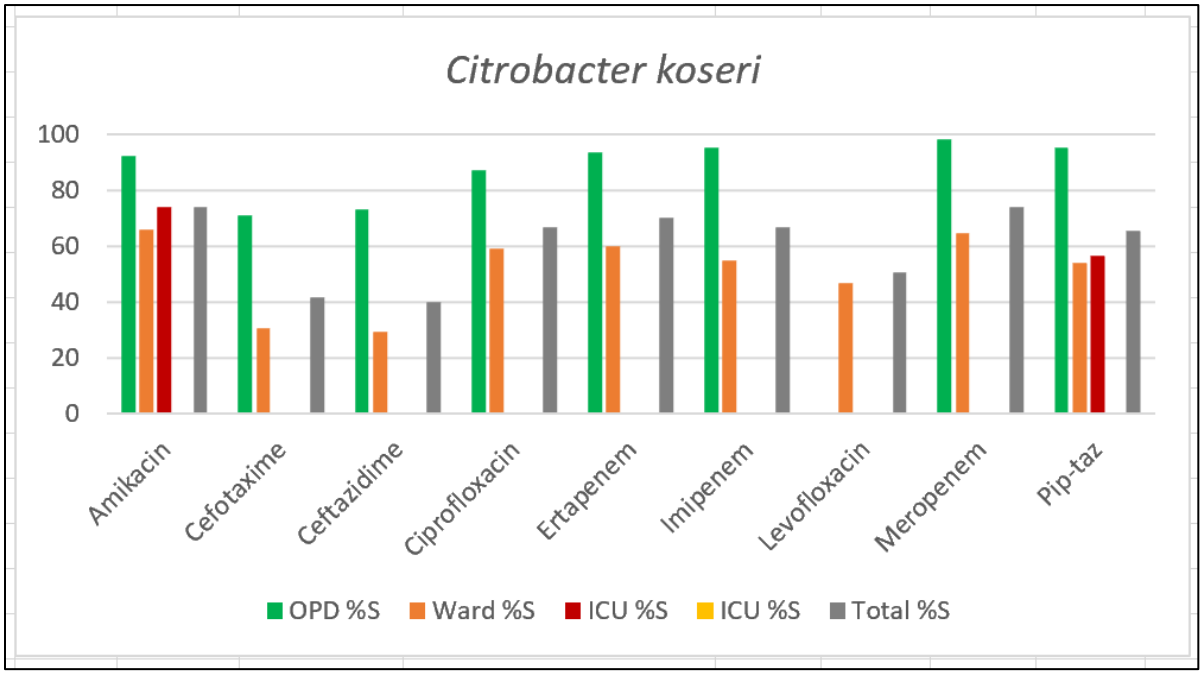


Figure 3.5. Comparison of susceptibility of *Citrobacter koseri* isolated from OPD, ward and ICU.

Table 3.5. Comparison of susceptibility of *Enterobacter cloacae* isolated from OPD, ward and ICU.

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	193	85	539	78	140	69	872	78
Cefotaxime	158	49	472	38	109	37	739	40
Ceftazidime	100	46	329	38	60	18	489	37
Ciprofloxacin	182	73	512	66	118	59	812	67
Ertapenem	129	88	329	73	101	67	559	75
Imipenem	188	82	535	72	140	59	863	72
Levofloxacin	70	69	184	71	57	47	311	66
Meropenem	179	84	514	77	121	57	814	75
Pip-taz	182	74	522	62	135	51	839	63

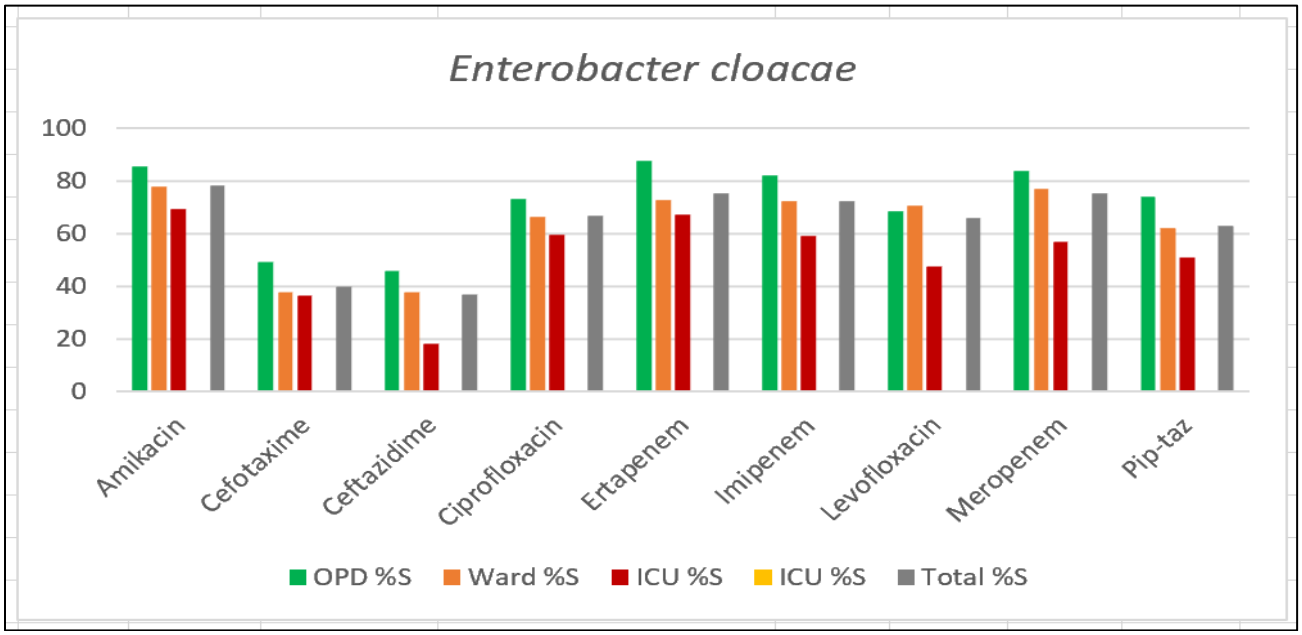


Figure 3.6. Comparison of susceptibility of *Enterobacter cloacae* isolated from OPD, wardand ICU.

Susceptibility trends of various species over time: Over the last five years, imipenem susceptibility of *E. coli* dropped steadily from 86% in 2016 to 63% in 2019 and showed slight recovery to 72% in 2020 (Table 3.6, Figure 3.7) and that of *Klebsiella pneumoniae* dropped steadily from 65% in 2016 to 46% in 2019 and remained at 45% in 2020 (table 3.7, figure 3.8). The drop in meropenem susceptibility was modest and inconsistent.

Piperacillin-tazobactam susceptibility of *Citrobacter* species dropped from 65% in 2016 to 60% in 2019 and remained at 59% in 2020 (Table 3.8, Figure 3.9). There was an increase in susceptibility to amikacin from 53% in 2016 to 71% in 2020 and to ciprofloxacin from 37% in 2016 to 62% in 2020. There was an increase in susceptibility of *Enterobacter* species to ciprofloxacin from 46% in 2016 to 65% in 2020 (Table 3.9, Figure 3.10). Susceptibility to other antibiotics didn't show much change.

Relative susceptibilities of carbapenem susceptible and carbapenem resistant isolates of *E. coli* and *K. pneumoniae*: Overall, carbapenem susceptible isolates showed higher susceptibility to all the antibiotics tested, than carbapenem resistant (resistant to at least one of the carbapenems tested) isolates (Table 3.10). The difference was more marked in *K. pneumoniae* than *E. coli* indicating that carbapenem resistant *K. pneumoniae* isolates were more resistant to all the antibiotics than carbapenem resistant *E. coli* isolates. In *E. coli*, the differences in susceptibility were high for piperacillin-tazobactam, carbapenems and amikacin and moderate for cefotaxime, ceftazidime, ciprofloxacin, and levofloxacin (range of differences 20%-83%). In *K. pneumoniae*, the differences were high for all the antibiotics tested (range of differences 43%-93%).

Table 3.6. Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=1018	Year-2017 (S%) Total n=6282	Year-2018 (S%) Total n=9187	Year-2019 (S%) Total n=13133	Year-2020 (S%) Total n=8198
Piperacillin-tazobactam	607/1009 (60.2)	3424/6030 (56.8)	4857/8961 (54.2)	6620/12121 (54.6)	4211/7890 (53.4)
Cefazolin	*0/0	*0/8	*2/6	*0/1	*0/4
Cefotaxime	165/928 (17.8)	879/5747 (15.3)	1274/7817 (16.3)	1537/10646 (14.4)	1063/6835 (15.6)
Ceftazidime	244/977 (25)	1295/5513 (23.5)	1398/5956 (23.5)	1501/7540 (19.9)	943/5072 (18.6)
Ertapenem	514/705 (72.9)	3104/4605 (67.4)	4528/6877 (65.8)	6633/9335 (71.1)	4067/5729 (71)
Imipenem	699/814 (85.9)	4699/5773 (81.4)	6453/8874 (72.7)	6497/10254 (63.4)	5176/7191 (72)
Meropenem	792/981 (80.7)	4158/5678 (73.2)	5873/8404 (69.9)	9110/12167 (74.9)	5683/7499 (75.8)
Amikacin	796/961 (82.8)	4788/6048 (79.2)	7071/8912 (79.3)	9936/12549 (79.2)	6451/7935 (81.3)
Ciprofloxacin	151/745 (20.3)	1028/5368 (19.2)	1889/8451 (22.4)	2427/11700 (20.7)	1580/7092 (22.3)
Levofloxacin	*2/4	140/889 (15.7)	600/3493 (17.2)	1145/6050 (18.9)	717/3762 (19.1)

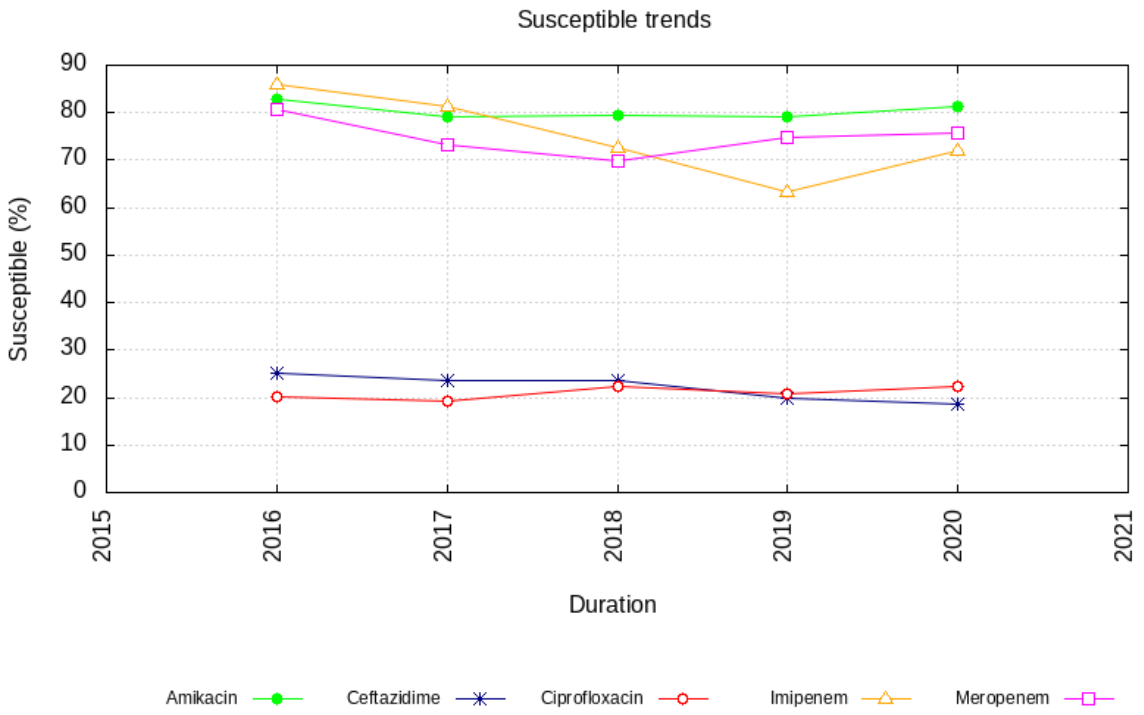


Figure 3.7.Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

Table 3.7. Yearly susceptibility trend of *Klebsiella pneumoniae* isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=875	Year-2017 (S%) Total n=5389	Year-2018 (S%) Total n=8394	Year-2019 (S%) Total n=13381	Year-2020 (S%) Total n=8932
Piperacillin-tazobactam	364/871 (41.8)	2207/5179 (42.6)	3256/8223 (39.6)	4872/12502 (39)	3165/8669 (36.5)
Cefazolin	*0/0	*0/3	*0/0	*0/1	*0/3
Cefotaxime	170/831 (20.5)	1109/5092 (21.8)	1577/7158 (22)	2400/11292 (21.3)	1472/7658 (19.2)
Ceftazidime	213/853 (25)	1320/4790 (27.6)	1488/5503 (27)	1985/7908 (25.1)	1147/5334 (21.5)
Ertapenem	317/690 (45.9)	2022/4456 (45.4)	3189/6667 (47.8)	4362/9650 (45.2)	2560/6255 (40.9)
Imipenem	566/874 (64.8)	3136/5360 (58.5)	4257/8223 (51.8)	5039/11031 (45.7)	3771/8392 (44.9)
Meropenem	436/847 (51.5)	2478/5147 (48.1)	3832/7591 (50.5)	6081/12164 (50)	3660/7771 (47.1)
Amikacin	396/848 (46.7)	2583/5286 (48.9)	4204/8276 (50.8)	6507/13018 (50)	4171/8828 (47.2)
Ciprofloxacin	243/838 (29)	1667/5213 (32)	2766/7688 (36)	4144/11560 (35.8)	2420/7218 (33.5)
Levofloxacin	*1/1	254/898 (28.3)	967/3333 (29)	2596/7432 (34.9)	1391/4913 (28.3)

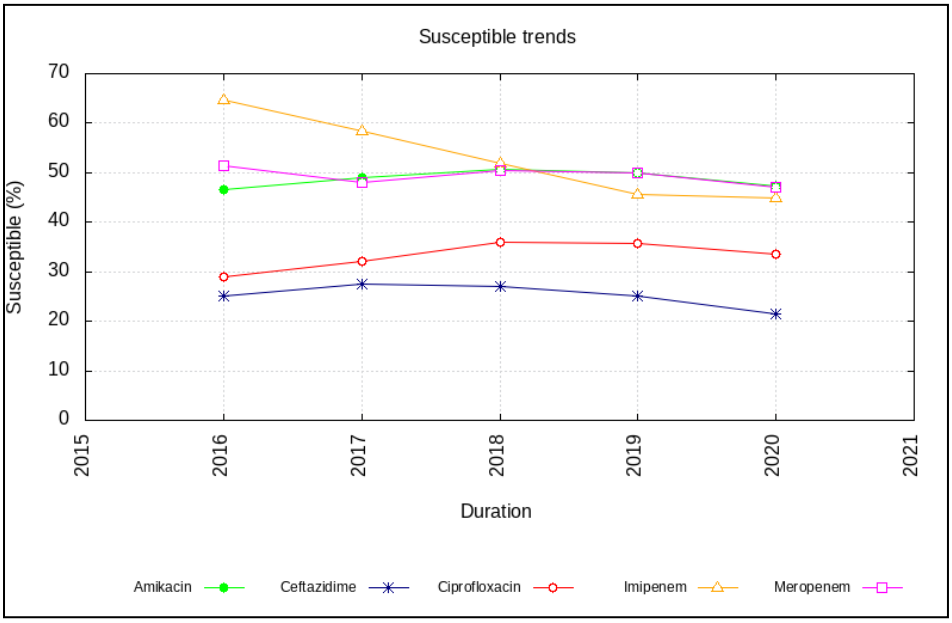


Figure 3.8. Yearly susceptibility trend of *Klebsiella pneumoniae* isolated from all samples (except faeces and urine)

Table 3.8. Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine)

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	(S%)	(S%)	(S%)	(S%)	(S%)
	Total n=49	Total n=321	Total n=613	Total n=796	Total n=447
Piperacillin-tazobactam	31/48 (64.6)	178/308 (57.8)	365/603 (60.5)	458/760 (60.3)	252/427 (59)
Cefazolin	*0/0	*0/0	*0/0	*0/0	*0/0
Cefotaxime	5/46 (10.9)	94/306 (30.7)	193/556 (34.7)	228/654 (34.9)	144/388 (37.1)
Ceftazidime	13/47 (27.7)	110/285 (38.6)	168/474 (35.4)	201/577 (34.8)	105/295 (35.6)
Ertapenem	25/46 (54.3)	161/263 (61.2)	336/522 (64.4)	381/597 (63.8)	224/334 (67.1)
Imipenem	39/46 (84.8)	198/303 (65.3)	369/594 (62.1)	403/679 (59.4)	270/421 (64.1)
Meropenem	33/49 (67.3)	187/284 (65.8)	396/580 (68.3)	505/765 (66)	299/427 (70)
Amikacin	25/47 (53.2)	212/318 (66.7)	416/604 (68.9)	509/763 (66.7)	312/438 (71.2)
Ciprofloxacin	18/49 (36.7)	138/295 (46.8)	324/599 (54.1)	430/740 (58.1)	256/410 (62.4)
Levofloxacin	*0/0	44/86 (51.2)	145/319 (45.5)	296/512 (57.8)	132/236 (55.9)

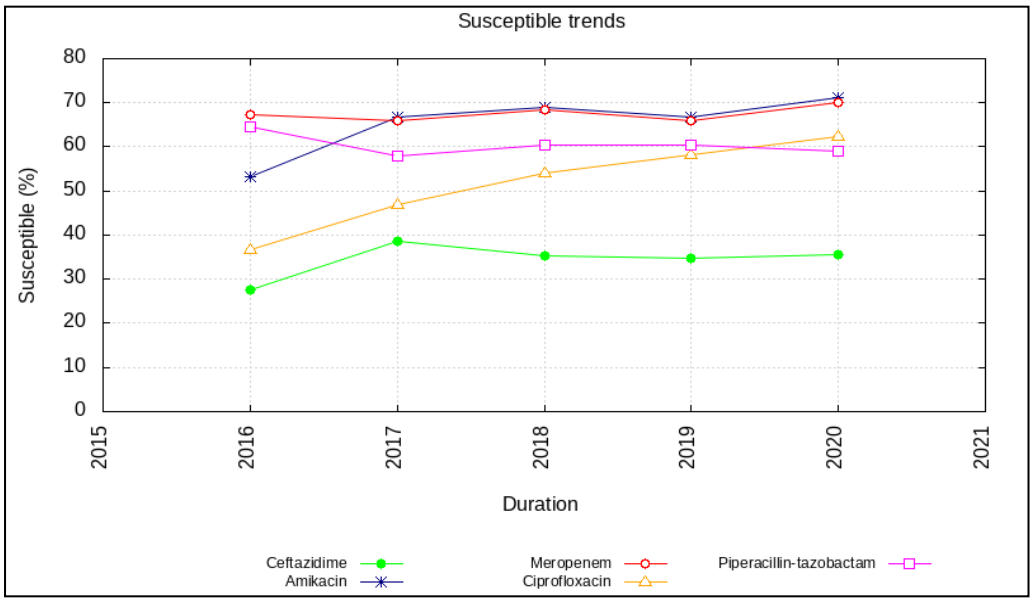


Figure 3.9. Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine)

Table 3.9. Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=222	Year-2017 (S%) Total n=1140	Year-2018 (S%) Total n=1600	Year-2019 (S%) Total n=2071	Year-2020 (S%) Total n=1287
Piperacillin-tazobactam	123/216 (56.9)	682/1092 (62.5)	961/1567 (61.3)	1253/1908 (65.7)	781/1225 (63.8)
Cefazolin	*0/0	*0/0	*0/0	*0/0	*0/0
Cefotaxime	55/214 (25.7)	310/1093 (28.4)	448/1423 (31.5)	576/1590 (36.2)	391/1094 (35.7)
Ceftazidime	71/216 (32.9)	363/1013 (35.8)	424/1159 (36.6)	494/1305 (37.9)	281/823 (34.1)
Ertapenem	117/187 (62.6)	613/929 (66)	855/1170 (73.1)	950/1281 (74.2)	562/783 (71.8)
Imipenem	174/219 (79.5)	851/1133 (75.1)	1111/1575 (70.5)	1117/1662 (67.2)	826/1148 (72)
Meropenem	150/215 (69.8)	735/1051 (69.9)	1068/1503 (71.1)	1497/1990 (75.2)	918/1211 (75.8)
Amikacin	139/193 (72)	734/1059 (69.3)	1119/1572 (71.2)	1446/1965 (73.6)	948/1250 (75.8)
Ciprofloxacin	98/213 (46)	578/1088 (53.1)	837/1369 (61.1)	1147/1836 (62.5)	699/1080 (64.7)
Levofloxacin	*0/0	93/150 (62)	289/550 (52.5)	587/959 (61.2)	334/554 (60.3)

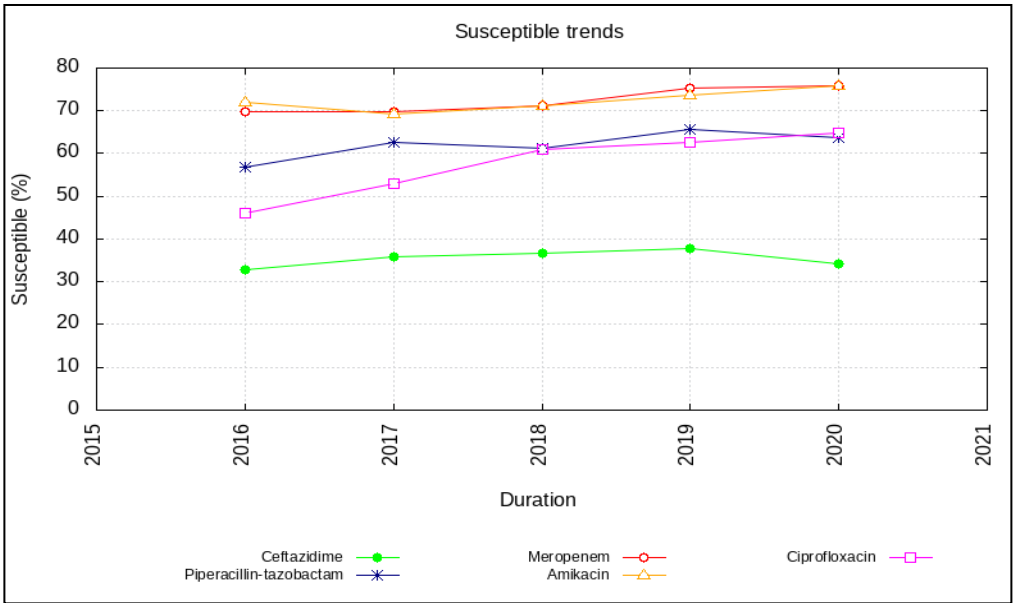


Figure 3.10. Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine)

Table 3.10: Susceptible pattern of Carbapenem-resistant and susceptible records for *E.coli* and *Klebsiella pneumoniae* isolated from all (except faeces and urine) specimens

	E. coli		***Klebsiella pneumoniae***	
AMA	CR n=12474	CS n=25975	CR n=20748	CS n=16979
Piperacillin-tazobactam	2076/12088 (17.2)	17943/24541 (73.1)	1037/20127 (5.2)	13047/16069 (81.2)
Cefotaxime	212/10603 (2)	4797/21895 (21.9)	199/17523 (1.1)	6633/15158 (43.8)
Ceftazidime	339/9241 (3.7)	5127/16228 (31.6)	293/13537 (2.2)	5969/11395 (52.4)
Ertapenem	1394/9539 (14.6)	17845/18286 (97.6)	598/15901 (3.8)	12059/12438 (97)
Imipenem	2550/11632 (21.9)	21314/21802 (97.8)	2369/19440 (12.2)	14660/15144 (96.8)
Meropenem	2591/11537 (22.5)	23416/23776 (98.5)	1096/18207 (6)	15638/15979 (97.9)
Amikacin	6260/12120 (51.7)	23228/24871 (93.4)	3351/20378 (16.4)	14787/16626 (88.9)
Ciprofloxacin	461/11094 (4.2)	6713/22803 (29.4)	884/17708 (5)	10544/15456 (68.2)
Levofloxacin	370/7314 (5.1)	2307/7297 (31.6)	955/11370 (8.4)	4381/5763 (76)
Trimethoprim-sulfamethoxazole	*4/16 (-)	20/40 (50)	-	-
Nitrofurantoin	*8/14 (-)	26/31 (83.9)	-	-

Analysis of results from individual Regional Centers: 21 Regional Centers (RCs) from various parts of the country, both public and private sectors, participated in surveillance. The results of all centers for the designated organisms and the designated antibiotics were used for overall susceptibility but only those drug-pathogen combinations where the number tested was 30 or more were used for RC wise analyses. The susceptibility profiles showed considerable variation between the RCs.

Species wise susceptibility of Enterobacterales isolated from urine: Fosfomycin showed 98% susceptibility to *E. coli* isolated from urine (Table 3.11 and figure 3.11 and 3.12). Overall, the isolates from urine showed good susceptibility to meropenem (77%), amikacin (77%), imipenem (73%) and ertapenem (72%), followed by nitrofurantoin (68%) and piperacillin-tazobactam (63%). Species wise, *C. koseri* was the most susceptible followed by *E. cloacae* and *E. coli*. *P. rettgeri*was the least susceptible showing susceptibility of 22 percent or less to all antibiotics tested. Comparison of overall susceptibilities of urinary isolates and non-urinary isolates of Enterobacterales showed marginally better susceptibility in the former (Figure 3.13).

Table 3.11. Susceptibility of species of Enterobacterales isolated from urine to antibiotics, overall and species wise

	E. coli		K. pneumoniae		K. oxytoca		Klebsiella spp		E. cloacae		Enterobacter spp		P. mirabilis		C. koseri		C. freundii		M. morganii		P. rettgeri		Overall	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
Pip-taz	7660	68	2655	47	74	51	40	30	165	66	49	67	270	89	172	77	47	55	72	85	38	21	11242	63
Cefazolin	2720	22	893	21																			3690	22
Cefotaxime	6664	24	2250	26	66	32	40	18	153	46	42	33	238	56	166	58	31	23	61	52	28	14	9739	26
Ertapenem	6541	78	2304	53	52	71	41	29	131	79	22	68	231	86	152	78	39	64	69	72	25	16	9607	72
Imipenem	7785	80	2708	59	77	68	42	55	168	71	48	69	256	55	178	79	46	48	73	53	34	12	11415	73
Meropenem	7279	83	2419	62	74	64	42	33	156	78	50	76	276	88	172	80	44	57	78	79	36	14	10626	77
Amikacin	8131	84	2823	59	81	78	42	50	174	79	54	72	275	72	181	83	47	64	81	79	38	11	11927	77
Ciprofloxacin	7364	29	2461	40	83	31	41	34	159	69	40	65	242	50	171	75	44	43	72	39	38	11	10715	33
Levofloxacin	3676	26	1348	33	39	46	35	29	52	65	28	57	131	41	73	53	31	55	29	38	20	5	5462	29
Cotrimoxazole	6656	43	2330	41	73	48	33	45	123	67	44	59	228	40	128	70	42	50	60	40	32	22	9749	43
Fosfomycin	3691	98																					3691	98
NFT	7935	83	2716	33	83	70	38	26	167	44	52	48	208	16	178	65	42	45	63	22	29	7	11511	68

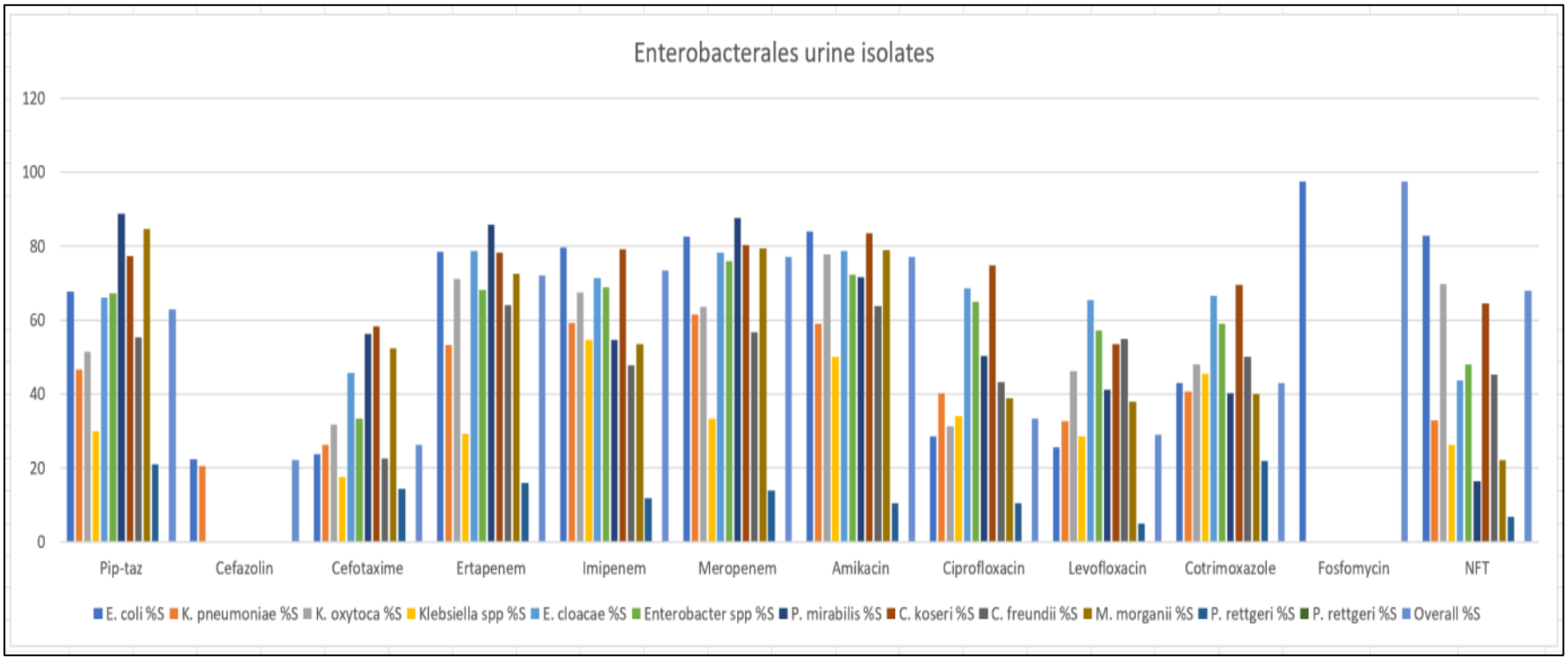


Figure 3.11. Susceptibility of Enterobacterales isolated from urine, antibiotic wise

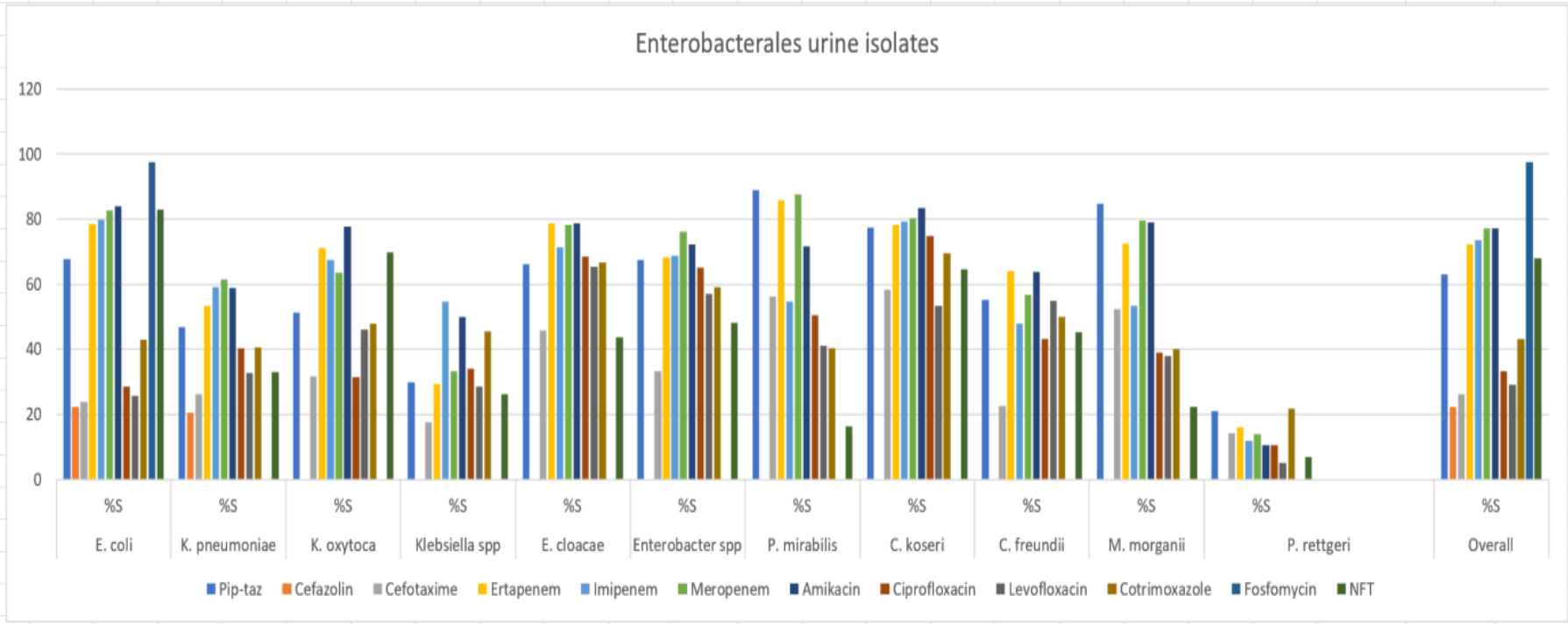


Figure 3.12. Susceptibility of Enterobacterales isolated from urine, overall and species wise

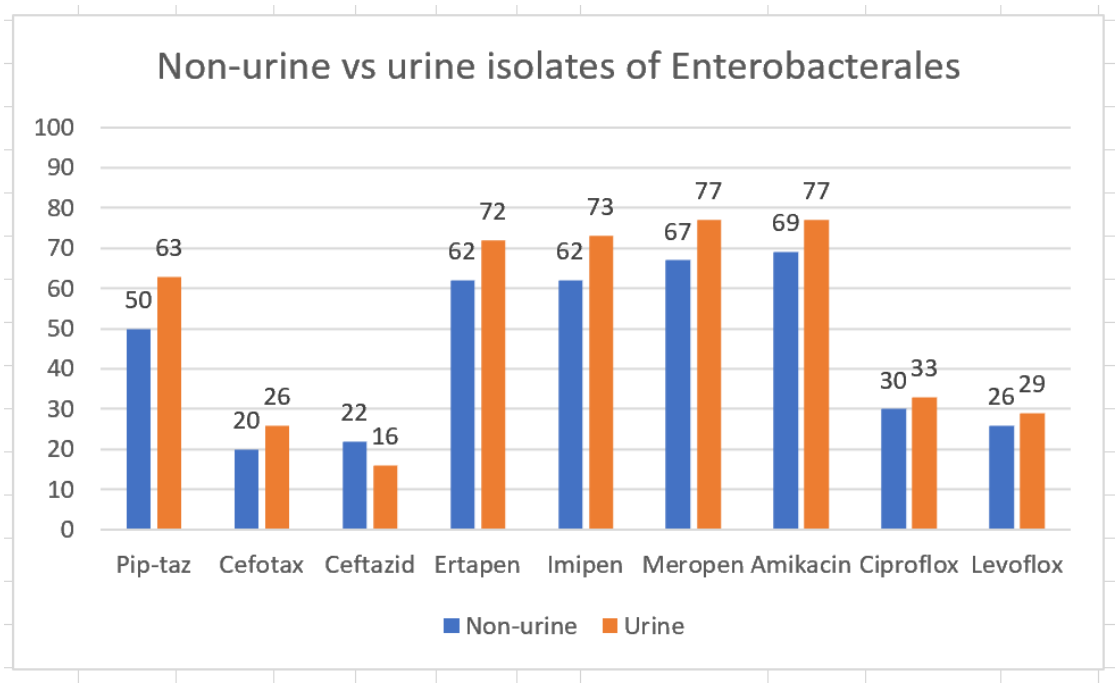


Figure 3.13. Overall susceptibility of non-urinary versus urinary isolates of Enterobacterales to the common antibiotics tested.

Comparison of susceptibilities of *E. coli* and *K. pneumoniae* showed that the former is more susceptible than the latter to all antibiotics except fluoroquinolones (table 3.12 and figure 3.14)

Table 3.12. Comparison of susceptibility of *E. coli* and *K. pneumoniae* from urine

	E. coli	K. pneumoniae
Pip-taz	68	47
Cephazolin	22	21
Cefotaxime	24	26
Ertapen	78	53
Imipen	80	59
Meropen	83	62
Amikacin	84	59
Ciproflox	29	40
Levoflox	26	33
Cotrimox	43	41
NFT	83	33

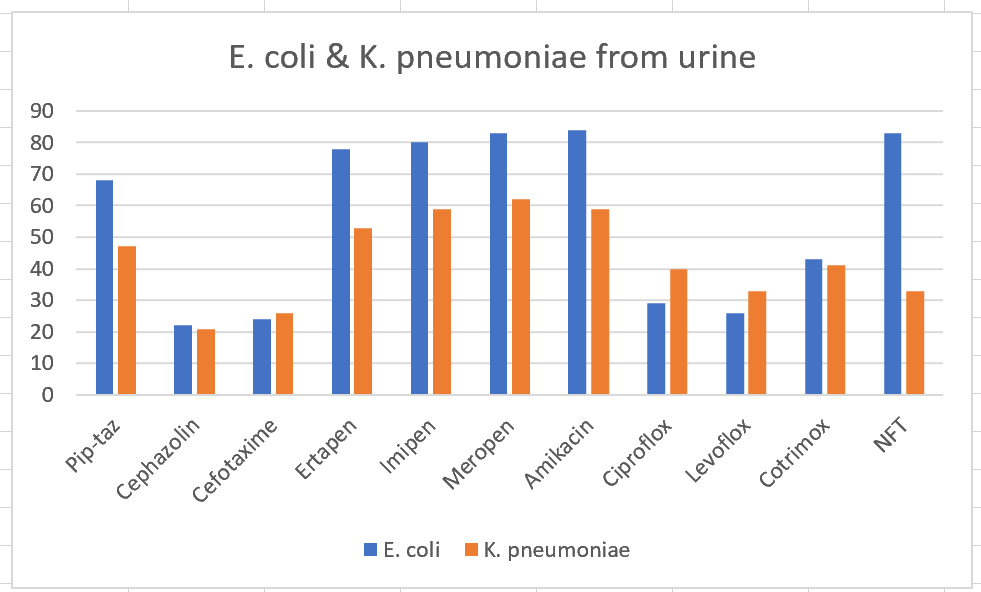


Figure 3.14.Comparison of susceptibility of *E. coli* and *K. pneumoniae* from urine.

RC wise susceptibility of *E. coli* and *K. pneumoniae* showed similar variations as the non-urine isolates except in *E. coli* for fosfomycin and nitrofurantoin. RC 21 showed unusually low susceptibility for most antibiotics tested (table 3.13 and 3.14).

Table 3.13. Susceptibility of *E. coli* isolated from urine, overall and RC wise.

	Pip-taz		Cephazolin		Cefotaxime		Ertapen		Imipen		Meropen		Amikacin		Ciproflox		Levoflox		Cotrimox		Phosphomycin		NFT	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
RC 01	586	51	584	27	586	18	585	61	586	69	586	61	586	61	586	18	586	19	584	35	581	95	583	78
RC 04	774	64			776	24			764	87	776	86	777	86	777	29							741	88
RC 05	384	77			411	31	406	89	411	91	411	92	410	95	411	31			411	53			409	81
RC 06	517	62			553	20	553	79	553	81			552	87			553	20	553	33			553	75
RC 07	668	62	82	32	195	19	582	80	533	79	538	76	761	87	724	34	242	33	618	45	269	93	694	78
RC 08	50	76			39	13	45	84	56	84	56	86	55	96	56	27	56	23	54	48	50	100	31	90
RC 09	323	79	316	35	324	37	321	76	325	82	325	71	324	90	307	41	318	41	322	47	289	99	312	93
RC 10	842	83			915	35	838	92	963	93	959	93	947	94	935	36			458	49	827	99	896	83
RC 11	43	51					31	68					45	67	44	16			43	33			36	67
RC 12	210	66	274	25	135	15	285	76	288	83	304	82	295	82	285	28	228	30	259	41			303	84
RC 13	52	69			15	7	66	70	51	43	50	70	67	81	81	19			76	39	81	99	81	93
RC 14	890	89			940	31	940	97	940	99	940	98	940	99	937	40			940	54			940	91
RC 15	166	70	168	22	170	22			171	86	171	92	171	87			171	29	149	37			167	98
RC 16	346	66			317	24	87	77	278	71	324	84	352	86	350	24	208	31	348	44	312	99	349	89
RC 17	529	71					552	84	564	87	564	88	564	88	563	19			563	52			561	78
RC 18	456	45	456	31	456	19	456	64	456	49	456	68	456	64	456	32	456	38	456	43	456	98	456	84
RC 19	213	62	212	11	217	7	211	64	217	66	214	68	217	77	212	14	212	22	211	31	215	99	215	94
RC 20	350	57	349	9	350	9	312	85	347	30	327	92	350	88	350	16	350	18	345	27	318	97	348	89
RC 21	261	46	260	3	263	8	263	13	263	74	260	47	262	29	264	9	261	13	259	23	237	98	260	37
Overall	7660	68	2720	22	6664	24	6541	78	7785	80	7279	83	8131	84	7364	29	3676	26	6656	43	3691	98	7935	83

Table 3.14. Susceptibility of *K. pneumoniae* isolated from urine, overall and RC wise

	Pip-taz		Cephazol		Cefotax		Ertapen		Imipen		Meropen		Amikacin		Ciproflox		Levoflox		Cotrimox		NFT	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
RC 01	233	22	233	14	233	9	231	28	233	50	233	29	233	30	233	16	233	15	233	14	233	39
RC 04	219	53			219	32			214	64	218	64	219	64	218	38					208	47
RC 05	159	59			166	37	166	64	166	66	166	65	164	66	166	49			166	48	165	15
RC 06	279	22			294	14	294	31	294	34			294	39			294	21	294	24	294	3
RC 07	343	48	33	33	96	20	290	68	302	66	304	67	385	71	373	49	123	48	310	53	345	37
RC 08																						
RC 09	93	57	89	33	94	45	91	60	94	63	94	57	93	66	89	54	92	54	94	53	89	55
RC 10	312	54			337	35	307	58	362	61	360	59	349	64	339	42			174	43	322	31
RC 11	28	11											31	42	32	9						
RC 12	63	41	94	35	45	13	86	56	93	49	102	56	93	52	97	36	69	42	86	48	101	29
RC 13	40	45					43	40	33	24	35	46	48	42	50	32			44	41	50	30
RC 14	202	86			210	61	210	93	210	99	210	99	210	98	210	66			210	73	210	44
RC 15	54	70	55	25	56	32			56	82	56	88	56	73			56	41	49	43	53	55
RC 16	89	34			85	20			77	48	79	67	90	56	88	26	50	28	90	34	87	36
RC 17	135	63					140	70	144	72	144	71	145	68	145	50			145	50	145	23
RC 18	121	31	121	33	121	17	121	55	121	43	121	54	121	64	121	46	121	54	121	45	121	55
RC 19	83	47	84	14	84	7	81	47	84	44	84	49	84	51	83	29	84	39	83	29	84	65
RC 20	68	40	66	17	70	21	61	66	69	45	63	84	70	57	69	28	69	41	67	28	68	31
RC 21	116	39	113	2	120	5	117	9	120	67	115	39	119	25	120	18	115	23	114	20	114	14
Overall	2655	47	893	21	2250	26	2304	53	2708	59	2419	62	2823	59	2461	40	1348	33	2330	41	2716	33

Clinical relevance:

The relative frequency of isolation of various species and their susceptibility trends has an important role in deciding empiric antibiotic policies in hospitals. The trends of change in susceptibility indicate behavior of organisms over time and alert us to take appropriate preventive measures.

Colistin, as expected, was the most effective antibiotic with an overall susceptibility of 96% with *E. coli* showing complete susceptibility and *Klebsiella* and *Enterobacter* species showing more than 90% susceptibility. With increasing use over the last five years, colistin resistance is emerging and the recent removal by CLSI of susceptible category from colistin indicates that there are strains of organisms without any detectable resistance mechanism (wild strains) which may not respond to therapy with this drug. Systemic therapy with colistin has also been mentioned as not adequate for treating respiratory tract infections. The fact that, in tertiary care facilities, many isolates from hospital-acquired and ventilator-associated pneumonias are carbapenem resistant, colistin therapy, if required, should be supplemented with nebulized colistin through inhalation. The removal of susceptible category from colistin also indicates that, in all situations, therapy with colistin may have unpredictable outcome and therefore should be highly restricted.

Carbapenem resistance was very high in *Klebsiella pneumoniae* (47%), *K. oxytoca* (55%), *Citrobacter freundii* (56%), and *K. aerogenes* (59%) with an overall all-species susceptibility of 67%. Carbapenems have been mainstay in empiric therapy in tertiary care ICU settings. Though there was good susceptibility in *Serratia marcescens* (90%), *Proteus mirabilis* (85%), *Morganella morganii* (76%), *E. coli* (76%), and *Enterobacter cloacae*

(75%), the efficacy of this drug as empiric therapy protocol should depend on relative distribution of the various species in a particular set up. This also demands regular surveillance of carbapenem-resistant Enterobacterales by molecular detection of various genes.

Piperacillin-tazobactam susceptibility overall was alarmingly low at 50%. Though the drug showed good susceptibility in *Proteusmirabilis* (90%), *Serratia marcescens* (83%), and *P. rettgeri* (74%) it showed high resistance in commonly isolated species like *Klebsiella* species (susceptibility 37%), *Citrobacter freundii* (susceptibility 41%) and *E. coli* (susceptibility 53%) and therefore should be used only when an isolate is tested susceptible. Third generation cephalosporins and fluoroquinolones have susceptibilities far below the level to consider them appropriate for use in serious patients. Extensive use and abuse of these two groups over the last three decades have resulted in high prevalence of extended-spectrum beta-lactamases and carbapenemases against oxyimino-cephalosporins and multiple mutations in organisms against fluoroquinolones making them nearly unusable as empiric therapy in seriously ill patients in tertiary care practices.

The differences in susceptibility of various organisms isolated from patients in OPD, indoor wards and ICU practices are clearly an outcome of the extent of use of the antibiotics in these areas and the consequent selection pressure. While OPD patients are usually put on oral antibiotics, the indoor patients are frequently on parenteral antibiotics and the ICU patients are usually exposed to the highest and broad-spectrum antibiotics, often multiple.
Resistance of an organism to an antibiotic is a direct outcome of the frequency of isolation of the organisms and the selection pressure of the antibiotic load used to treat it. Over the last two decades, use of carbapenems have increased many folds and the same is reflected in imipenem susceptibility of *E. coli* dropping steadily from 86% in 2016 to 63% in 2019 (slightly recovering to 72% in 2020) and that of *Klebsiella pneumoniae* dropping steadily from 65% in 2016 to 45% in 2020. The marginal increase in susceptibility of amikacin and ciprofloxacin in *Citrobacter* species and ciprofloxacin in *Enterobacter* species may reflect drop in use of the same.

Molecular tests

Materials and methods

Molecular mechanism of antimicrobial resistance in clinical isolates

Three multiplex PCRs were performed (as described by Dallenne*et al*.) to detect resistance mechanisms in representative indicator organisms (*E. coli*, *K. pneumoniae*).

Table 3.15 PCR gene targets and primers used

PCR name	Beta lactamase targeted	Primers	Product size (bp)
Multiplex I TEM,SHV and OXA-1	Tem variants including TEM1 and TEM 2 Oxa1,4 and 30	F:CATTTCCGTGTCGCCCTTATTC R:CGTTCATCCATAGTTGCCTGAC F:AGCCGCTTGAGCAATTAAAC R:ATCCCGCAGATAAATCACCAC F:GGCACCAGATTCAACTTTCAAG R:GACCCCAAGTTTCCTGTAAGTG	800 713 564
Multiplex II CTXM1,2 and 9	Variants of CTXM group 1, M3 and 15 Variants of CTXM group 2 and variants of CTXM group 9 and CTXM14	F:TTAGGAARTGTGCCGCTGYA R:CGATATCGTTGGTGGTRCCCAT F:CGTTAACGGCACGATGAC R:CGATATCGTTGGTGGTRCCAT F:TCAAGCCTGCCGATCTGGT R:TGATTCTCGCCGCTGAAG	688 404 561
Multiplex IV Metallo beta lactamases and carbapenamases	IMP,VIM and KPC	F:TTGACACTCCATTTACDG R:GATYGAGAATTAAGCCACYCT F:GATGGTGTTTGGTCGCATA R:CGAATGCGCAGCACCAG F:CATTCAAGGGCTTTCTTGCTGC R:ACGACGGCATAGTCATTTGC	139 390 538
Simplex	NDM-1 CTXM-15	F:GGTTTGGCGATCTGGTTTTC R:CGGAATGGCTCATCACGATC F:AGAATAAGGAATCCCATGGTT R:ACCGTCGGTGACGATTTTAG	621 913

E. coli: Total three hundred and twenty-seven *E. coli* isolates were subjected to three multiplex PCRs and two monoplex PCRs for CTXM-15 and NDM.Overall, CTXM-1 (35%) was the most common, followed by OXA-1 (32%), VIM (27%) and NDM (25%) (table 3.16 and figures 3.15 and 3,16). In RC-02, *E. coli* isolates positive for OXA-1 were maximum (47%), followed by CTXM1 (33%) and VIM (23%). In RC-8 isolates, NDM (44%) was the most common, followed by OXA-48 (37%), OXA-1 (34%) and VIM (27%).RC-01 isolates showedOXA-1 (60%) followed by CTXM-1 (53%) and NDM (37%).In RC-04, CTXM-1 was detected in 32% isolates whereas other genes were in low prevalence.In RC-12 isolates, CTXM-1 was the commonest (42%) followed by TEM (40%), OXA-1 (38%), VIM (34%) and NDM (26%). In RC-14, CTXM-1 was commonest (34%) followed by VIM (34%) and OXA-48 (32%).

Table 3.16. Showing positivity of various genes in *E. coli* isolates from various centers, center wise and overall

	RC-02		RC-08		RC-01		RC-04		RC-12		RC-14		Overall	
	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+
NDM	30	17	41	44	30	37	56	16	53	26	38	8	327	25
IMP	30	7	41	5	30	7	56	13	53	0	38	5	327	23
VIM	30	23	41	27	30	13	56	5	53	34	38	34	327	27
KPC	30	3	41	2	30	3	56	7	53	0	38	11	327	6
TEM	30	20	41	24	30	13	56	18	53	40	38	11	327	24
SHV	30	7	41	5	30	27	56	7	53	19	38	32	327	14
OXA-1	30	47	41	34	30	60	56	11	53	38	38	26	327	32
CTXM-1	30	33	41	24	30	53	56	32	53	42	38	34	327	35
CTXM-2	30	0	41	2	30	0	56	2	53	0	38	3	327	2
CTXM-9	30	7	41	0	30	3	56	2	53	4	38	5	327	5
CTXM-8/25	30	0	41	0	30	0	56	2	53	0	38	0	327	1
OXA-48	30	10	41	37	30	23	56	14	53	4	38	32	327	21

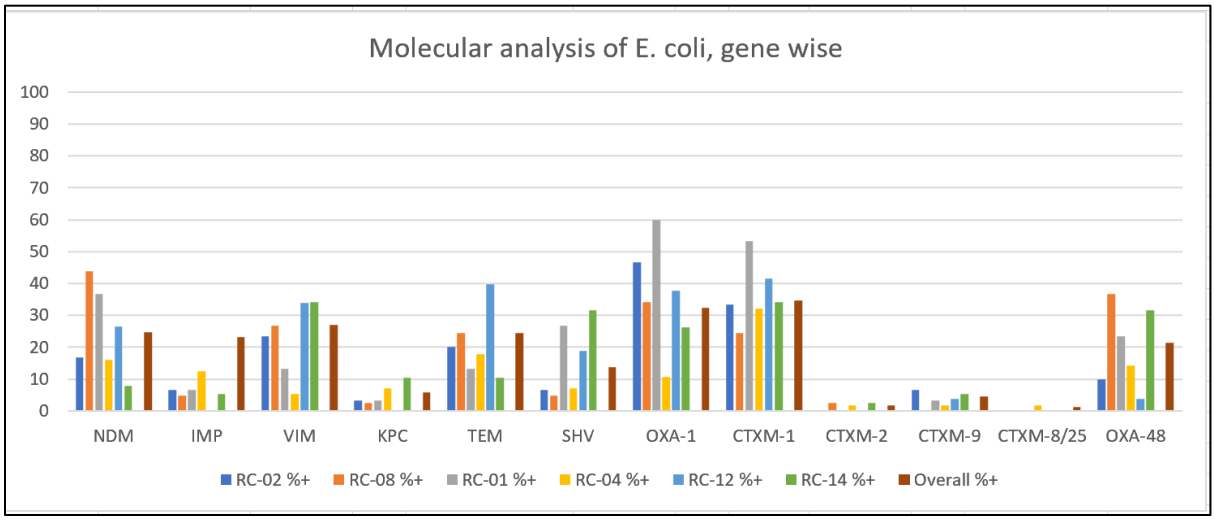


Figure 3.15. Showing positivity of various genes in *E. coli* isolates from various centers, gene wise and overall

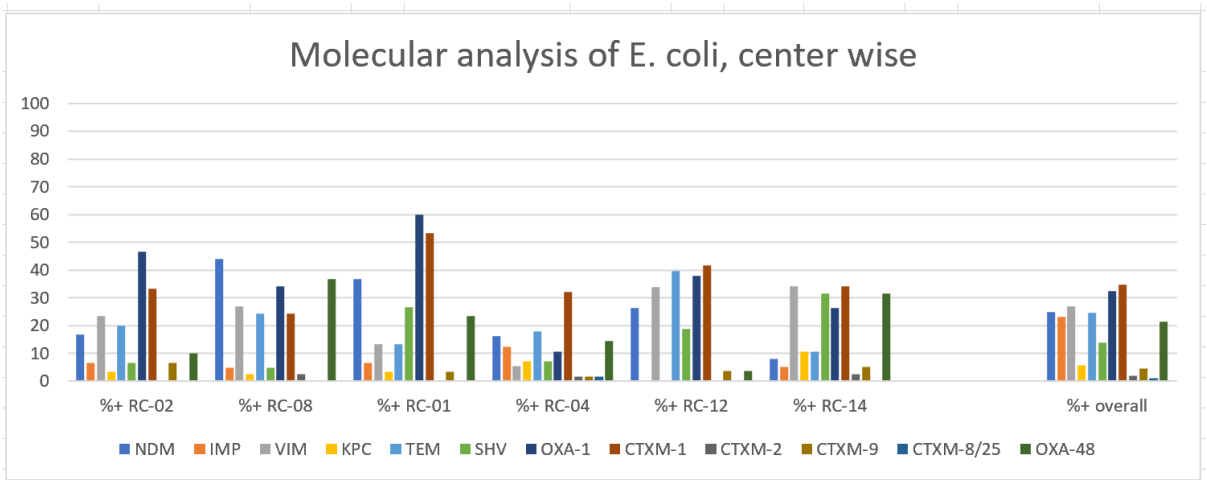


Figure 3.16. Showing positivity of various genes in *E. coli* isolates from various centers, gene wise and overall

K. pneumoniae: Three hundred and eight *K. pneumoniae* isolates were subjected to three multiplex PCRs and two monoplex PCRs for CTXM-15 and NDM as mentioned under *E. coli*.Overall, OXA-48 (48%) was the most commonly detected, followed by VIM (47%), CTXM-1 (45%), SHV (42%), TEM (41%), OXA-1 (39%) and NDM (37%) (Table 3.17 and figure 3.17 and 3.18). In RC-02VIM (63%) was the commonest, followed by TEM (60%), CTXM-1 (53%), SHV (50%) and NDM (47%). In RC-08, NDM (50%) was the most prevalent, followed by VIM (45%), IMP (43%), OXA-1 (40%), CTXM-1 (38%) and SHV (36%). In RC-01, OXA-48 (63%) was the most prevalent, followed by OXA-1 (47%), TEM (43%), SHV (40%), NDM (37%) and VIM (37%). In RC-04, VIM and KPC (54% each) was followed by TEM (53%) and SHV (42%). In RC-13, VIM was the most prevalent (77%), followed by OXA-48 (63%), CTXM-1 (57%) and NDM (47%). RC-12 showed CTXM-1 and OXA-48 at 56% each, followed by TEM (46%), SHV (44%) and OXA-1 (44%).

The center wise distribution of genes in *E. coli* and *K. pneumoniae* is shown in tables 3.18 to 3.21.

Table 3.17. Showing positivity of various genes in *K. pneumoniae* isolates from various centers, center wise and overall

	RC-02		RC-08		RC-01		RC-04		RC-13		RC-12		Overall	
	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+
NDM	30	47	42	50	30	37	59	17	30	47	59	34	308	37
IMP	30	33	42	43	30	10	59	10	30	20	59	7	308	25
VIM	30	63	42	45	30	37	59	54	30	77	59	27	308	47
KPC	30	17	42	12	30	7	59	54	30	10	59	2	308	18
TEM	30	60	42	29	30	43	59	53	30	7	59	46	308	41
SHV	30	50	42	36	30	40	59	42	30	17	59	44	308	42
OXA-1	30	23	42	40	30	47	59	20	30	40	59	44	308	39
CTXM-1	30	53	42	38	30	30	59	27	30	57	59	56	308	45
CTXM-2	30	0	42	0	30	3	59	2	30	3	59	2	308	1
CTXM-9	30	3	42	2	30	3	59	2	30	0	59	2	308	2
CTXM-8/25	30	0	42	0	30	0	59	0	30	0	59	0	308	0
OXA-48	30	53	42	38	30	63	59	24	30	63	59	56	308	48

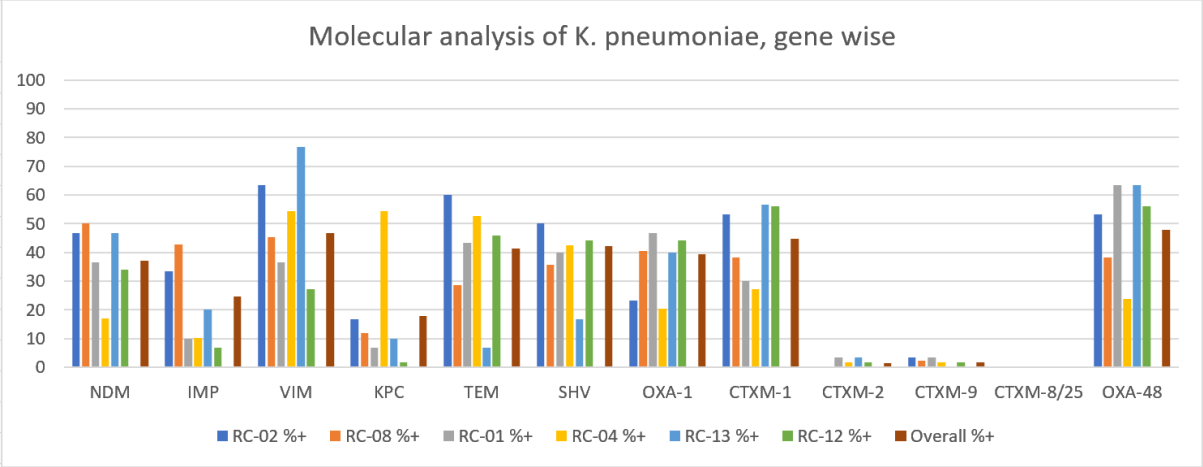


Figure 3.17. Showing positivity of various genes in *K. pneumoniae* isolates from various centers, gene wise and overall.

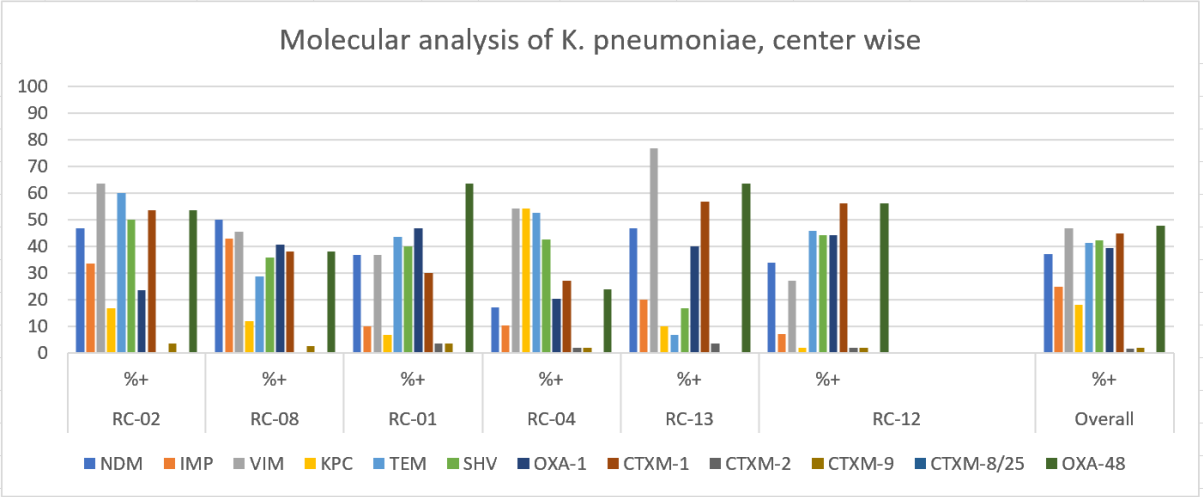


Figure 3.18. Showing positivity of various genes in *E. coli* isolates from various centers, gene wise and overall

Table 3.18. Relative prevalence of genes in *K. pneumoniae*, gene wise

Gene	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
NDM	RD-08	RC-04
IMP	RD-08	RC-01, RC-04, RC-12
VIM	RC-02, RC-13	RC-12
KPC	RC-04	RC-12
TEM	RC-02	RC-13
SHV		RC-13
OXA-1		RC-02, RC-04
CTXM-1	RC-13	RC-01, RC-04
OXA-48	RC-01, RC-13	RC-04

Table 3.19. Relative prevalence of genes in *K. pneumoniae*, center wise

Center	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
RC-02	VIM, TEM	KPC
RC-08	NDM, IMP	KPC
RC-01	OXA-48	IMP, CTXM-1
RC-04	KPC	NDM, IMP, OXA-1, CTXM-1, OXA-48
RC-13	VIM, CTXM-1, OXA-48	TEM, SHV
RC-12		IMP, VIM, KPC

Table 3.20. Relative prevalence of genes in *E. coli*, gene wise

Gene	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
NDM	RC-08	RC-14
IMP		All centers
VIM		RC-01, RC-04
KPC		
TEM	RC-12	RC-01, RC-14
SHV	RC-01, RC-14	
OXA-1	RC-01	RC-04
CTXM-1	RC-01	RC-08
OXA-48	RC-08	RC-12

Table 3.21. Relative prevalence of genes in *E. coli*, center wise

Gene	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
RC-02		IMP
RC-08	NDM, OXA-48	IMP, CTXM-1
RC-01	SHV, OXA-1, CTXM-1	IMP, VIM, TEM
RC-04		IMP, VIM
RC-12	TEM	IMP, OXA-48
RC-14	SHV	NDM, IMP, TEM

Chapter 4 Typhoidal Salmonella

Enteric fever is one of the most common febrile illnesses, if not treated well may result in high mortality rate. Enteric fever is caused by *Salmonella* Typhi of *Salmonella* Paratyphi A, B or C (human restricted bacterium) and poses serious health problem mainly in India and South East Asia, as developed countries have improved amenities for food and water. Most cases in developed countries are due to the travel to endemic areas. Enteric fever is mainly spread via fecal oral route by the use of contaminated food or water (flies, fomites, feces, and fingers) and directly associated with sanitation and drinking water treatment.
Invention of antibiotic was the major breakthrough in human history as multiple diseases can be treated with antibiotics. Although antibiotic treatment was the mainstay in case of enteric fever but it has been complicated by the emergence of multiple antibiotic resistant strains in *Salmonella* Typhi and Paratyphi A. Initially emergence of resistance to multiple first line drug (ampicillin, chloramphenicol, cotrimoxazole) used for the treatment shifted complete treatment burden on fluoroquinolones (FQ) due to less fever clearance time and bactericidal activities which resulted in decrease in MDR cases and increase in fluoroquinolone resistance with reports of clinical failure. Although these strains were sensitive in vitro but become resistant while patient was on treatment.

After the emergence of MDR and FQ resistance, third generation cephalosporin's, became the first choice of treatment in complicated cases and azithromycin in uncomplicated cases. But now there are reports of increased minimum inhibitory concentration (MIC) and ceftriaxone resistant cases from different parts of world worsening the situation. In 2017, WHO ranked FQ resistant *Salmonella* as a high priority pathogen in a priority list of antibiotic resistant bacteria for the research and development of new antibiotics. Antibiotic resistance in typhoidal *Salmonella* varies geographically and also there is no new antibiotic in the horizon as no new classes of antibiotics have been discovered since the 1980s. All the antibiotics which are introduced in to market in the past three decades, all are variations of already discovered antibiotics over a period of time. This is because of limited or non interest of pharamaceuticals in the antibacterial research or development. This declination is based on long period (ten to fifteen years) to bring a drug (from phase wise clinical trial to product launch and limited chance of approvals of new drug over the previous year's antibiotics) in to the market along with a requirement of huge investment. Even after this the risks of post approval side effects of antibiotic is also there. There are only 40 and 50 antibiotics in development as per World Health Organization and many of these have limited benefit as compare to available antibiotics. Out of these antibiotics, few target the most dangerous Gram- negative bacteria. So continuous monitoring of antibiotic resistance pattern, trend of disease and risk factor analysis is important to know the actual burden of disease and outbreak to design strategy for effective prevention and control

efforts of enteric fever and to keep life saving drugs as reservoir till the discovery of any new drug which can be used in case of extensively resistant typhoidal *Salmonella*.

To summarize *S*. Typhi is the most common etiological agent followed by *S.* Paratyphi A in India. The ciprofloxacin susceptibility is only 5% in *S.* Typhi and 3% in *S.* Paratyphi A from all over India. Although maximum number of *S.* Typhi shows intermediate sensitivity against ciprofloxacin but these were also considered as resistant. MIC trend for ciprofloxacin shows increase in MIC50 and MIC90 values over time. MIC50 has increased from 0.38 µg/ml (2013) to 0.5 µg/ml (2019) followed by 0.38µg/ml in 2020 and MIC90 has increased from 8µg/ml (2013 – 2018) to 16µg/ml (2019) and 24 µg/ml in 2020 in *S.* Typhi. *Salmonella* Paratyphi A shows 97% resistance to ciprofloxacin though 91% *S*. Paratyphi A was intermediate. Overall Fluoroquinolone resistance in *S*. Paratyphi A is higher as compared to *S*. Typhi but ciprofloxacin MIC value is higher in *S.* Typhi. This is just observation from this data and all over the literature. Fluoroquinolone resistance was mainly associated with DNA gyrase mutations. The reason for this may be the emergence of H58 MDR haplotype dominance over the other *S*. Typhi lineage in Asia and Africa showing FQ resistance associated with QRDR mutations (mainly Ser83Phe, mutation in codon 83, resulting in a serine to phenylalanine amino acid change). As per a study from south India, H58 haplotype emerged since 1991 in India. So, it is no longer empirical choice. MDR is decreasing from 6% in 2017 to 4% in 2019 and 2% in 2020 in *S.* Typhi. Third generation cephalosporins are most commonly used for the treatment but MIC_{50} and MIC_{90} is showing increasing trend. Although maximum number of *S.* Typhi and *S.* Paratyphi A are sensitive but creeping MIC is towards higher value and Ceftriaxone resistance also has been started to appear and maximum number of isolates show 0.023 µg/ml to 0.38 µg/ml MIC range. The MICs against azithromycin in the *Salmonella* Typhi isolates were normally distributed and ranged from 0.125 to 32 µg/ ml, with MIC_{50} and MIC_{90} values of 8 and 16µg/ ml respectively.

For the treatment of typhoid fever combination therapy is still under debate but most of the hospitalized patient were treated with combination of ceftriaxone and azithromycin. At present ceftriaxone and cefixime remains the first line of drug to treat severe infections of enteric fever in hospitalized patients while azithromycin continues to be used as drug of choice in outpatient without any associated complications but the limitation is absence of CLSI guidelines in *S*. Paratyphi A for azithromycin. Therefore, in the absence of culture positive cases, we still lack evidence of its appropriateness in clinical use.

***Clinical relevance*:**

Total 258 typhoidal *Salmonella*e were reported online during 2020. Overall ampicillin sensitivity was 98%, chloramphenicol sensitivity was 97% followed by 96% sensitivity for tromethoprim sulfmethoxazole in *Salmonella* Typhi. Cephalosporins were 99% sensitive

and azithromycin was 98% sensitive. Ciprofloxacin was 5% sensitive as compare to pefloxacin which was 12% sensitive in *Salmonella* Typhi (**Table 4.1**). This discordance between ciprofloxacin and pefloxacin was not observed when we tested the isolates sent by regional centers to our Nodal Center. The reason could be due to not all the isolates being transported to our center and secondly could be due to disk variation when comparing oxoid verses Hi-media disks for pefloxacin.
In *Salmonella* Paratyphi A, ampicilllin was 91% sensitive, chloramphenicol was 98% sensitive and trimethoprim sulfmethoxazole was 96% sensitive. Cephalosporins sensitivity was 100% while ciprofloxacin was 3% sensitive (**Table 4.1).**

***Salmonella* Typhi:** As per the data entered online there is an increase in isolation of *Salmonella* Typhi over the years e.g. total isolation was 3.6% in 2017 which was increased to 4.1% in 2018 and 4.2% in 2019 and 4.3% in 2020 from all over India irrespective of COVID-19 pandemic. Same pattern has been observed from West and South parts of India. (**Table 4.2, Fig 4.1).**
Maximum number of *S.* Typhi was isolated from South India followed by North India and west India. Minimum isolation was reported from East India and Central India. In reference to the sensitivity across different parts of Indian subcontinent, ampicillin and chloramphenicol was 100% sensitive from West India, 99% from South and 98% from North India. While nationally, sensitivity was 97% for ampicillin (**Table 4.3**). Trimethoprim sulfmethoxazole sensitivity was same as ampicillin and chloramphenicol from North and West India while it shows 96% sensitivity as cumulative sensitivity from all over India and individually from South India.

Cephalosporin sensitivity was 100% from North and West India respectively while some reports of resistance were reported from South India making sensitivity to 99% from South and all over India. In case of azithromycin from all different parts of India, 100% sensitivity has been reported from North India, 99% from South India and 95% from west India, restricting its sensitivity to 98% from all over India. Overall ciprofloxacin sensitivity was 5% nationally and 5% from West, followed by 3% from south India. Isolation of MDR (multiple drug resistance) strains has decreased over the period of 2016 to 2020 from 8% to 3% (**Table 4.4, Figure 4.2**). Ampicillin sensitivity has increased from 92% in 2016 to 98% in 2020 while chloramphenicol sensitivity has increased from 91% in 2016 to 97% in 2020 followed by 92% sensitivity of trimethoprim sulfmethoxazole in 2016 to 96% sensitivity in 2020.

Ceftriaxone and cefixime susceptibility was also almost equal during studied period. It was 98.5% (329/334) in 2017 and 98.1% (531/541) in 2018 followed by 98% (645/658) in 2019 and 99% (192/193) in 2020. Ciprofloxacin sensitivity has decreased from 18% (6/33) in 2016, 11.6% (35/302) in 2017 to 6.6% (29/440) in 2018 and again increased in 2019 to 7.2% (35/501) followed by 5% (8/162S) in 2020. Levofloxacin sensitivity was 9%

(3/35) in 2019 and 6% in 2020. Pefloxacin sensitivity decreased from 20.2% (36/178) in 2017 to 19.6% (39/199) in 2018, 15.3% (47/307) in 2019 and 12% (13/108). Azithromycin susceptibility was 95.7% (266/278) in 2017, 98.4% (497/506) in 2018, 96.3% (547/568) in 2019 and 98% (163/166). Overall there was a sudden increase in resistance in 2018 and 2020 as compare to 2019.

Table 4.1: Susceptibility pattern of *Salmonella* species from blood

AMA	*Salmonella* Typhi Total n=206 (S %)	*Salmonella* ParatyphiA Total n=52 (S %)
Ampicillin	192/197 (97.5)	42/46 (91.3)
Ceftriaxone	192/193 (99.5)	47/47 (100)
Cefixime	157/158 (99.4)	32/32 (100)
Azithromycin	163/166 (98.2)	*0/0
Ciprofloxacin	8/162 (4.9)	1/31 (3.2)
Levofloxacin	*4/12	*0/9
Ofloxacin	*0/0	*0/0
Pefloxacin	13/109 (11.9)	*3/14
Trimethoprim-sulfamethoxazole	194/202 (96)	47/49 (95.9)
Chloramphenicol	180/185 (97.3)	48/49 (98)

*Azithromycin sensitivity cutoff values are not given in CLSI for *Salmonella* Paratyphi A

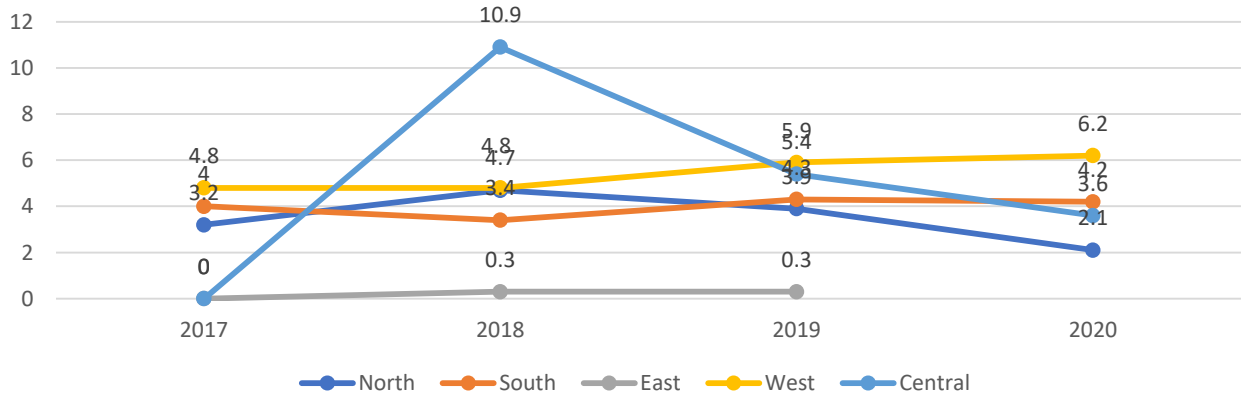


Figure 4.1 Yearly-isolation trend of *Salmonella* Typhi from All Samples across different regions of India (except Faeces)

Table 4.2. Yearly-isolation trend of *Salmonella* Typhi from different part of India

Years	2017	2018	2019	2020
Total Culture	**n=9491**	**n=14091**	**n=17108**	**n=11728**
North	138/4272 (3.2%)	246/5247 (4.7%)	174/4415 (3.9%)	111/2962 (2.1%)
Central	0/0* (-)	12/110 (10.9%)	30/551 (5.4%)	28/411 (3.6%)
East	0/171* (0%)	2/712 (0.3%)	4/1443 (0.3%)	--
West	31/648 (4.8%)	115/2010 (5.7%)	160/2694 (5.9%)	97/1605 (6.2%)
South	176/4400 (4%)	204/6012 (3.4%)	342/8005 (4.3%)	256/6039 (4.2%)
National	345/9491 (3.6%)	579/14091 (4.1%)	710/17108 (4.2%)	31/711 (4.3%)

https://amr.icmr.org.in/amr/amr_analysis/rc/tc_resis_gph.php

Table 4.3: Susceptibility pattern of *Salmonella* Typhi from Blood across different regions of India

Antibiotic	National (n=206)	North (n=47)	Central (n=14)	East (n=1)	West (n=41)	South (n=103)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Ampicillin	192/197 (97.5)	46/47 (97.9)	8/11 (-)	1/1 (-)	35/35 (100)	102/103 (99)
Ceftriaxone	192/193 (99.5)	46/46 (100)	12/12 (-)	1/1 (-)	33/33 (100)	100/101 (99)
Cefixime	157/158 (99.4)	47/47 (100)	10/10 (-)	1/1 (-)	24/24 (100)	75/76 (98.7)
Azithromycin	163/166 (98.2)	47/47 (100)	10/10 (-)	0/0 (-)	39/41 (95.1)	67/68 (98.5)
Ciprofloxacin	8/162 (4.9)	2/18 (-)	1/14 (-)	0/1 (-)	2/40 (5)	3/89 (3.4)
Trimethoprim-sulfamethoxazole	194/202 (96)	46/47 (97.9)	9/12 (-)	1/1 (-)	41/41 (100)	97/101 (96)
Chloramphenicol	180/185 (97.3)	46/47 (97.9)	7/10 (-)	1/1 (-)	41/41 (100)	85/86 (98.8)

Table 4.4: Yearly susceptibility trends of *Salmonella* Typhi from Blood

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=37	Total n=345	Total n=580	Total n=728	Total n=206
	(S%)	(S%)	(S%)	(S%)	(S%)
Ampicillin	34/37 (91.9)	305/332 (91.9)	551/576 (95.7)	658/703 (93.6)	192/197 (97.5)
Ceftriaxone	37/37 (100)	329/334 (98.5)	531/541 (98.2)	645/658 (98)	192/193 (99.5)
Cefixime	*15/15	221/223 (99.1)	344/349 (98.6)	434/448 (96.9)	157/158 (99.4)
Azithromycin	24/24 (100)	266/278 (95.7)	497/506 (98.2)	547/568 (96.3)	163/166 (98.2)
Ciprofloxacin	6/33 (18.2)	35/302 (11.6)	29/440 (6.6)	35/501 (7)	8/162 (4.9)
Levofloxacin	*0/0	*0/3	*5/18	3/35 (8.6)	*4/12
Trimethoprim-sulfamethoxazole	34/37 (91.9)	322/341 (94.4)	552/575 (96)	693/718 (96.5)	194/202 (96)
Chloramphenicol	31/34 (91.2)	267/278 (96)	541/560 (96.6)	582/611 (95.3)	180/185 (97.3)
Pefloxacin	0/0	36/178 (20.2)	39/199 (19.6)	47/307 (15.3)	13/108 (12)

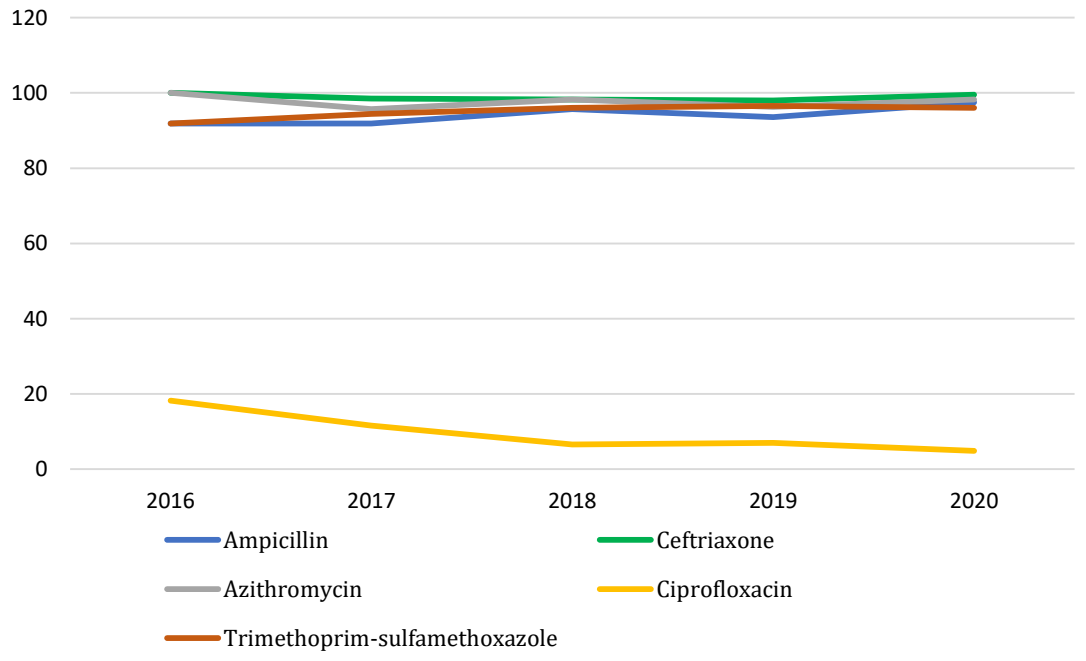


Figure 4.2: Yearly susceptibility trends of *Salmonella* Typhi from Blood

***Salmonella* Paratyphi A:** *Salmonella* Paratyphi A antibiotic susceptibility pattern from 2017 to 2019 shows that ampicillin was 95% (38/40) sensitive in 2017 and 97.6% (122/125) in 2018. There was an increase in ampicillin resistance in 2019 as total sensitivity was 90.6% (125/138) less than previous years and 91.3% (42/46) in 2020 while chloramphenicol and trimethoprim - sulmethoxazole was 100% sensitive in 2017 and 2018 but decreased to 99.3% susceptibility in 2019 followed by 95.8% in 2020. Ciprofloxacin sensitivity has decreased from 2017 to 2019 as it was 10% (4/40) in 2017 and only 1% in 2018 and 2019 but due to the less number of isolates it increased to 3.2% (1/31) in 2020 as only one isolate was sensitive to ciprofloxacin (**Table 4.5) (Figure 4.3).** Ceftriaxone antimicrobial susceptibility has increased from 95% (38/40) in 2017 to 97.6% (121/124) in 2018 and 97.9% (139/142) in 2019 and reached up to 100% susceptibility by 2020. Cefixime was 96.3% (26/27) susceptible in 2017 followed by 100% (105/105) in 2018, 98.1% (105/107) in 2019 and again 100% (31/31) in 2020. Azithromycin was not analysed as azithromycin susceptibility cutoff for *Salmonella* Paratyphi A are not given in CLSI.

Table 4.5: Yearly susceptibility trends of *Salmonella* Paratyphi A from Blood

AMA	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=41	Total n=125	Total n=147	Total n=52
	(S%)	(S%)	(S%)	(S%)
Ampicillin	38/40 (95)	122/125 (97.6)	125/138 (90.6)	42/46 (91.3)
Ceftriaxone	38/40 (95)	121/124 (97.6)	139/142 (97.9)	47/47 (100)
Cefixime	26/27 (96.3)	105/105 (100)	105/107 (98.1)	32/32 (100)
Ciprofloxacin	4/40 (10)	1/111 (0.9)	1/86 (1.2)	1/31 (3.2)
Levofloxacin	*0/2	*0/5	0/25 (0)	*0/9
Trimethoprim-sulfamethoxazole	41/41 (100)	123/123 (100)	144/145 (99.3)	47/49 (95.9)
Chloramphenicol	30/30 (100)	121/121 (100)	128/128 (100)	48/49 (98)

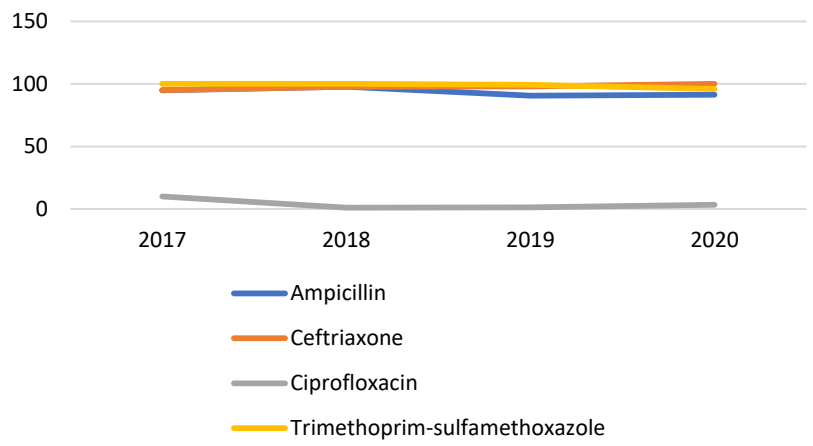


Figure 4.3: Yearly susceptibility trends of *Salmonella* Paratyphi A from Blood

Creeping MICs for cephalosporins, azithromycin and fluoroquinolones – data from the isolates processed at AIIMS, Nodal Center: In 2020 from January to December total isolation of *Salmonella* was 37 at AIIMS, New Delhi. Out of which 7 were *Salmonella* Paratyphi A and 27 were *Salmonella* Typhi. All the strains were confirmed by standard biochemical and serological tests using specific antisera according to the manufacturer guidelines. Antibiotic susceptibility was done by Kirby – Bauer disk diffusion method as per CLSI guidelines (2020) for Amoxicillin, Co-trimoxazole (Trimethoprim-sulfamethoxazole), Ciprofloxacin, Chloramphenicol, Ceftriaxone and Cefixime. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 was used as reference strains for quality control and *Enterococcus faecalis* ATCC 29212 was used as reference strain for QC of Co-trimoxazole susceptibility.

MIC to ciprofloxacin in *S.*Typhi and *S.* Paratyphi A is presented in **Figure 4.4, Figure 4.5** and **Table 4.6.** MIC to ceftriaxone is presented in **Figure 4.6, Figure 4.7** .MIC to azithromycin is presented in Fig. 4.9,Fig 4.10. Regarding fluoroquinolones, levofloxacin MIC range from 0.016 µg/ml to >32 µg/ml while ofloxacin MIC ranged from 0.032 µg/ml to >32 µg/ml. The maximum range was similar in case of levofloxacin and ofloxacin but MIC50 and MIC90 value were higher for ofloxacin. While in case of ciprofloxacin, maximum range reached up to >256 µg/ml followed by 0.38 µg/ml and 24 µg/ml value as MIC50 and MIC90 (Table 4).

MIC for ceftriaxone varies from 0.023 to 0.38 µg/ml. Minimum range of ceftriaxone MIC has been incresed over the time, reached up to 0.064 from 0.004 µg/ml in previous years report. Still maximum number of strains are sensitive but resistant strain also start to appear there in the community. Azithromycin MIC was reported form o.125 µg/ml to 32 µg/ml .

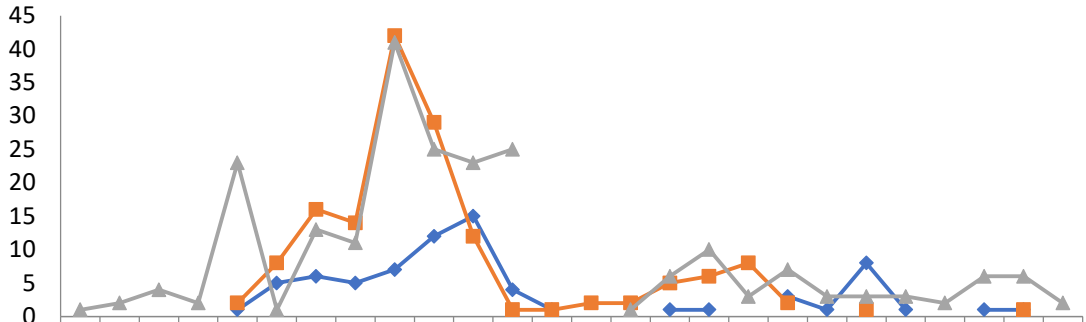
To study ciprofloxacin MIC trends over six year, time has been grouped in to two groups of three year each (2014-2016 and 2017-2019) and 2020 has been added as single year (**Figure 4.4, Figure 4.5).** The minimum MIC value (0.016 µg/ml to 0.047 µg/ml) was not reported from 2014 to 2019 but reported in the strains isolated in 2020. The maximum MIC range (256µg/ml) was also reported in 2020.

Although maximum number of *S.* Typhi 45/77 (58%) show intermediate sensitivity against ciprofloxacin in 2014-2016 and 113/160 (71%) in 2017-2019 these were considered as resistant which makes total ciprofloxacin resistance 92% (71/77) in 2014- 2016 and 93% (149/160) in 2017-2019 and 98.4% (191/194) in 2020 in typhoidal *Salmonella* .

During 2014-2016, 28% *S.* Paratyphi A was sensitive for ciprofloxacin and 54% were intermediate followed by 18% of resistant strains. This sensitivity pattern has been changed over time and only 4% of *S.* Paratyphi A were sensitive and maximum number of *S.* Paratyphi A (91%) shows intermediate pattern followed by 4% resistance during 2020 **(Figure 4.5).**

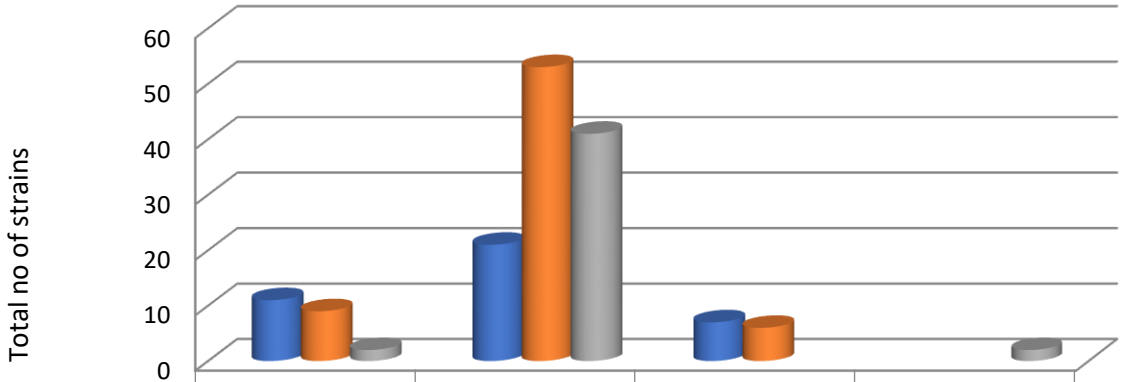
Table 4.6: Minimum, maximum MIC range along with MIC50 and MIC 90 in *Salmonella* Typhi to fluoroquinolones, cephalosporin's and Macrolide received from all centres in 2020

	Min (MIC**(µg/ml)**	Maxi (MIC**(µg/ml)**	MIC 50	MIC 90
Levofloxacin	0.016	>32	0.5	12
Oflofloxacin	0.032	>32	1	24
Ciprofloxacin	0.016	>256	0.38	24
Ceftriaxone	0.023	0.38	0.125	0.25
Cefixime	0.094	>256	0.38	0.5
Azithromycin	0.125	32	8	16



	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	1	1.5	3	4	6	8	12	16	24	32	48	50	64	96	256
2014-2016					1	5	6	5	7	12	15	4	1			1	1		3	1	8	1		1	1	
2017-2019					2	8	16	14	42	29	12	1	1	2	2	5	6	8	2		1				1	
2020	1	2	4	2	23	1	13	11	41	25	23	25			1	6	10	3	7	3	3	3	2	6	6	2

Figure 4.4: Ciprofloxacin MIC trends in S. Typhi at AIIMS, New Delhi over a period of seven years



	0.032-0.064	0.125-0.75	1-3	4-6
2014-2016	11	21	7	
2017-2019	9	53	6	
2020	2	41		2

Figure 4.5: Year-wise ciprofloxacin MIC in *S.* Paratyphi A isolated at AIIMS, New Delhi

Though maximum number of strains still falls in sensitive range from 0.125 to 0.19 µg/ml but few no. of strains started to show increased MIC.

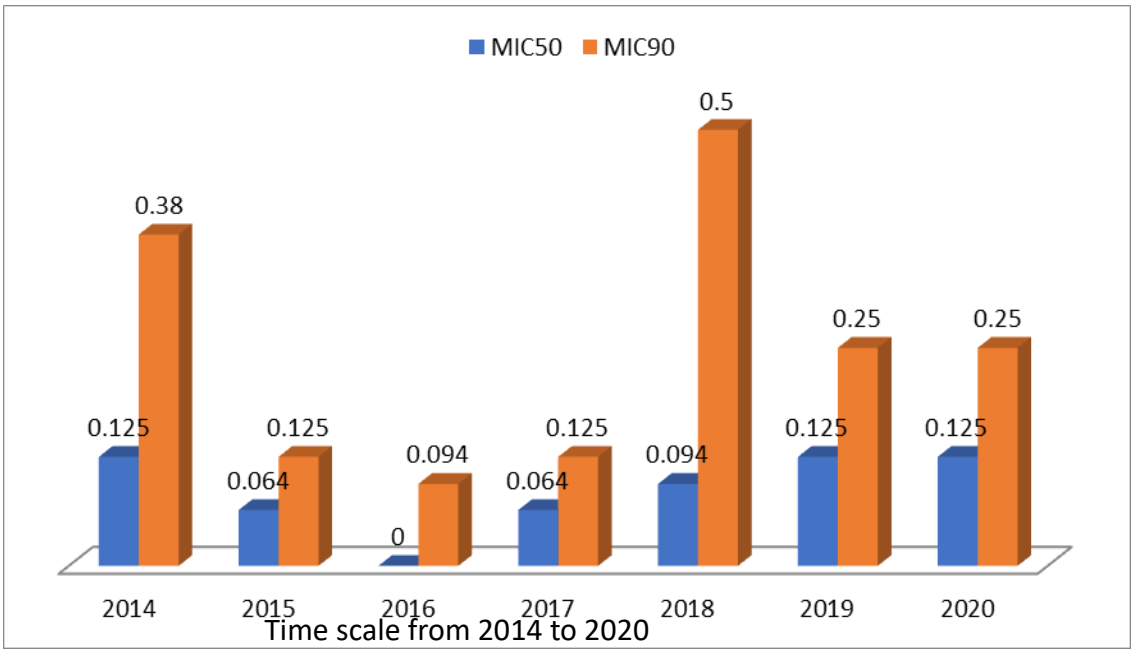
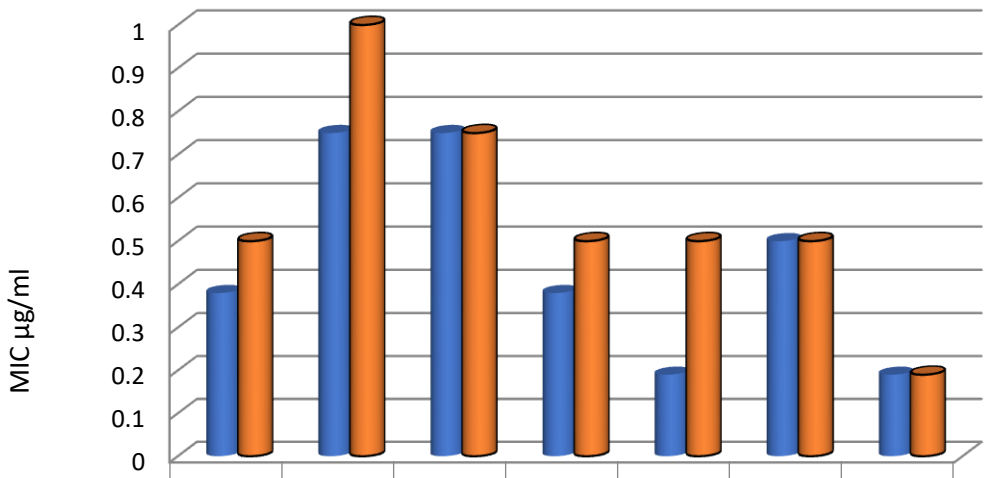


Figure 4.6 Comparison of ceftriaxone MIC50 and MIC 90 for S. Typhi at AIIMS over a period of seven years

During 2014, MIC 50 and MIC 90 for *Salmonella* Paratyphi A was 0.38 µg/ml and 0.5 µg/ml while MIC 50 has increased overv the years and in 2020 it was 0.5 while MIC90 has decreased up to 0.19 µg/ml from 0.5 µg/ml in 2014 (Figure 4.7)



	2014	2015	2016	2017	2018	2019	2020
MIC50 SPA	0.38	0.75	0.75	0.38	0.19	0.5	0.19
MIC90 SPA	0.5	1	0.75	0.5	0.5	0.5	0.19

Figure 4.7: Ceftriaxone MIC in *S.* Paratyphi A from 2014-2020 isolated at AIIMS

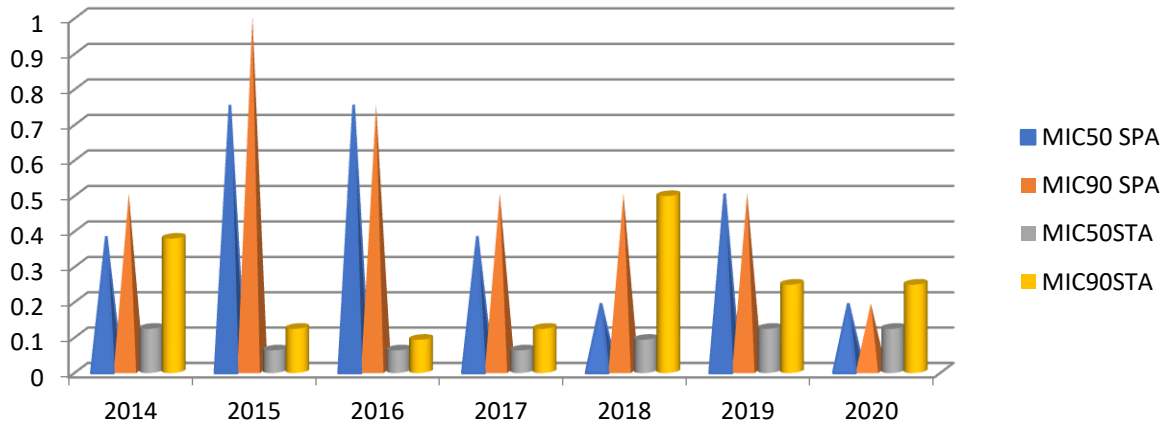
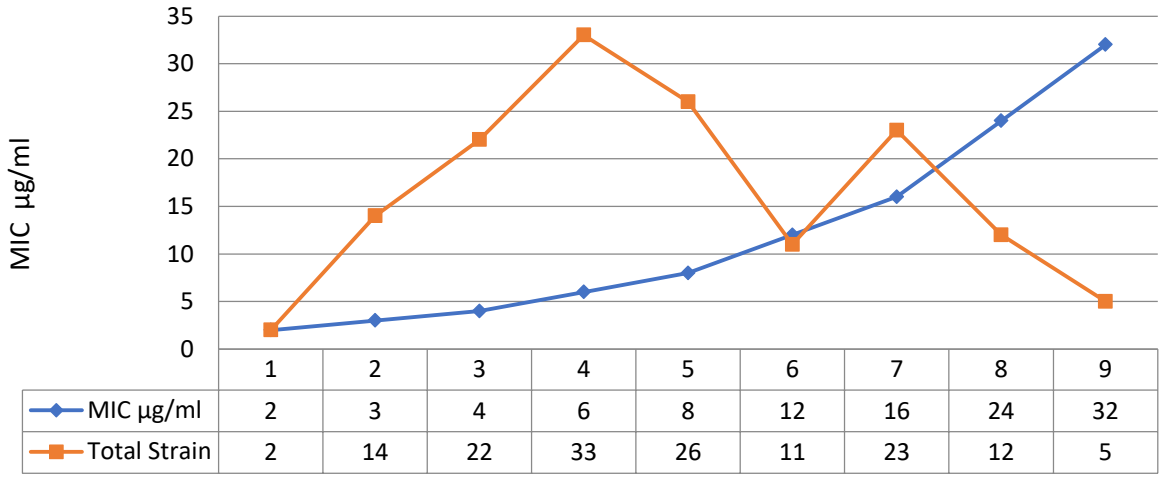


Figure 4.8. Comparison of MIC50 and MIC90 for ceftriaxone in typhoidal *Salmonellae* over the years

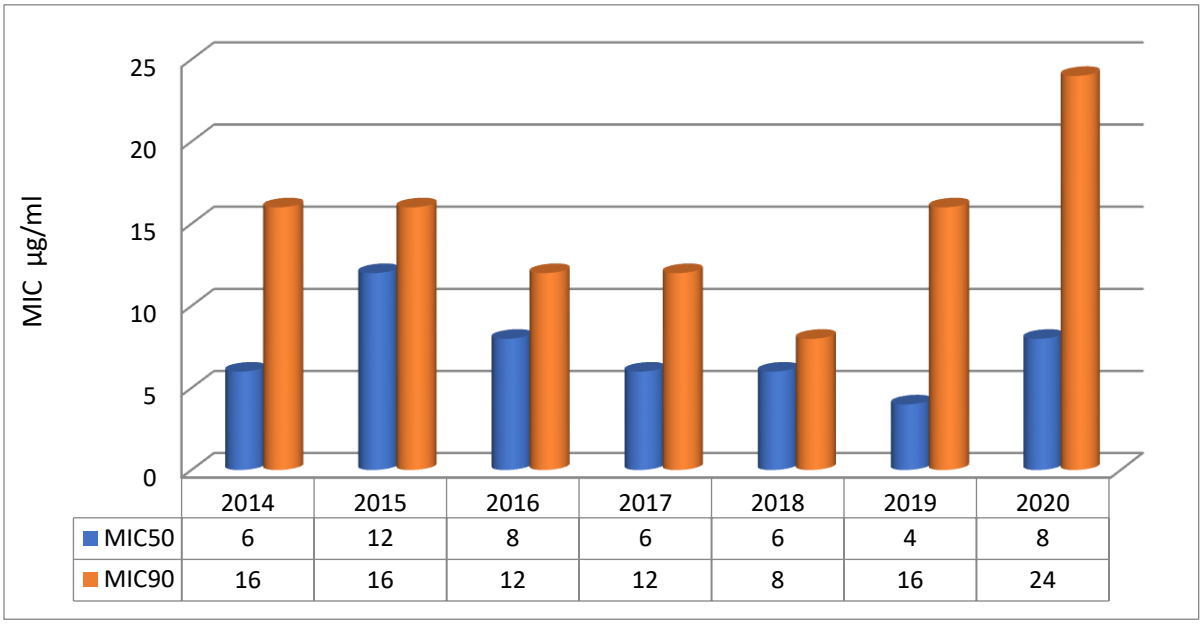
From all the data available from 2014 to 2020, it was observed that none of the isolate was azithromycin resistant but in 2020 few strains with MIC (32 µg/ml) towards higher side were noted (Figure 4.9)



	1	2	3	4	5	6	7	8	9
MIC µg/ml	2	3	4	6	8	12	16	24	32
Total Strain	2	14	22	33	26	11	23	12	5

Figure 4.9: Azithromycin MIC in *S.*Typhi from all centers received at AIIMS in 2020

MIC50 and MIC90 have been increased from 6 µg/ml and 16 µg/ml respectively in 2014 to 8 µg/ml and 24 µg/ml respectively in 2020 (Figure 4.10).



	2014	2015	2016	2017	2018	2019	2020
MIC50	6	12	8	6	6	4	8
MIC90	16	16	12	12	8	16	24

Figure 4.10: Comparison of Azithromycin MIC50 and MIC90 in *S.* Typhi over a period of seven years at AIIMS

Whole Genome Sequencing: *Salmonella* Typhi & *S.* Paratyphi A

For whole genome sequencing total 44 strains were selected initially based on selection criteria described below.

First strains were selected on the basis of AMR pattern with following antibiotic resistance: Azithromycin, Ceftriaxone, first line drug (Chloramphenicol, Ampicillin and Co-trimoxazole) and Ciprofloxacin. Of the remaining strains, rests were selected to complete 25% by selecting month-wise and batch wise. We present WGS results for 36 strains selected during the present year by using above mentioned criteria **(Table 4.7).**

Briefly, Genomic DNA isolated from freshly grown overnight culture (*QIAamp* DNA minikit; *Qiagen*, Germany) was quantified using Qubit fluorometer (Life Technologies, USA). Sequencing library preparation was by Illumina Nextera XT DNA sample preparation kit (Illumina, USA). Genomic DNA samples were fragmented using Covaris M-series (M220) at temperature of 5.5 to 6 °C for 40 seconds. DNA fragments were end repaired using dA bases before ligation with Illumina indexed adapters, amplified for 10 cycles of PCR and sequenced employing v2 and v3 chemistry with paired-end 2 × 151 bp reads on Illumina MiSeq (Illumina, USA). Output data files were de-multiplexed and tra nsformed with Casava v.1.8.2. in to FASTQ files (Illumina, Inc, USA). The selected sequence paired-end reads from Fast QC v0.11.4 were pre-processed and assembled *de novo* with A5-miseq

pipeline. Sequence adapters and low-quality (<Q30) regions were filtered with trimmomatic v0.36. Read errors were corrected by SGA's k-mer-based error correction algorithm. Paired and unpaired reads were assembled utilizing IDBA-UD algorithm and quality of genome assembly evaluated by quality assessment tool (QUAST) (http://quast.sourceforge.net/quast). The redundant homologues with identity cut-off of 0.9 were removed from assembled *de novo* contigs by Cluster database at high identity with tolerance (CD-HIT). The assembled genomes were aligned to a reference genome of *S*. Typhi to avoid the risk of cross-contamination. The assembled bacterial genomes were annotated with Prokka v1.12 http://www.vicbioinformatics.com/software.prokka.shtml). The total numbers of coding sequence regions (CDS) in annotated genomes were compared among all strains to filter out outlier strains. To identify strain-specific genomic features and genomic diversity among *S*.Typhi isolates, the pan-genome was constructed using computational pipeline BPGA. The resistance genes in the assembled *Salmonella* genomes were predicted through the resistance gene identifier (RGI) from the Comprehensive Antibiotic Resistance Database (CARD, available at https://card.mcmaster.ca/analyze/rgi) and Pathogen watch from the Center for Genomic Pathogen Surveillance (CGPS, available at https://pathogen.watch) databases of antimicrobial resistance genes. For sequence identity cut-off criteria was ≥ 50% and for query coverage it was ≥70%. RGI (RGI 4.2.0, CARD 2.0.3) prediction of resistome was determined based on homology and SNP models, where the "perfect and strict hits only" criteria were chosen for the prediction. ResFinder webserver 3.0 (https://cge.cbs.dtu.dk/services/ResFinder/) was used to pinpoint the acquired antimicrobial resistance genes and genes associated with chromosomal point mutations.

Whole- genome sequencing showed that amoxicillin resistance was associated with the presence of beta-lactam genes which were observed in 13/36 strains by WGS. The most common beta-lactam resistance gene *blaTEM-1B* was observed in 11 strains, *blaOXA-232 in 1* strains and *blaTEM116* in one strain. The resistance genes encode for the predominant plasmid-mediated β-lactamases of *Enterobacterales*. Overall, antimicrobial resistance was observed in 16/36 strains by phenotypic method. Earlier reports for amoxicillin resistance in *Salmonella* strains isolated pan-India was 3%.

Chloramphenicol resistance determinants were observed in 11/36 strains by WGS. All non-susceptible strains harboured *catA1* gene which encodes chloramphenicol acetyltransferase enzyme causing chloramphenicol resistance by chemical modification of the drug molecule, whereas ten isolates harboured the *catI* genes. Our findings are consistent with other studies reporting chloramphenicol susceptibility in *S. enterica*. Antimicrobial resistance to chloramphenicol was 9/36 by disk diffusion method. Similar findings have been reported by other studies where resistance gene carriage rate was higher than phenotypically reported resistance.

Trimethoprim-sulfamethoxazole is available in combination for treatment but WGS analyze these as two separate antimicrobial agents as trimethoprim and sulfamethoxazol. Out of 36 strains, trimethoprim resistance determining genes were found in 11/36 isolates. Likewise, gene *sul1* and *sul2*, encoding dihydropteroate synthases known to disseminate sulfamethoxazole resistance, were also detected in 11/36 isolates. Overall, antimicrobial resistance to co-trimoxazole was detected in 9/36 isolates by phenotypic method.

Molecular determinants of resistance to fluoroquinolone including ciprofloxacin and pefloxacin antibiotics encoded by *gyr*Aand *parC,* genes were detected in 35/36 strains by WGS. Mutations in *gyrA* and *parC,* was observed in 29/36 and 7/36 of strains, respectively **(Tables 4.7).** Total 6/36 isolates were observed with double mutation in *gyrA* and *parC* gene. The identified genes were associated with mutations in Quinolone Resistance Determining Region of DNA gyrase enzyme, the binding site for fluoroquinolone. Antimicrobial resistance to fluoroquinolones was 23/36 by both disc diffusion and E-test method. MIC distribution ranged between 2–24 mg/L and peaked at 12 mg/L. DNA Gyrase A mutations at position 83 (Ser-83→Phe, Ser-83→Tyr and Asp 87→ Phe) are the most prevalent resistance mechanisms for Fluoroquinolone in India, followed by Ser-80→Ile and Glu 84→ Lys substitution in *parC* gene. Highly non-susceptible strains (with ciprofloxacin MIC > 8 mg/L) were found to be double or triple mutants with mutations in *gyrA*83, *gyrA*87 and *parC*80. Strains with moderate resistance to ciprofloxacin possessed single mutations in DNA *gyrA* gene at Ser83 position.

Antimicrobial susceptibility to antibiotics, cefixime and ceftriaxone, observed for all strains is consistent with other studies from India. Though all the strains were susceptible, however, a gradual increase in median MIC values was perceived over a time period. Mutations in PBP3 gene at D350N, S357N, Escherichia coli ampC1 beta-lactamase, and Escherichia coli ampH beta-lactamase gene were present in all tested isolates.This clearly raises an alarm towards the judicial use of these antibiotics.None of the isolate was resistant to azithromycin byphenotypic method. But nalD, KpnE, CRP gene responsible for macrolide resistance were observed by WGS.

S. Typhi can demonstrate resistance to multiple antibiotics by acquiring new resistance genes through horizontal genes transfer (HGT). The acquired antimicrobial resistance genes including *aac(6')-Iaa, AAC(6')-Iy, aadA1, aph(3")-Ib, aph(6)-Id, strA*, and *strB* that provided resistance to aminoglycosides were observed in 100% (36/36) isolates. Tetracycline resistance encoded by mdfA gene which belongs to major facilitator superfamily (MFS) antibiotic efflux pump was detected in 5 isolates while *tet(B)* and *tet(R)* genes for tetracycline efflux pumps was detected in one isolate of *S*. Typhi. In addition, *S*. Typhi isolates harboured the genes *baeR, emrb, H-NS, marA, mdfA, mdtK, msbA, acrA, emrR, kpnE, kpnF, marR, sdiA, crp, soxR*, and *soxS* that could confer multidrug resistance and were detected in all strains.

Table 4.7: Complete detailed results of WGS

Center Name	AMR ID	S. No.	MLST	Plasmid	Plasmid	Plasmid	Plasmid	streptomycin	kanamycin	ampicillin	chloramphenicol	trimethoprim	ciprofloxacin I/R, nalidixic acid		sulfisoxazole		tetracycline	
RC13	240974	32	ST1										parC (S80I)					
RC13	190042	34	ST1				IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2		
RC12	124002	47	ST1										parC (S80I)					
RC7	124588	36	ST85											gyrA (S83Y)				
RC10	196286	12	ST1		IncFII(K)								parC (S80I)					
RC10	154452	13	ST85											gyrA (S83F)				
RC10	157993	14	ST85											gyrA (S83F)				
RC10	158887	15	ST129											gyrA (S83F)				
RC10	41399	16	ST1		IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83F)	sul1	sul2		
RC5	137237	7	ST129											gyrA (S83F)				
RC5	137238	8	ST129	ColRNAI										gyrA (S83F)				
RC5	136401	9	ST1										parC (S80I)					
RC5	145476	10	ST1				IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2		
RC5	113595	11	ST1		IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2		
RC14	B-124	28	ST1				IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7			sul1	sul2		
RC14	B-125	29	ST85							blaTEM-116				gyrA (S83F)				
RC14	B-268	31	ST129											gyrA (S83F)				
RC15	140517	42	ST1	ColRNAI	IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7	parC (E84K)	gyrA (S83Y)	sul1	sul2		
RC15	170556	44	ST129	ColKP3						blaOXA-232				gyrA (S83F)				ARR-2
RC17	202691	40	ST1	Col440II	IncFIB (pHCM2)	IncR	IncQ1	aph(3")-Ib aac(6')-Ib-Hangzhou aac(6')-Ib-cr	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83F)	sul1	sul2		

RC20	BT-3481/19	18	ST85											gyrA (S83F)			
RC20	BT-4973/19	19	ST1											gyrA (S83F)			
RC20	BT-4795/19	20	ST1										parC (S80I)				
RC20	BT-5819/19	21	ST85											gyrA (S83F)			
RC20	BT-6556/19	22	ST129											gyrA (S83F)			
RC20	106158	25	ST1											gyrA (D87N)			
RC20	106824	26	ST85											gyrA (S83F)			
RC20	106871	27	ST2		IncFIB (pHCM2)									gyrA (S83F)			
RC6	9406	1	ST1	IncHI1A	IncHI1B (R27)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83F)	sul1	sul2	tet(B)
RC6	18449	2	ST1		IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2	
RC6	19272	3	ST1		IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2	
RC6	201663	4	ST1		IncFIB (pHCM2)		IncQ1	aph(3")-Ib	aph(6)-Id	blaTEM-1B	catA1	dfrA7		gyrA (S83Y)	sul1	sul2	
RC6	10874 (201663)	5	ST1										parC (S80I)				
RC6	11221	6	ST129											gyrA (S83F)			
RC8	202673	23	ST2											gyrA (S83F)			
RC8	184945	24	ST129											gyrA (S83F)			

MLST: Multi-locus sequence typing (MLST) profile disclosed low genetic variation in housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*) among 36 Salmonella *isolates*. Two different sequence types (STs) including ST1 and ST2 were observed in *Salmonella* Typhi while *Salmonella* Paratyphi A was divided in ST 85 and ST129 respectively. ST1 was the predominant type, accounting for 19/36 of examined strains, whereas ST2 was observed in 2/36 of the strains. In case of S Para A, ST85 was observed in 7 and ST129 was observed in 5 isolates. Complete detail of MLST is presented in **Table 4.8**. By WGS it has been revealed that apart from the drugs used to treat typhoid *Salmonella Typhi* also harbor the resistant genes for other antibiotics.

Table 4.8 Center wise complete details of MLST from 2019-2020

Center Name	***Salmonella* Typhi**		***Salmonella* Paratyphi A**	
	ST 1	**ST2**	**ST 85**	**ST 129**
RC1	10			
RC3	10	1		
RC2	25	3		
RC4	13	1		
RC5	20	1		2
RC6	20	1		1
RC10	2		2	1
RC14	15	2	1	1
RC12	3			
RC7	1		1	
RC13	1			
RC15				1
RC17	1			
RC20	3	1	3	1
RC8		1		1
Total	**124**	**11**	**7**	**8**

Chapter 5.Non fermenting Gram Negative Bacteria (NFGNB)

Among the Non-fermenting Gram -negative bacilli collected during Jan-Dec 2020 across all AMRSN sites, *Pseudomonas aeruginosa* was the most commonly isolated pathogen followed by *Acinetobacter baumannii*, *Burkholderia cepacia*and *Stenotrophomonas maltohphilia*. However, differences in the isolation rates based on the clinical settings were observed. Notably, *P. aeruginosa* was predominantly isolated in wards (53.7%) and OPD (24.7%) compared to ICU (21.4%), while *A. baumannii* was predominant in ward (50.8%), followed by ICU (40.9%) and OPD (8.1%) respectively.

Clinical implications

Pseudomonas aeruginosa is the most common pathogen constituting for about 20 % of NFGNB during 2019 across all AMRSN sites. The isolation rates were higher from wards (12.1%), followed by ICU (11.9%) and OPD (10.8%) settings. Notably, the overall isolation rates were found to decline this year, compared to previous years. Antimicrobial susceptibility profile showed the least susceptibility rates to anti-pseudomonal for isolates from ICU settings (45-55%) and wards (40-60%), compared to OPD (70-80%). Among the anti-pseudomonal, the least susceptibility of 40% was observed for fluoroquinolones; 60-70% to cephalosporins, carbapenems, and aminoglycosides; highest susceptibility being 90% to colistin. There were also differences in the susceptibility rates for isolates from different settings. For instance, 50% carbapenem susceptibility in ICU isolates compared to 80% susceptible in OPD. This is critical to choose an empiric therapy based on the hospital location, where carbapenems may not be a better option for ICU settings with high resistance rates, rather best suitable for OPD and Wards.

Based on the susceptibility profile, it would be ideal to choose agents with high susceptibility such as piperacillin/tazobactam (70-80%), amikacin (60-70%), and colistin (90-95%) as an empiric therapy. Nevertheless, choosing the right antibiotics essentially depends on the settings where the isolates are likely from, as the profile varies with different settings. Combination agents of any two-antipseudomonal could be preferred to overcome the high resistance rates, which are always recommended for pseudomonal infections. Moreover, the newer antimcirobial agents evaluated here such as ceftazidime/avibactam, aztreonam/avibactam, ceftolozane/tazobactam and imipenem/relebactam could be an alternative with good *in-vitro* suscpetiblity profile, including against Metallo-beta-lactamase (MBL) producers.

In *A. baumannii* increased resistance to almost all the available drugs has been observed and found to be involved in nosocomial infections and hospital outbreaks. Reduced susceptibility of 10-20% was observed against cephalosporins, carbapenems, monobactams and β-lactam-β-lactamase inhibitors across all the specimen types. Among BSIs, susceptibility only to minocycline was retained up-to 40%. Among the tested antibiotics, only colistin showed >90% susceptibility. Though colistin-based combinations can be considered for treating carbapenem-resistant *A. baumannii* infections, both CLSI and EUCAST revised the interpretive criteria for *in-vitro* polymyxin susceptibility testing and suggested to prefer non-polymyxin agents for treating *Acinetobacter* infections. Such revision would effectively be helpful in considering polymyxin as a treatment option in selected cases. Currently, none of the newly available drug combinations have clinical activity against carbapenem-resistant *A. baumannii* infections except for the two novel agents, cefepime-zidebactam and sulbactam-durlobactam.

Detailed analysis of antimicrobial susceptibility profile:

Pseudomonas aeruginosa: The overall isolation rates of *Pseudomonas aeruginosa* was found to decline in the last one year (Jan-Dec, 2020) with a total of 7839 isolates, as compared to the previous year (Jan-Dec, 2019) with 12,507 isolates. There was a clear demarcation with an increase in isolation rate in ICU settings by 7% and reduction in OPD (by 6%) **(Table 5.1)**. Additionally, isolation rates among the different clinical specimens showed increase in *P. aeruginosa* from lower respiratory tract (by 2%) and blood stream (by 3%) infections, while the rates from other specimens remained the same as previous years **(Table 5.2)**. This could be due to the increased hospital-acquired infections in COVID-19 patients resulting in elevated ICU rates and LRT infections. Antimicrobial susceptibility profile ranged from 55-70% to all anti-pseudomonal agents, with colistin being the highest (95%). Among the settings, susceptibility from OPD isolates showed highest, followed by Wards and ICU. However, isolates from urine showed the least susceptibility, followed by CSF, LRT, blood, deep and superficial infections. Notably, carbapenem resistance was observed at slightly higher rates in ICU infections and in particular urine, CSF and LRT specimens, that could probably be a reflection of hospital acquired infections of resistant phenotypes. Among all the agents, amikacin and piperacillin/tazobactam showed highest susceptibility (other than colistin) invariably in all specimen sources/settings **(Table 5.2)**. Trend analysis of antimicrobial susceptibility profile showed an overall reduction of 2-3% in the susceptible rates of all anti-pseudomonal beta-lactams, while aminoglycosides and fluoroquinolones remains nearly the same in comparison with the previous years **(Table 5.3; Figure 5.1)**.

Table 5.1: Location-wise susceptible percentage of *Pseudomonas aeruginosa* isolated from all samples (except faeces) across OPD, Ward and ICU.

AMA	Total n=7839	OPD n=1941	Ward n=4213	ICU n=1685
	(S %)	(S %)	(S %)	(S %)
Piperacillin-tazobactam	5012/7418 (67.6)	1437/1853 (77.5)	2639/3963 (66.6)	936/1602 (58.4)
Cefepime	4497/7355 (61.1)	1260/1796 (70.2)	2355/3930 (59.9)	882/1629 (54.1)
Ceftazidime	4647/7635 (60.9)	1337/1897 (70.5)	2428/4102 (59.2)	882/1636 (53.9)
Imipenem	4411/7036 (62.7)	1228/1696 (72.4)	2466/3897 (63.3)	717/1443 (49.7)
Meropenem	4955/7661 (64.7)	1422/1878 (75.7)	2661/4130 (64.4)	872/1653 (52.8)
Colistin*	1291/1355 (95.3%)	224/232 (96.6)	724/758 (95.5)	343/365 (94)
Amikacin	5276/7723 (68.3)	1461/1922 (76)	2779/4133 (67.2)	1036/1668 (62.1)
Gentamicin	3241/5341 (60.7)	949/1406 (67.5)	1601/2679 (59.8)	691/1256 (55)
Tobramycin	2907/4331 (67.1)	756/1012 (74.7)	1646/2500 (65.8)	505/819 (61.7)
Ciprofloxacin	3768/6541 (57.6)	1037/1649 (62.9)	2026/3524 (57.5)	705/1368 (51.5)
Levofloxacin	3771/6743 (55.9)	981/1631 (60.1)	2084/3725 (55.9)	706/1387 (50.9)

*Colistin represents percentage Intermediate susceptibility

Table 5.2: Sample-wise susceptible percentage of *Pseudomonas aeruginosa*

AMA	Blood	LRT	Superficial Infection	Deep Infection	CSF	Urine
	n=788	n=2335	n=2181	n=565	n=51	n=1114
Piperacillin-tazobactam	524/745 (70.3)	1504/2241 (67.1)	1460/2047 (71.3)	377/538 (70.1)	29/50 (58)	535/1057 (50.6)
Cefepime	474/731 (64.8)	1405/2244 (62.6)	1354/2105 (64.3)	295/498 (59.2)	23/50 (46)	445/1034 (43)
Ceftazidime	498/757 (65.8)	1430/2293 (62.4)	1358/2154 (63)	344/551 (62.4)	24/49 (49)	441/1055 (41.8)
Imipenem	419/652 (64.3)	1048/1844 (56.8)	1529/2137 (71.5)	335/553 (60.6)	23/45 (51.1)	534/1073 (49.8)
Meropenem	506/769 (65.8)	1428/2287 (62.4)	1540/2146 (71.8)	362/555 (65.2)	27/50 (54)	522/1075 (48.6)
Colistin*	595/629 (94.6%)	1665/1724 (96.6%)	1676/1796 (93.3%)	0/0	59/59 (100%)	812/844 (96.2%)
Amikacin	557/784 (71)	1685/2325 (72.5)	1523/2175 (70)	389/561 (69.3)	24/38 (63.2)	555/1100 (50.5)
Gentamicin	416/637 (65.3)	893/1446 (61.8)	838/1304 (64.3)	276/433 (63.7)	15/32 (46.9)	464/986 (47.1)
Tobramycin	278/401 (69.3)	1121/1538 (72.9)	970/1400 (69.3)	120/178 (67.4)	12/23 (52.2)	180/477 (37.7)
Ciprofloxacin	402/614 (65.5)	1065/1783 (59.7)	1111/1813 (61.3)	312/537 (58.1)	19/37 (51.4)	432/1067 (40.5)
Levofloxacin	424/669 (63.4)	1329/2163 (61.4)	1166/1993 (58.5)	198/400 (49.5)	19/40 (47.5)	303/874 (34.7)

*Colistin represents percentage Intermediate susceptibility

Table 5.3: Yearly susceptibility trend of *Pseudomonas aeruginosa* isolated from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=1056	Total n=5687	Total n=8880	Total n=12634	Total n=7839
	(S%)	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	705/1036 (68.1)	3757/5450 (68.9)	6034/8499 (71)	8416/11430 (73.6)	5012/7418 (67.6)
Cefepime	585/981 (59.6)	3074/5003 (61.4)	5259/8284 (63.5)	7660/12038 (63.6)	4497/7355 (61.1)
Ceftazidime	624/1035 (60.3)	3602/5504 (65.4)	5663/8598 (65.9)	7545/11977 (63)	4647/7635 (60.9)
Imipenem	809/1016 (79.6)	4059/5514 (73.6)	5627/8377 (67.2)	6425/10230 (62.8)	4411/7036 (62.7)
Meropenem	650/969 (67.1)	3490/5083 (68.7)	5736/8292 (69.2)	8255/12242 (67.4)	4955/7661 (64.7)
Colistin*	711/723 (98.3)	1727/1738 (99.4)	983/1075 (91.4)	1767/1899 (93)	1291/1355 (95.3)
Amikacin	693/1030 (67.3)	3864/5609 (68.9)	6019/8747 (68.8)	8340/12329 (67.6)	5276/7723 (68.3)
Gentamicin	402/776 (51.8)	2526/4249 (59.4)	4077/6462 (63.1)	5820/9383 (62)	3241/5341 (60.7)
Tobramycin	579/957 (60.5)	2954/4365 (67.7)	3809/5603 (68)	4627/6783 (68.2)	2907/4331 (67.1)
Ciprofloxacin	436/842 (51.8)	2930/5069 (57.8)	4814/8026 (60)	6281/10945 (57.4)	3768/6541 (57.6)
Levofloxacin	536/958 (55.9)	3236/5351 (60.5)	4794/8217 (58.3)	6148/10922 (56.3)	3771/6743 (55.9)

*Colistin represents percentage Intermediate susceptibility

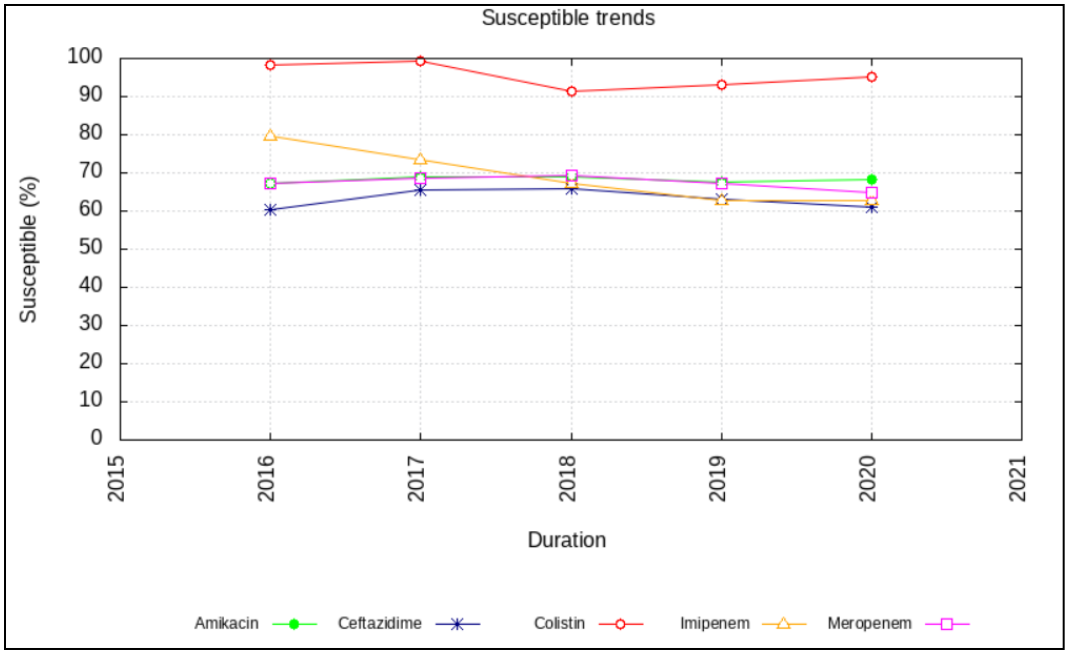


Figure 5.1: Yearly Susceptibility trend of *Pseudomonas aeruginosa* isolated from all samples.

Acinetobacter baumannii: *Acinetobacter baumannii* is a nosocomial Gram-negative pathogen that has become a critical challenge for common antibiotic treatments. *A.baumannii* is resistant to almost all the available drugs and none of the newly available drug is active against *A. baumannii*. Increased antimicrobial resistance due to multi-drug resistant, extensively drug resistant and pan drug-resistant strains have also been implicated in nosocomial infections and hospital outbreaks. Antimicrobial susceptibility profile of isolates of *A. baumannii* collected from ICU showed reduced susceptibility rates (<10%) to all the tested antibiotics **(Table 5.4)**, except for minocycline which showed susceptible rate of 49%. At least 5% and 10% increased susceptibility was observed among the isolates collected from the ward and the OPD respectively **(Table 5.4).** Of all the agents tested, minocycline was the only agent which showed highest susceptibility of 60% compared to other agents. Among the various specimens tested against different classes of antibiotics, susceptible rates are < 15% among specimens like LRT, deep infections, superficial infections and CSF except for minocycline. Isolates from blood and urine showed to have much better susceptibility profile compared to other specimens (20-30%) **(Table 5.5).**Trend analysis of susceptibility profile of different classes of antibiotics against isolates collected between 2016 and 2020 were less for ceftazidime and cefepime followed by piperacillin-tazobactam, imipenem, meropenem and amikacin. There has been reduced susceptibility to all these antibiotics from 2016 to 2020. Overall, 5% - 10% decreased susceptibility was observed in the trend during the year 2020 compared to 2019 **(Table 5.6: Figure 5.2)**.

Table 5.4: Location-wise susceptible percentage of *A. baumannii* isolated from all samples except faeces across OPD, Ward and ICU

AMA	Total n=6849	OPD n=559	Ward n=3482	ICU n=2808
	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	770/6724 (11.5)	134/547 (24.5)	433/3409 (12.7)	203/2768 (7.3)
Cefepime	587/6571 (8.9)	106/532 (19.9)	330/3291 (10)	151/2748 (5.5)
Ceftazidime	546/6441 (8.5)	108/526 (20.5)	300/3329 (9)	138/2586 (5.3)
Imipenem	744/6702 (11.1)	140/549 (25.5)	419/3398 (12.3)	185/2755 (6.7)
Meropenem	779/6747 (11.5)	141/545 (25.9)	452/3429 (13.2)	186/2773 (6.7)
Colistin*	91/94 (96.8)	*8/8	53/55 (96.4)	30/31 (96.8)
Amikacin	1014/5863 (17.3)	150/495 (30.3)	563/2865 (19.7)	301/2503 (12)
Minocycline	2794/5139 (54.4)	181/426 (42.5)	1610/2684 (60)	1003/2029 (49.4)
Levofloxacin	825/6181 (13.3)	120/489 (24.5)	486/3112 (15.6)	219/2580 (8.5)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

Table 5.5: Sample-wise susceptible percentage of *A. baumannii*

AMA	Blood	LRT	Superficial infection	Deep infection	CSF	Urine
	n=1473	n=2915	n=1290	n=288	n=140	n=195
Piperacillin-tazobactam	245/1446 (16.9)	191/2887 (6.6)	135/1264 (10.7)	47/281 (16.7)	18/137 (13.1)	60/188 (31.9)
Cefepime	195/1401 (13.9)	161/2865 (5.6)	99/1253 (7.9)	27/265 (10.2)	14/138 (10.1)	35/169 (20.7)
Ceftazidime	187/1410 (13.3)	143/2727 (5.2)	85/1230 (6.9)	24/269 (8.9)	11/118 (9.3)	44/179 (24.6)
Imipenem	222/1443 (15.4)	186/2875 (6.5)	163/1267 (12.9)	43/284 (15.1)	13/134 (9.7)	54/177 (30.5)
Meropenem	220/1448 (15.2)	198/2892 (6.8)	179/1270 (14.1)	44/286 (15.4)	15/139 (10.8)	62/186 (33.3)
Colistin*	27/29 (93.1%)	*13/13 (-)	33/33 (100%)	*0/0	*3/3 (-)	*3/3 (-)
Amikacin	311/1254 (24.8)	265/2565 (10.3)	213/1096 (19.4)	51/250 (20.4)	18/102 (17.6)	69/174 (39.7)
Minocycline	726/1267 (57.3)	943/2110 (44.7)	640/940 (68.1)	150/223 (67.3)	41/111 (36.9)	69/125 (55.2)
Levofloxacin	259/1310 (19.8)	213/2774 (7.7)	178/1191 (14.9)	30/216 (13.9)	21/123 (17.1)	43/147 (29.3)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

Table 5.6: Yearly susceptible trend of *A. baumannii* isolated from all samples except faeces

AMA	Year -2016 Total=396	Year -2017 Total=3359	Year -2018 Total=4549	Year -2019 Total=8531	Year -2020 Total=6849
	(S%)	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	94/335 (28.1)	484/3187 (15.2)	760/4494 (16.9)	1245/8010 (15.5)	770/6724 (11.5)
Cefepime	67/318 (21.1)	368/3300 (11.2)	587/4457 (13.2)	1040/8271 (12.6)	587/6571 (8.9)
Ceftazidime	56/328 (17.1)	355/3202 (11.1)	575/4164 (13.8)	905/7453 (12.1)	546/6441 (8.5)
Imipenem	104/334 (31.1)	501/3346 (15)	818/4517 (18.1)	1098/7272 (15.1)	744/6702 (11.1)
Meropenem	100/331 (30.2)	615/3287 (18.7)	953/4178 (22.8)	1742/8399 (20.7)	779/6747 (11.5)
Colistin*	*0/0	28/31 (90.3)	36/38 (94.7)	103/108 (95.4)	91/94 (96.8)
Amikacin	102/347 (29.4)	638/3312 (19.3)	877/3795 (23.1)	1429/7016 (20.4)	1014/5863 (17.3)
Minocycline	*0/0	926/1380 (67.1)	2393/3725 (64.2)	3893/6431 (60.5)	2794/5139 (54.4)
Levofloxacin	104/312 (33.3)	886/3040 (29.1)	959/4047 (23.7)	1500/7841 (19.1)	825/6181 (13.3)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

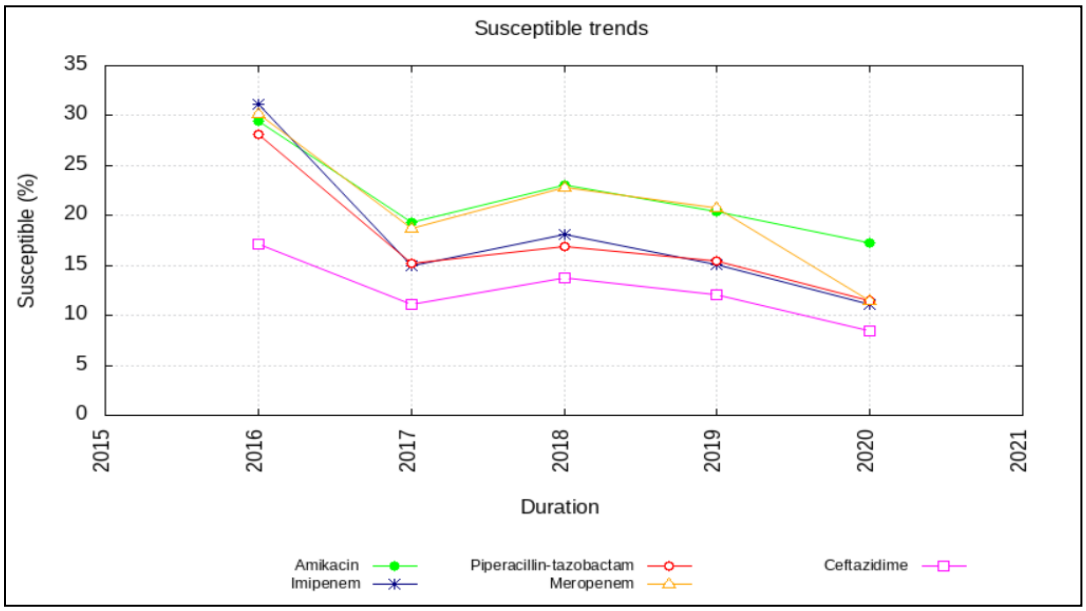


Figure 5.2: Yearly susceptible trend of *A. baumannii* isolated from all samples except faeces

Stenotrophomonas maltophilia: *Stenotrophomonas maltophilia* is the third most common pathogen among non-fermenting gram negative bacilli. The incidence of nosocomial and community-acquired *S. maltophilia* infections/isolation rates appeared to be similar as of previous years. The preferred treatment for *S. maltophilia* infections has been the use of trimethoprim-sulfamethoxazole and minocycline. **Table 5.7** shows the location-wise susceptible trend of *S. maltophilia* across OPD, ward and ICU. There was a decrease in susceptibility noted for ceftazidime, with the susceptibility rates for other agents like levofloxacin, minocycline and trimethoprim-sulfamethoxazole (TMP-SMX) being similar as of previous year. In case of trimethoprim sulfamethoxazole, susceptibility was less in ICU patients (85%) in comparison to ward and OPD (≥90%). In contrast, ceftazidime susceptibility was higher in ICU isolates (60%). Decreased susceptibility to minocycline in isolates from OPD, as compared to ICE and ward was notable. **Table 5.8** depicts sample-wise susceptible trend of *S. maltophilia* which shows that among LRT samples, ceftazidime had least susceptible rate (55%), whereas other agents were >80% susceptible. Overall, minocycline, trimethoprim-sulfamethoxazole and levofloxacin had high susceptible rate of 96%, 90% and 89% respectively. Among blood samples, minocycline and levofloxacin showed highest susceptibility of 96.6%. **Table 5.9** and **Figure 5.3** shows year-wise susceptible trend of *S. maltophilia* from all samples. There were minor changes observed between the years 2017 and 2020.The isolates exhibit susceptibility of 70 – 90% to ticarcillin-clavulanate over the last four years. Ticarcillin-clavulanate has been proposed as an alternate therapy to TMP-SMX, but resistance to ticarcillin-clavulanate has also being reported. The isolates exhibit susceptibility of 70 – 90% to ticarcillin-clavulanate over the last four years. Ticarcillin-clavulanate has been proposed as an alternate therapy to TMP-SMX, but resistance to ticarcillin-clavulanateis also being reported. More number of isolates has to be tested for susceptibility to derive significance of any change in the trend over time. Susceptibility to ceftazidime was found to decline by 7%, which needs to be monitored.

***Burkholderia cepacia* complex:** *Burkholderia cepacia* complex (BCC) is a significant opportunistic pathogen and its intrinsic resistance to commonly used antibiotic classes like aminoglycosides, first-and second-generation cephalosporins, anti-pseudomonal penicillins and polymyxins are being a significant concern. Also, BCC rapidly develops resistance to β-lactams due to presence of inducible chromosomal β-lactamases and altered penicillin-binding proteins which makes the treatment challenging. **Table 5.10** shows the location-wise susceptibilities of BCC across OPD, ward and ICU. For ceftazidime, susceptibility rates were comparable across OPD, ward and ICU (86%, 87% & 86% respectively) whereas for meropenem increased susceptibility was observed among ward (88%) followed by OPD and ICU. BCC isolates from OPD showed higher susceptibility to minocycline (95.5%) whereas ICU isolates were highly susceptible to trimethoprim-sulfamethoxazole (90.5%).

Table 5.11 shows sample-wise susceptible rates for BCC. For blood isolates, most of the antibiotics (ceftazidime, meropenem, minocycline and trimethoprim-sulfamethoxazole) showed >80% susceptibility. In contrast, for LRT isolates, only minocycline and trimethoprim-sulfamethoxazole showed >90% susceptibility. Yearly susceptible trends of BCC depicted in **Table 5.12** and **Figure 5.4** showed improved susceptibility between the years 2019 and 2020 for minocycline and levofloxacin, which is in contrast for meropenem and trimethoprim-sulfamethoxazole, where there was a 5% decline in susceptibility rate from 2019 (89%) to 2020 (83%) and from (92%) to (87%) respectively. Ceftazidime and trimethoprim-sulfamethoxazole are considered to be first line choice of drugs for BCC infections. However, increased resistance to trimethoprim-sulfamethoxazole has been observed which needs continuous monitoring.

Table 5.7: Location-wise susceptible percentage of *Stenotrophomonas maltophilia* isolated from all samples across OPD, Ward and ICU.

AMA	Total n=360	OPD n=45	Ward n=199	ICU n=116
	(S %)	(S %)	(S %)	(S %)
Ticarcillin-clavulanic acid	28/33 (84.8)	*7/7	*10/14	*11/12
Ceftazidime	41/73 (56.2)	*8/9	12/29 (41.4)	21/35 (60)
Minocycline	332/346 (96)	39/44 (88.6)	187/193 (96.9)	106/109 (97.2)
Levofloxacin	324/358 (90.5)	40/45 (88.9)	184/199 (92.5)	100/114 (87.7)
Trimethoprim-sulfamethoxazole	318/359 (88.6)	40/45 (88.9)	179/198 (90.4)	99/116 (85.3)
Chloramphenicol	*8/9	*4/4	*3/4	*1/1

Table 5.8: Sample-wise susceptible percentage of *Stenotrophomonas maltophilia*

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection
	n=360	n=90	n=164	n=56	n=*13
Ticarcillin-clavulanic acid	28/33 (84.8)	*7/7 (-)	*7/10 (-)	*4/4 (-)	*2/3 (-)
Ceftazidime	41/73 (56.2)	*10/18 (-)	18/33 (54.5)	*3/5 (-)	*3/3 (-)
Minocycline	332/346 (96)	85/88 (96.6)	157/159 (98.7)	50/54 (92.6)	*12/13 (-)
Levofloxacin	324/358 (90.5)	86/89 (96.6)	141/163 (86.5)	51/56 (91.1)	*12/13 (-)
Trimethoprim-sulfamethoxazole	318/359 (88.6)	83/90 (92.2)	140/163 (85.9)	50/56 (89.3)	*12/13 (-)
Chloramphenicol	*8/9 (-)	*0/0 (-)	*2/2 (-)	*0/0 (-)	*2/2 (-)

Table 5.9: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples

AMA	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=157	Total n=310	Total n=374	Total n=360
	(S%)	(S%)	(S%)	(S%)
Ticarcillin-clavulanic acid	19/26 (73.1)	45/60 (75)	59/68 (86.8)	28/33 (84.8)
Ceftazidime	15/27 (55.6)	42/63 (66.7)	46/73 (63)	41/73 (56.2)
Minocycline	143/151 (94.7)	272/299 (91)	331/350 (94.6)	332/346 (96)
Levofloxacin	126/152 (82.9)	225/257 (87.5)	225/261 (86.2)	324/358 (90.5)
Trimethoprim-sulfamethoxazole	132/150 (88)	255/308 (82.8)	333/372 (89.5)	318/359 (88.6)
Chloramphenicol	*0/0	*1/2	*3/3	*8/9

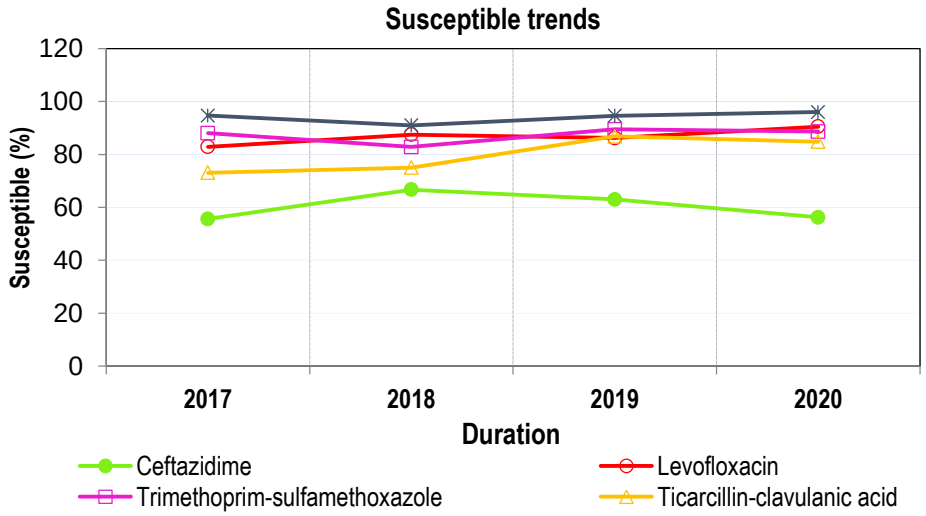


Figure 5.3: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples

Table 5.10: Location-wise susceptible percentage of *Burkholderia cepacia* isolated from all samples across OPD, Ward and ICU

AMA	Total n=200	OPD n=22	Ward n=104	ICU n=74
	(S %)	(S %)	(S %)	(S %)
Ticarcillin-clavulanic acid	36/80 (45)	*3/8	18/33 (54.5)	15/39 (38.5)
Ceftazidime	172/198 (86.9)	19/22 (86.4)	89/102 (87.3)	64/74 (86.5)
Meropenem	166/198 (83.8)	17/21 (81)	91/103 (88.3)	58/74 (78.4)
Minocycline	163/191 (85.3)	21/22 (95.5)	84/97 (86.6)	58/72 (80.6)
Levofloxacin	81/125 (64.8)	*8/13	41/63 (65.1)	32/49 (65.3)
Trimethoprim-sulfamethoxazole	174/200 (87)	18/22 (81.8)	89/104 (85.6)	67/74 (90.5)
Chloramphenicol	*4/4	*1/1	*0/0	*3/3

Table 5.11: Sample-wise susceptible percentage of *Burkholderia cepacia*

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection	Urine
	n=200	n=107	n=41	n=*10	n=*10	n=*11
Ticarcillin-clavulanic acid	36/80 (45)	18/44 (40.9)	*3/15 (-)	*2/2 (-)	*3/5 (-)	*2/6 (-)
Ceftazidime	172/198 (86.9)	89/105 (84.8)	35/41 (85.4)	*8/10 (-)	*10/10 (-)	*10/11 (-)
Meropenem	166/198 (83.8)	90/106 (84.9)	29/41 (70.7)	*9/10 (-)	*10/10 (-)	*9/11 (-)
Minocycline	163/191 (85.3)	84/102 (82.4)	35/38 (92.1)	*10/10 (-)	*10/10 (-)	*7/10 (-)
Levofloxacin	81/125 (64.8)	42/67 (62.7)	13/22 (59.1)	*5/5 (-)	*6/7 (-)	*1/6 (-)
Trimethoprim-sulfamethoxazole	174/200 (87)	91/107 (85)	38/41 (92.7)	*7/10 (-)	*10/10 (-)	*9/11 (-)
Chloramphenicol	*4/4 (-)	*0/0 (-)	*0/0 (-)	*0/0 (-)	*2/2 (-)	*0/0 (-)

Table 5.12: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples

AMA	Year-2017 Total n=112	Year-2018 Total n=197	Year-2019 Total n=181	Year-2020 Total n=200
	(S%)	(S%)	(S%)	(S%)
Ticarcillin-clavulanic acid	*0/9	4/51 (7.8)	36/103 (35)	36/80 (45)
Ceftazidime	73/101 (72.3)	137/192 (71.4)	156/178 (87.6)	172/198 (86.9)
Meropenem	83/111 (74.8)	140/171 (81.9)	161/181 (89)	166/198 (83.8)
Minocycline	89/104 (85.6)	146/185 (78.9)	133/174 (76.4)	163/191 (85.3)
Levofloxacin	*4/13	34/66 (51.5)	70/124 (56.5)	81/125 (64.8)
Trimethoprim-sulfamethoxazole	84/109 (77.1)	179/192 (93.2)	164/177 (92.7)	174/200 (87)
Chloramphenicol	*0/0	*1/1	*3/3	*4/4

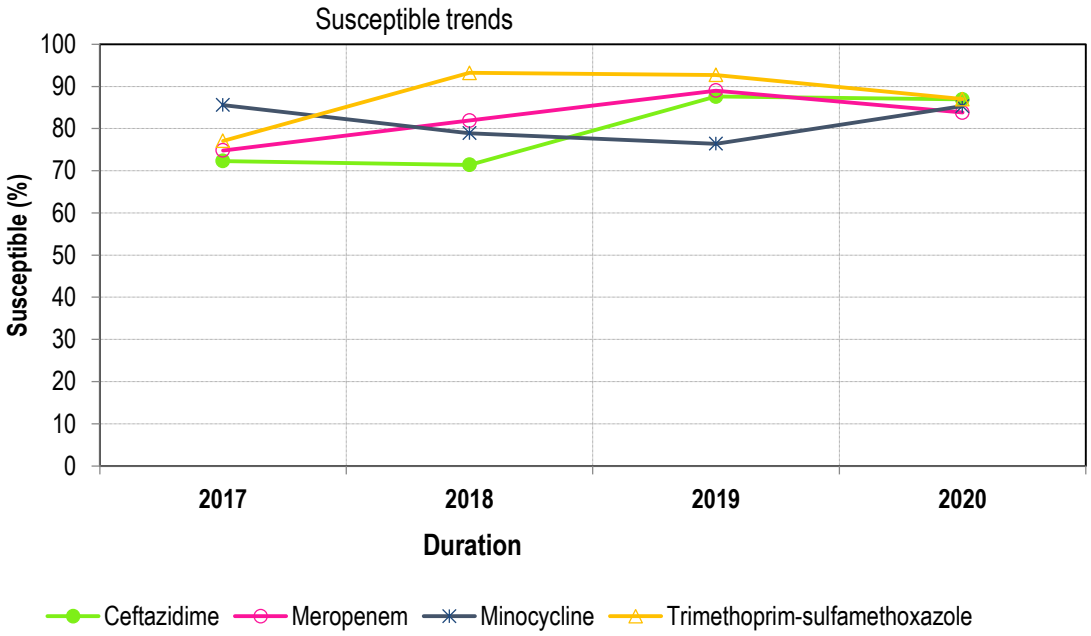


Figure 4.4: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples

Molecular studies

A total of 760 *P. aeruginosa* isolated from various clinical specimens were received at the reference laboratory for molecular characterization of antimicrobial resistant determinants. Of which, 174 were identified as carbapenem resistant and were screened for the presence of beta lactamases by multiplex PCR (ESBLs and carbapenemases). Of the entire beta lactamases screened, bla_{VEB} was the most common ESBL and few bla_{TEM} genes were identified; bla_{SHV} and bla_{PER} were absent in all the isolates. Similarly, among the carbapenemases, bla_{NDM} was the most common Metallo-beta lactamase (carbapenemase) identified, followed by bla_{VIM} and few bla_{IMP} were identified. Rates of co-producers of dual Metallo-beta-lactamaseswere observed to increase, particularly *bla*NDM co-carried with ESBLs such as bla_{VEB}andbla_{TEM}. Trend analysis over the last two years highlights that there has been a shift from bla_{VIM} to bla_{NDM} producers across different geographical location during the year 2018 and 2020.

A total of 451 isolates from various regional centers were subjected to PCR for characterization of antimicrobial resistance genes. All the isolates harbored the bla_{OXA-51} like gene, which is intrinsic to *Acinetobacter baumannii*. As expected, bla_{OXA-23} like only was the predominant carbapenemase across all the centers contributing to 41% of the

carbapenem resistance. *bla*$_{NDM}$ like only was observed among four isolates; two from Assam Medical Center (AMC) and two from Rajendra Institute of Medical Sciences (RIMS). Co-producers of various AMR genes like ESBLs with carbapenemases and dual carbapenemases were observed across all the centers. Of which, co-producers of *bla*$_{OXA-23}$ like with *bla*$_{TEM}$ like n=35 7.7% (4.6%) or *bla*$_{PER}$ like n=60 13.3% (16.8%) and *bla*$_{OXA-23}$ like with *bla*$_{NDM}$ like n=137 30.3% (18.7%) were found to be predominant. Two isolates from RC5 carried *bla*$_{OXA-58}$ like with *bla*$_{NDM}$ like and three isolates from RC15 carried *bla*$_{OXA-23}$ like with *bla*$_{OXA-58}$ like. None of the isolates had *bla*$_{OXA-24}$ like, *bla*$_{IMP}$ like, *bla*$_{VIM}$ like, *bla*$_{SIM}$ like, *bla*$_{KPC}$ like and *bla*$_{GES}$ like carbapenemases. Among *A. baumannii* isolates, the molecular profile was found to be consistent across all the centers with*bla*$_{OXA-23}$ like as the predominant carbapenemase followed by *bla*$_{NDM}$ like. Also, sporadic presence of *bla*$_{OXA-58}$ like were observed. Trend analysis showed increased prevalence of co-producers of *bla*$_{OXA-23}$ like and *bla*$_{NDM}$ like during the year 2020 compared to 2019. Therefore, the choice of treatment should be based on both phenotypic and molecular profile, mainly for the management of infection caused by NDM producing strains of *A. baumannii*.

The treatment options for CRAB infection are limited and include sulbactam, colistin, tigecycline, minocycline based combinations. Among the tested antibiotics, only minocycline and colistin showed better susceptibility. Though colistin-based combinations can be considered for treating carbapenem-resistant *A. baumannii* infections, both CLSI and EUCAST revised the interpretive criteria for *in-vitro* polymyxin susceptibility testing and suggested to prefer non-polymyxin agents for treating *Acinetobacter* infections. Such revision would effectively helpful in considering polymyxin as a treatment option in selected cases. Minocycline had bactericidal activity against CRAB and synergistic effects with other antibiotics. Though increased susceptibility rates were reported, additional study may be needed to evaluate the efficacy of minocycline. Currently, none of the newly available drug combinations have clinical activity against carbapenem-resistant *A. baumannii* infections except for the two novel agents in the pipeline, sulbactam-durlobactam and cefepime-zidebactam.

Table 5.13: Molecular characterization of acquired beta-lactamases (ESBLs and Carbapenemases) identified in *P. aeurignosa* collected across India during Jan-Dec 2020

Centres	*P.aeruginosa*	ESBL				Class A Carbapenemase		Class B carbapenemase (MβLs)				Combination genes
	Total (R tested)	SHV	TEM	VEB	PER	KPC	GES	SPM	IMP	VIM	NDM	Co-producers
RC3	60(42)	-	4	6	-	-	-	-	-	3	12	TEM+IMP&NDM-3 VEB+IMP&NDM-1 VIM&NDM-1 TEM&NDM-1 VEB&NDM-9 VEB&VIM&NDM-1 VEB&VIM-1
RC1	43 (9)	-	-	1	-	-	-	-	-	1	-	VEB&NDM-3 VEB&TEM+VIM&NDM-1 VIM&NDM-1 VEB&VIM-1 TEM&NDM-1
RC4	59 (19)	-	2	5	-	-	-	-	1	-	6	TEM&IMP-1 GES&NDM-1 TEM&NDM-1 VEB&NDM-2
RC2	39(5)	-	-	-	-	-	-	-	-	3	1	VEB&NDM-1
RC8	17 (12)	-	-	-	-	-	1	-	-	-	1	TEM&NDM-1 VEB&NDM-9
RC6	60 (19)	-	-	4	-	-	-	-	-	-	7	VEB&NDM-3 VEB&VIM-1 TEM&MDM-1 VEB&VIM&NDM-1 VEB&NDM-1 VEB&TEM+VIM&NDM-1
RC9	67 (6)	-	-		-	-	-	-	-	3	1	VEB&NDM-1 VIM&NDM-1
RC10	56 (6)	-	-	-	-	-	1	-	-	-	-	VEB&TEM&NDM-2 VEB&NDM-3
RC5	90 (13)	-	-	3	-	-	-	-	-	1	1	VEB&VIM-1 VEB&NDM-2 VEB&VIM&NDM-1 TEM&NDM-2 SHV&NDM-1 TEM&VIM&NDM-1
RC17	56(6)	-	1	-	-	-	-	-	-	-	4	VEB&NDM-1
RC20	-	-	-	-	-	-	-	-	-	-	-	-
RC14	22 (4)	-	-	-	-	-		-	-	1	2	TEM&NDM-1
RC18	29 (6)	-	-	1	-	-	-	-	-		3	TEM&NDM-1 VEB&NDM-1
RC7	23 (4)	-	-	1	-	-	-	-	-	-	2	TEM&NDM-1
RC16	64 (20)	-	-	2	-	-	-	-	-	6	5	VEB&NDM-1 VIM&NDM-1 VEB&TEM&NDM-1 VEB&TEM+VIM&NDM-1

												GES&NDM-2 VEB&GES+VIM&NDM-1
RC19	7 (0)	-	-	-	-	-	-	-	-	-	-	-
RC15	68(3)	-	-	2	-	-	-	-	-	-	-	SHV&GES&TES-1
Total	**760(174)**	**-**	**7**	**25**	**-**	**-**	**2**	**-**	**1**	**18**	**45**	**76**

Table 5.14: Molecular characterization of carbapenem resistant *A. baumannii* collected across India during the year 2020

Centres	*A.baumannii*	ESBL				Class A Carbapenemase		Class B carbapenemase (MβLs)				Class D carbapenemase			Combination genes
	Total (R tested)	SHV	TEM	VEB	PER	KPC	GES	IMP	VIM	NDM	SIM	OXA-23	OXA-24	OXA-58	Co-producers
RC3	69(63)	-	2	-	-	-	-	-	-	-	-	29	-	-	OXA23&NDM=15 OXA23&PER=3 OXA23&TEM=12 OXA23,NDM&TEM=1 OXA23,NDM,&PER=1
RC1	57(50)	-	-	-	-	-	-	-	-	-	-	23	-	-	OXA23&NDM=11 OXA23&PER=10 OXA23&TEM=2 OXA23,NDM&TEM=4
RC4	62(29)	-	-	-	-	-	-	-	-	-	-	16	-	-	OXA23&NDM=8 OXA23&PER=1 OXA23&TEM=3 OXA23,NDM&TEM=1
RC21	66(41)	-	-	-	-	-	-	-	-	-	-	25	-	-	OXA23&NDM=4 OXA23&PER=7 OXA23&TEM=4 OXA23,NDM,&PER=1
RC8	28(17)	-	-	-	-	-	-	-	-	-	-	9	-	-	OXA23&NDM=5 OXA23&PER=2 OXA23,NDM,&PER=1
RC6	60(54)	-	-	-	-	-	-	-	-	-	-	26	-	-	OXA23&NDM=13 OXA23&PER=10 OXA23&TEM=2 OXA23,NDM&TEM=2 OXA23,NDM,&PER=1
RC9	40(24)	-	-	-	-	-	-	-	-	-	-	9	-	-	OXA23&NDM=8 OXA23&PER=3 OXA23&TEM=1 OXA23,NDM&TEM=1 OXA23,NDM,&PER=2
RC5	50(21)	-	-	-	-	-	-	-	-	-	-	6	-	-	OXA23&NDM=5 OXA23&PER=1

															OXA23&TEM=3 OXA23,NDM&TEM=4 OXA58&NDM=2
RC17	58(45)	-	-	-	-	-	-	-	-	-	-	16	-	-	OXA23&NDM=13 OXA23&PER=11 OXA23&TEM=5
RC20	3(NG)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RC14	32(29)	-	-	-	-	-	-	-	-	-	-	9	-	-	OXA23&NDM=15 OXA23&PER=2 OXA23&TEM=2 OXA23,NDM&TEM=1
RC18	27(19)	-	-	-	-	-	-	-	-	2	-	3	-	-	OXA23&NDM=12 OXA23&PER=2
RC7	17(8)	-	1	-	-	-	-	-	-	-	-	1	-	-	OXA23&NDM=3 OXA23&PER=3
RC16	35(29)	-	-	-	-	-	-	-	-	2	-	8	-	-	OXA23&NDM=18 OXA23&PER=1
RC19	15(10)	-	-	-	-	-	-	-	-	-	-	3	-	-	OXA23&NDM=2 OXA23&PER=3 OXA23&TEM=1 OXA23,NDM&PER=1
RC15	72(12)	-	-	-	-	-	-	-	-	-	-	2	-	-	OXA23&NDM=5 OXA23&PER=1 OXA23&OXA58=3 OXA23,PER&OXA58=1
Total	**688 (451)**	**-**	**3**	**-**	**-**	**-**	**-**	**-**	**-**	**4**	**-**	**185**	**-**	**-**	**-**

Chapter 6 Diarrheal pathogens

The isolation percentage of diarrheal pathogens in the year 2020 was comparatively lesser than the previous year which could be due to the COVID19 pandemic. However, there is no significant change in the pathogen isolation trend and overall antimicrobial susceptibility among these pathogens. Considering the common pathogens causing bacterial gastroenteritis, such as *Aeromonas*, *Salmonella, Shigella, E. coli* or *Vibrio* species, third generation cephalosporins or azithromycin can still be the drug of choice for severe gastroenteritis except for cholera for which tetracycline or doxycycline is recommended.

Aeromonas spp: The susceptibility profile of *Aeromonas spp* in the year 2020 showed more than 70% susceptibility to tetracycline and norfloxacin and are highly resistant to ciprofloxacin (95%) (**Table 6.1)**. The five-year susceptibility trend showed that tetracycline and norfloxacin susceptibility seems to be consistent, whereas, decreasing susceptibility to ciprofloxacin was observed over the years (10 – 5%). The year-wise antibiotic susceptibility percentage was given in **Table 6.2** and year-wise trend was shown in **Figure 6.1**. Aeromonas-associated gastroenteritis in immunocompetent persons is usually acute and self-limited. Therefore, antimicrobial therapy is not routinely recommended. The antimicrobial therapy may differ depending on the site of infection since *Aeromonas spp* is ubiquitous nature. Ideally, drug of choices should be tailored according to local prevalence of drug-resistance in aeromonads.

Shigella spp: *S. flexneri* and *S. sonnei* was the predominant sero group isolated with varying susceptibility profile*. S. flexneri* was highly resistant to ampicillin (17%), and fluroquinolones such as nalidixic acid and norfloxacin. However, they are >85% susceptible to third generation cephalosporins such as cefixime which indicates the increasing resistance to this class of antibiotics (**Table 6.3**). The trend analysis of *S. flexneri* showed that susceptibility to ampicillin seems to be decreasing. Whereas susceptibility to trimethoprim-sulfamethoxazole is slightly increased from 10% in 2017 to 16% in 2020, which could be due to the limited use of this antibiotic in the recent years. The antibiotic nalidixic acid and norfloxacin were tested only for few isolates. *S. flexneri* showed >80% susceptibility to cefixime over the last four years (**Table 6.4 and Figure 6.2**). Similar susceptibility profile was observed for *S. sonnei* except for ampicillin susceptibility which is higher (>65%) compared to *S. flexneri*, while S*. sonnei* showed >90% susceptibility to cefixime (**Table 6.5).** There was no significant change in the yearly susceptible trend was observed for *S. sonnei* **(Figure 6.3**).

A total of 24 *Shigella* isolates were characterized for the presence of AMR genes such as *dhfr*A, *sul*II, *bla*OXA, *bla*TEM, *bla*CTX-M-1, AmpCs and *qnr*A/B/S by PCR in the year 2020.

As expected, majority of the isolates carried *dhfr*A and *sul*II genes which confer resistant to trimethoprim/sulfamethoxazole. Among beta-lactamases, *bla*OXA, and *bla*TEMgene was predominantly seen which encodes resistance to ampicillin. While AmpC genes were identified in three isolates which can be responsible for cephalosporin resistance. Further, plasmid mediated quinolone resistance (PMQR) gene *qnr*S was identified only in one isolate. The molecular data correlates with the phenotypic susceptibility profile observed.

Vibrio spp: *V. cholerae* showed 42%, 39% and 76% susceptibility to trimethoprim-sulfamethoxazole, ampicillin and norfloxacin respectively (**Table 6.6**). Only a smaller number of isolates were tested for nalidixic acid. However, 100% susceptibility was observed for tetracycline. The year-wise susceptibility of *V. cholerae* was shown in **Table 6.7** and **Figure 6.4**. No change in the susceptibility of trimethoprim-sulfamethoxazole, norfloxacin and tetracycline was observed. However, ampicillin susceptibility decreased from 71% in 2017 to 39% in 2020 which needs routine monitoring. This data shows that tetracycline can still be the effective drug of choice for cholera since other antibiotics are widely used for other infections.

Diarrheagenic *E. coli* (DEC): The susceptibility of DEC showed that they are highly resistant to ampicillin and showed decreased susceptibility to other antibiotics such as nalidixic acid, norfloxacin and cefixime, whereas 30% susceptibility was observed for trimethoprim-sulfamethoxazole (**Table 6.8**). On the analysis of yearly susceptibility trend, the susceptibility of all antibiotics appears to be decreased compared to the last year (**Table 6.9 and Figure 6.5**). Further, molecular characterization of 20 fecal *E. coli* isolates showed the presence of AMR genes such as *dhfr*A and *qnr*S genes each in one isolate and two isolates were positive for AmpC genes. Antibiotic treatment is not routinely recommended for DEC infections unless the diarrhea is moderate or severe which is especially evident with EHEC infections, in which antimicrobials are considered harmful. Generally, supportive therapy without antibiotic is recommended but in certain cases, antibiotic treatment with fluoroquinolones such as ciprofloxacin and macrolides such as azithromycin are also indicated.

Table 6.1: Susceptibility pattern of *Aeromonas spp*

AMA	***Aeromonas spp.***
	Total n=77 (S %)
Cefixime	*0/0
Imipenem	*0/0
Meropenem	*0/0
Tetracycline	58/77 (75.3)
Ciprofloxacin	4/74 (5.4)
Norfloxacin	38/54 (70.4)

Table 6.2: Yearly susceptibility trend of *Aeromonas spp*

AMA	**Year-2016**	**Year-2017**	**Year-2018**	**Year-2019**	**Year-2020**
	Total n=21	**Total n=131**	**Total n=114**	**Total n=170**	**Total n=77**
	(S%)	**(S%)**	**(S%)**	**(S%)**	**(S%)**
Cefixime	*0/0	*0/0	23/36 (63.9)	*0/0	*0/0
Imipenem	*0/0	20/46 (43.5)	53/109 (48.6)	*1/2	*0/0
Meropenem	*0/0	26/48 (54.2)	71/109 (65.1)	*1/2	*0/0
Tetracycline	18/21 (85.7)	104/126 (82.5)	97/113 (85.8)	134/169 (79.3)	58/77 (75.3)
Ciprofloxacin	*0/0	8/78 (10.3)	11/112 (9.8)	20/169 (11.8)	4/74 (5.4)
Norfloxacin	19/21 (90.5)	28/29 (96.6)	*1/1	156/169 (92.3)	38/54 (70.4)

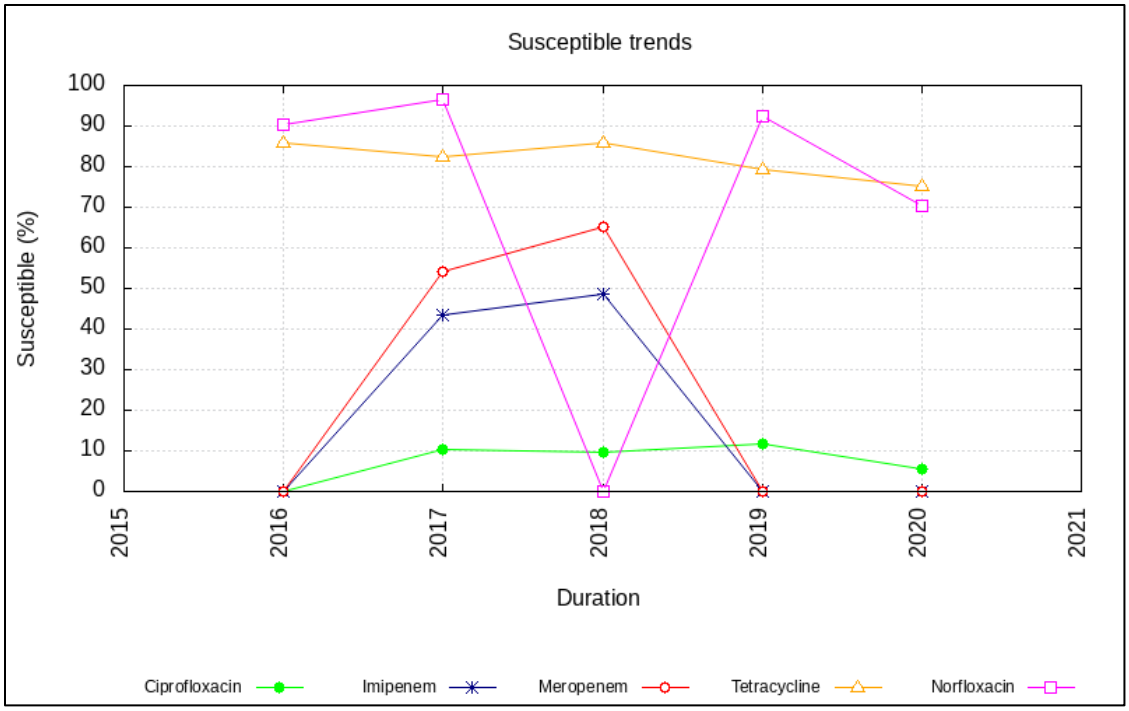


Figure 6.1: Yearly susceptible trends of *Aeromonas spp*

Table 6.3: Susceptibility pattern of *Shigella* species

AMA	**Faeces**		
	***Shigella flexneri* n=55**	***Shigella sonnei* n=*14**	***Shigella spp.* n=*12**
Ampicillin	9/54 (16.7)	*10/14 (-)	*7/12 (-)
Cefixime	45/51 (88.2)	*12/13 (-)	*8/11 (-)
Nalidixic acid	*2/13 (-)	*0/0 (-)	*0/2 (-)
Norfloxacin	*3/13 (-)	*1/2 (-)	*0/1 (-)
Trimethoprim-sulfamethoxazole	9/55 (16.4)	*1/13 (-)	*4/12 (-)

Table 6.4: Yearly susceptibility trend of *Shigella flexneri*

AMA	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=89	Total n=47	Total n=95	Total n=55
	(S%)	(S%)	(S%)	(S%)
Ampicillin	40/89 (44.9)	12/47 (25.5)	24/94 (25.5)	9/54 (16.7)
Cefixime	56/69 (81.2)	38/46 (82.6)	73/92 (79.3)	45/51 (88.2)
Nalidixic acid	0/24 (0)	*0/15	2/35 (5.7)	*2/13
Norfloxacin	12/24 (50)	*1/16	8/36 (22.2)	*3/13
Trimethoprim-sulfamethoxazole	7/72 (9.7)	14/47 (29.8)	22/95 (23.2)	9/55 (16.4)

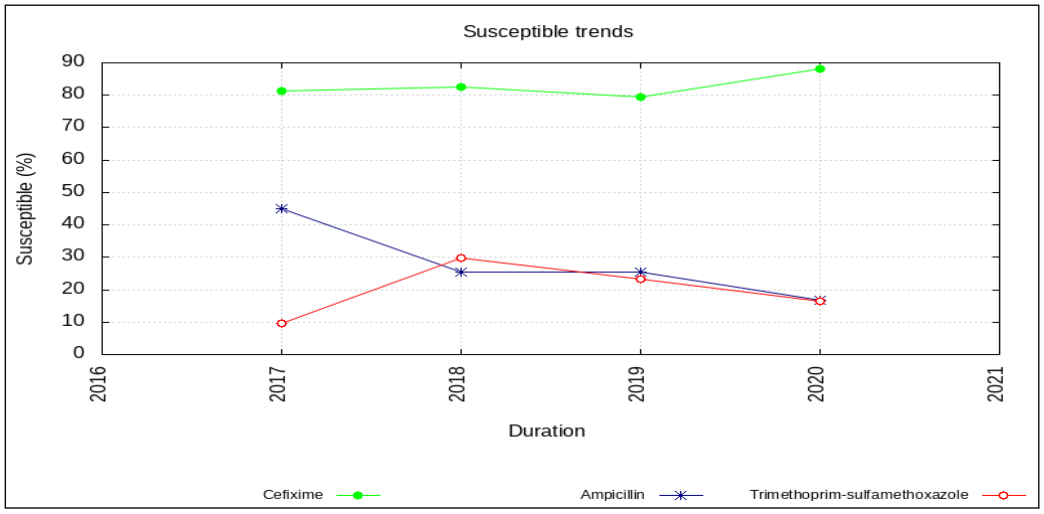


Figure 6.2: Yearly susceptible trends of *Shigella flexneri*

Table 6.5: Yearly susceptibility trend of *Shigella sonnei*

AMA	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=52	Total n=26	Total n=57	Total n=*14
	(S%)	(S%)	(S%)	(S%)
Ampicillin	35/52 (67.3)	18/24 (75)	42/57 (73.7)	*10/14
Cefixime	47/50 (94)	25/26 (96.2)	52/57 (91.2)	*12/13
Nalidixic acid	*0/8	*0/1	*0/8	*0/0
Norfloxacin	*2/8	*0/1	*3/9	*1/2
Trimethoprim-sulfamethoxazole	4/52 (7.7)	0/25 (0)	5/57 (8.8)	*1/13

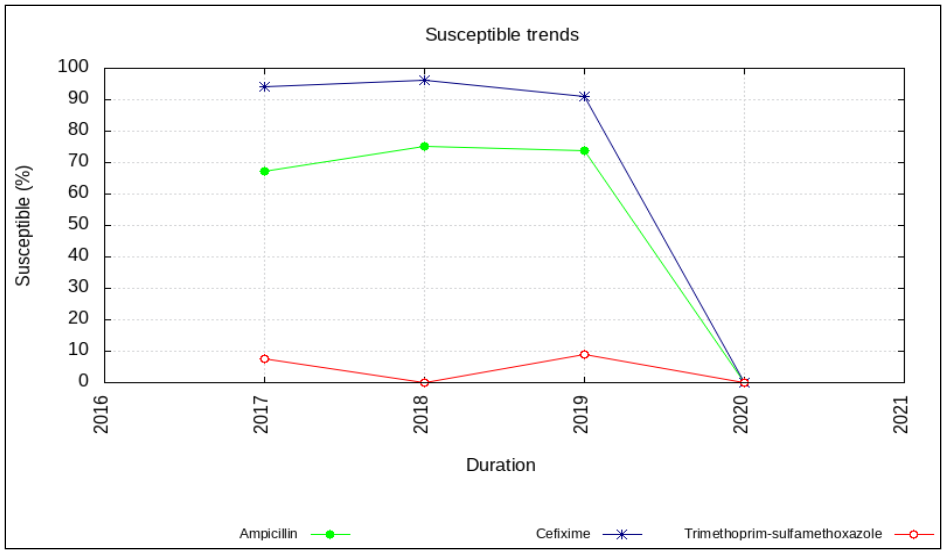


Figure 6.3: Yearly susceptible trends of *Shigella sonnei*

Table 6.6: Susceptibility pattern of *Vibrio cholerae* and *Vibrio spp*

AMA	Faeces
	Vibrio cholerae **n=31**
Ampicillin	11/28 (39.3)
Tetracycline	31/31 (100)
Nalidixic acid	*1/1 (-)
Norfloxacin	22/29 (75.9)
Trimethoprim-sulfamethoxazole	13/31 (41.9)

Table 6.7: Yearly susceptibility trend of *Vibrio cholerae*

AMA	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=24	**Total n=25**	**Total n=39**	**Total n=31**
	(S%)	**(S%)**	**(S%)**	**(S%)**
Ampicillin	17/24 (70.8)	17/24 (70.8)	22/39 (56.4)	11/28 (39.3)
Tetracycline	19/21 (90.5)	*7/10	36/38 (94.7)	31/31 (100)
Nalidixic acid	*1/8	*0/4	*0/5	*1/1
Norfloxacin	*9/14	*4/4	29/39 (74.4)	22/29 (75.9)
Trimethoprim-sulfamethoxazole	10/24 (41.7)	6/24 (25)	18/38 (47.4)	13/31 (41.9)

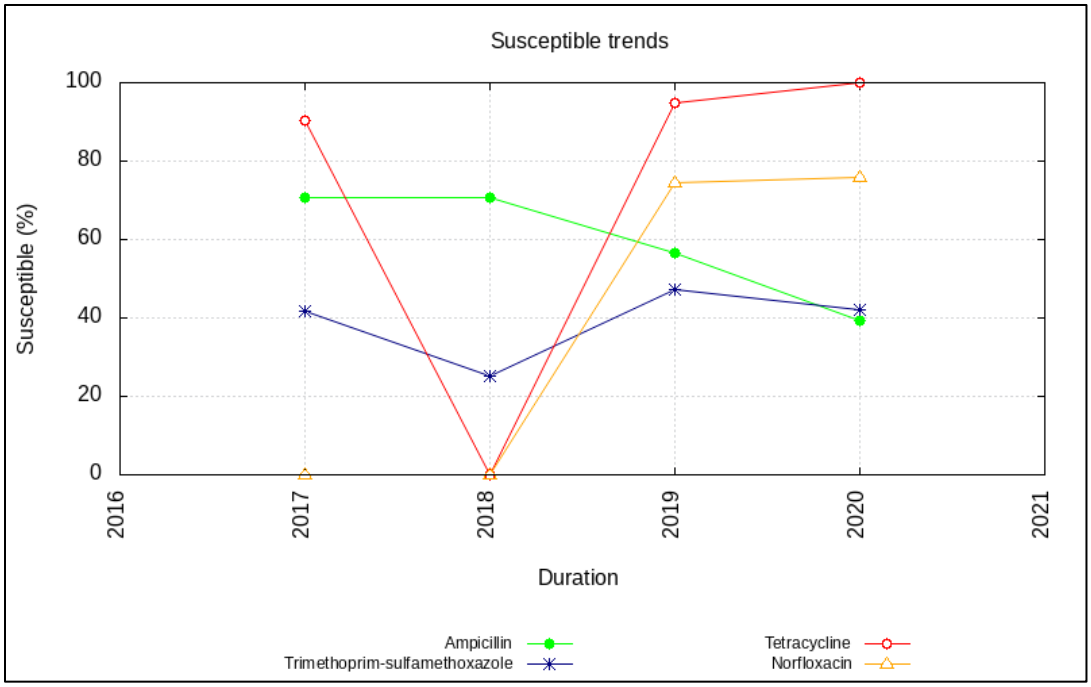


Figure 6.4: Yearly susceptibility trend of *Vibrio cholera*

Table 6.8 Susceptibility pattern of DEC in 2020

AMA	All Specimens
	Escherichia coli Diarrhoeagenic **n=102**
Ampicillin	1/102 (1)
Cefixime	11/100 (11)
Nalidixic acid	11/98 (11.2)
Norfloxacin	20/100 (20)
Trimethoprim-sulfamethoxazole	32/102 (31.4)

Table 6.9 Yearly susceptibility trend of DEC

AMA	Year-2019	Year-2020
	Total n=134 (S%)	Total n=102 (S%)
Ampicillin	6/132 (4.5)	1/102 (1)
Cefixime	17/129 (13.2)	11/100 (11)
Nalidixic acid	14/122 (11.5)	11/98 (11.2)
Norfloxacin	33/127 (26)	20/100 (20)
Trimethoprim-sulfamethoxazole	45/133 (33.8)	32/102 (31.4)

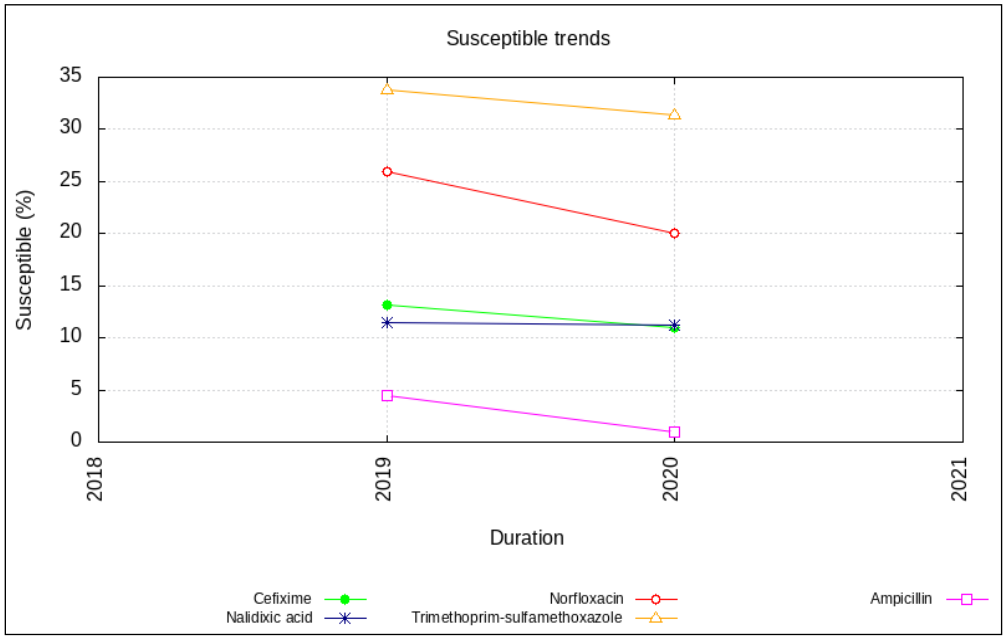


Figure 6.5 Yearly susceptible trend of DEC

Clinical relevance

Antimicrobial susceptibility profile varies from place to place thus definite therapy should be adjusted based on the local susceptibility profile. Among ***Vibrio spp***, decreased susceptibility to trimethoprim-sulfamethoxazole was observed. Therefore, this should be used for therapy only when the susceptibility is known. However, showed 100% susceptibility to tetracycline and >80% to third generation cephalosporins. Generally,

tetracycline/ doxycycline is being used for treating cholera infections. Also, azithromycin shown to have superior activity to tetracycline in treating cholera infections in children. Thus, tetracycline or azithromycin appears to be effective but the choice of which antibiotic to use will depend on local drug resistance.

Among ***Shigella spp***, increased resistance was observed to trimethoprim/sulfamethoxazole and ciprofloxacin but showed >90% susceptibility to third generation cephalosporins. Notably, azithromycin resistance seems to be increasing mainly in *S. sonnei*. These suggest that ampicillin, co-trimoxazole and ciprofloxacin should not be recommended unless susceptibility is known or expected based on local surveillance. Resistance to third generation cephalosporins and azithromycin needs to be monitored since these antibiotics are among the few therapeutic options commonly used for moderate to severe *Shigella* infections. Recently, CLSI has updated the clinical breakpoints for azithromycin for *Shigella spp* since only epidemiological cuttoff values are available till date. Azithromycin is used to treat Shigella infections, though clinical outcomes are uncertain. However, due to the increased reports of Non-wild type clinical strains with poor clinical outcome mainly higher in *S. sonnei*, there was an urge to establish unified breakpoints for azithromycin MIC and DD zone of inhibition against *Shigella spp*. Further, CLSI also recommended MIC by broth microdilution for azithromycin since hazy growth was reported in Disc diffusion test and makes the measurement difficult. Therefore, more isolates should be tested for azithromycin MIC. Among **Non-Typhoidal *Salmonella*** (NTS), *S*. Typhimurium was the predominant species. *Salmonella spp* was found to be highly resistant to ciprofloxacin and pefloxacin as expected and showed >80% susceptibility to other tested antibiotics such as ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, ceftriaxone, cefepime, and azithromycin.

For ***Aeromonas spp***, third generation cephalosporins, fluoroquinolones and aminoglycosides remain as a treatment option. The antimicrobial therapy of *Aeromonas spp* may differ depending on the site of infection. The current data showed decreasing susceptibility to fluoroquinolones among *Aeromonas spp* from diarrheal samples. Mostly, ***Campylobacter*** infections are acute and self-limited in nature and usually notrequire antibiotic treatment, unless infections occur in immunocompromised patients. Macrolides (erythromycin) and fluoroquinolones (ciprofloxacin) are considered as the first- and second- choice of antimicrobials, respectively for the treatment of human *Campylobacter* infections. To a less extent, tetracycline can be used as an alternative option.

Chapter 7 Staphylococci and Enterococci

A total of 6280 *Staphylococcus aureus*, 2018 CoNS and 4761 enterococci isolates collected across India were analysed in the year 2020. The total number of isolates available for analysis in 2020 was much less than that in 2019. This was due to the ongoing COVID 19 pandemic with most hospitals catering almost exclusively to these patients. Hence a comparison of resistance rates between these two years may not yield accurate results and the results need to be interpreted with caution. Phenotypic and genotypic characterization of antimicrobial resistance in these isolates was performed, similar to previous years. The added information this year is based on preliminary whole genome sequence analysis of some of the isolates.

***Staphylococcus aureus*:** A total of 6280 isolates of *S. aureus* and 2018 isolates of CoNS were reported from different centres across India **(Table 7.1).** The overall proportion of MRSA was 41.4%. Cefoxitin resistance, the surrogate marker for MRSA, was observed nearly twice as commonly among CoNS as *S.aureus* (74.5% vs 41.4%). There was a discrepancy in the MRSA rates detected by Oxacillin MIC (39% vs 41.4%). This discrepancy could be because of the smaller number of isolates tested against oxacillin than against cefoxitin. Moreover the same isolates may not have been tested by both the methods. Penicillin susceptibility was extremely low as expected (12% in MSSA and 9.6% among CoNS). Although *S. aureus*, overall, showed increasing trends of resistance to most antibiotics over the years, no such prominent trend could be observed with MSSA isolates. Susceptibility to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. The anti MRSA antibiotics such as vancomycin and tigecycline showed excellent in vitro activity (100% against MRSA isolates). Teicoplanin and linezolid resistance was encountered in MRSA isolates albeit at very low rates of 0.5 and 1 % respectively. Some of the teicoplanin results are based on disc diffusion testing which is no longer recommended by CLSI. This could explain the non susceptibility seen in some isolates of *S. aureus* and hence these results need to be interpreted with caution.

Table 7.2 shows the susceptibility pattern of *S. aureus* and CoNS across different hospital locations. Most of the *S. aureus* isolates were obtained from superficial infections followed by blood stream infections. MRSA rates differed based on the source of isolation with Blood isolates demonstrating highest rates (45.6%) while those from deep infections showed the lowest rates (38.6%). The overall MRSA proportion was 41.4%. As expected, the MRSA rates were lowest among OPD isolates (37.1%) while it was 43.1% among ward isolates and 48.8% among the ICU isolates. The susceptibility to most antibiotics was least among ICU isolates and highest among OPD isolates of *S. aureus* including MRSA and CoNS.

However, among MSSA, susceptibility to co-trimoxazole was slightly higher among ICU and ward isolates than OPD although the difference was not significant. Linezolid resistance among CoNS, MRSA, and MSSA isolates showed rates of 1%, 0.9 percent, and 0.2 percent, respectively.

Centerwise analysis

The centre wise susceptibility rates of *S. aureus* isolates were demonstrated in **Table 7.3.** Though the overall MRSA rate is 41.6%, there were significant differences observed between the various regional centres, the highest rate in the isolates from RC20 and RC07 (74.7% and 63.3%). The lowest MRSA rates were observed from the RC04 (26.8%) and RC05 (29.1%). However it should be noted that in RC 7, oxacillin resistance was used to identify MRSA rather than cefoxitin (140 vs 26 respectively). Ciprofloxacin susceptibility was extremely low across all centres. The susceptibility rate of other antibiotics varied widely between the centres for many of the antibiotics like erythromycin(1.6%in RC 21 to 60.2%in RC 04), tetracycline (57.9% in RC 21 to 97.1% in RC 10), clindamycin (16.8% in RC 21 to 97.4% in RC 14), co-trimoxazole (28.1% in RC 21 to 88.2% in RC 17). These unexpected differences could be a reflection of the methodologies employed (DD or MIC) or the pattern of antibiotic usage in the different regions.

The overall proportion of MRSA in 2020 across the country was 41.4%, which is marginally less than the rate reported in 2019 (42.4%). There were significant differences observed between the various zones of India, the highest in the North (54.1%), followed by east (48.9%), west (39.3%). Southern zone (33.4%) demonstrated much lower MRSA rates, with RC04 recording the lowest rate at 26.8% (Table 7.3). This variation may be indicative of the differences in the antibiotic prescription practices and usage in the different regions. It could also reflect different methodologies adopted across centres to identify MRSA. For example in RC07 which reported one of the highest rates of methicillin resistance, oxacillin MIC was used to identify MRSA rather than cefoxitin disc (140 vs 26 respectively) (Table 7.3).

Most laboratories depend on cefoxitin disc diffusion to identify MRSA. It has been observed that this test tends to misidentify a small number of isolates. This feature was noticed with both our isolates both from the nodal centre as well as those received as part of EQAS from regional centres. Some of the centres identified MRSA based on VITEK results. Here a discrepancy was found between cefoxitin and oxacillin results. As per the data shared by ICMR, MRSA rate based on cefoxitin DD results is 41.4% whereas, the rate was 39% based on oxacillin MIC results. This discrepancy could be due to the difference in the number of isolates being tested by both methods. Moreover the same isolates may not have been tested by both the methods (Table 7.3).

The MRSA phenotype was conferred by *mecA* gene as determined by PCR of randomly selected isolates from all centres. However in less than 1% of MRSA, *mec*A PCR was negative. PCR for *mec*C gene was also negative in these isolates. Recently plasmid mediated *mec*B and *mec*D genes have been reported in *S.aureus* which may complicate detection methods even further (Becker K, 2018, Lakhundi and Zhang 2018). These genes were not looked for in the 2020 isolates. On the other hand, a few randomly selected MSSA isolates were found to carry the *mec*A gene demonstrating the occurrence of dormant MRSA.
Among the non beta lactam antibiotics, macrolide resistance was conferred either through *erm*Aor *erm*Cgene, with *ermC*gene being more common. Erythromycin resistance may be mediated by either *erm* genes or *msr* genes, the former being more common among the isolates which show cMLS or iMLS phenotype. Resistance to the high level mupirocin (200 µg) was mostly conferred by *mupA*gene. None of the centres reported full blown resistance to vancomycin. However, hVISA (confirmed by PAP-AUC analysis) was encountered, albeit in small numbers. Among MRSA isolates from RC-4 and other centres combined, the VISA and hVISA prevalence was found to be 2.3% (8/347) and 4% (14/347). Our centre did not report any hVISA isolates this year although we found 2 VISA among the 60 MRSA isolates tested.

MIC creep

MIC creep for the anti MRSA antibiotics will be presented taking 2018 as the index year. MIC creep was observed for vancomycin in a few centres like RC03, RC05 and RC09, while RC01 isolates showed a slight reduction in median MIC. There was no change in the median MIC value among RC04 and RC06.In case of linezolid, RC04 isolates showed a threefold increase in median MIC while RC05 isolates showed a 2 fold decrease. The median MIC values of daptomycin showed a slight increase in all centres except RC03 where it decreased from 0.32 to 0.25µg/mL.

Table 7.1: Percentage susceptibility of *S. aureus*, MSSA, MRSA and CoNS isolated from all samples

AMA	All Specimens			
	S. aureus **n=6280**	**MSSA n=3655**	**MRSA n=2582**	**CoNS n=2018**
Cefoxitin	3394/5787 (58.6%)	3388/3388 (100%)	0/2399 (0)	487/1907 (25.5%)
Oxacillin	1140/1869 (61%)	1100/1100 (100%)	40/769 (5.2%)	*4/4 (-)
Penicillin	251/3608 (7%)	231/1931 (12%)	0/1652 (0)	134/1391 (9.6%)
Vancomycin	3846/3846 (100%)	2153/2153 (100%)	1676/1676 (100%)	890/890 (100%)
Teicoplanin	2043/2050 (99.7%)	1074/1075 (99.9%)	948/953 (99.5%)	229/238 (96.2%)
Erythromycin	2594/6096 (42.6%)	1962/3570 (55%)	621/2490 (24.9%)	396/1999 (19.8%)
Tetracycline	4734/5284 (89.6%)	2838/3047 (93.1%)	1885/2223 (84.8%)	1582/1916 (82.6%)
Tigecycline	1559/1559 (100%)	861/861 (100%)	694/694 (100%)	117/117 (100%)
Ciprofloxacin	1101/5845 (18.8%)	888/3386 (26.2%)	204/2417 (8.4%)	563/1597 (35.3%)
Clindamycin	4645/6084 (76.3%)	3021/3548 (85.1%)	1598/2497 (64%)	1057/2005 (52.7%)
Trimethoprim-sulfamethoxazole	3926/5821 (67.4%)	2425/3344 (72.5%)	1484/2449 (60.6%)	861/1935 (44.5%)
Linezolid	5846/5877 (99.5%)	3343/3349 (99.8%)	2476/2500 (99%)	1958/1978 (99%)
Mupirocin High Level	2563/2719 (94.3%)	1564/1600 (97.8%)	997/1117 (89.3%)	*0/0

Table 7.2: Location-wise susceptibility of *S. aureus*, MSSA, MRSA and CoNS from all samples

AMA	*Staphylococcus aureus*				*MSSA*				*MRSA*				*CoNS*			
	Total n=6008	**OPD n=2117**	**Ward n=3267**	**ICU n=624**	**Total n=3485**	**OPD n=1326**	**Ward n=1840**	**ICU n=319**	**Total n=2484**	**OPD n=777**	**Ward n=1410**	**ICU n=297**	**Total n=1985**	**OPD n=407**	**Ward n=1050**	**ICU n=528**
	(S %)	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**
Cefoxitin	3229/5525 (58.4)	1219/1933 (63.1)	1709/3003 (56.9)	301/589 (51.1)	3222/3223 (100)	1215/1215 (100)	1707/1707 (100)	301/301 (100)	0/2302 (0)	0/718 (0)	0/1296 (0)	0/288 (0)	481/1877 (25.6)	126/386 (32.6)	251/989 (25.4)	104/502 (20.7)
Oxacillin	1121/1842 (60.9)	485/733 (66.2)	555/960 (57.8)	81/149 (54.4)	1082/1082 (100)	459/459 (100)	542/542 (100)	81/81 (100)	39/760 (5.1)	26/274 (9.5)	13/418 (3.1)	0/68 (0)	*4/4 (-)	*1/1 (-)	*3/3 (-)	*0/0 (-)
Penicillin	229/3366 (6.8)	92/1204 (7.6)	108/1725 (6.3)	29/437 (6.6)	210/1785 (11.8)	84/672 (12.5)	103/900 (11.4)	23/213 (10.8)	0/1560 (0)	0/522 (0)	0/820 (0)	5/218 (2.3)	131/1360 (9.6)	47/342 (13.7)	61/698 (8.7)	23/320 (7.2)
Vancomycin	3784/3784 (100)	1374/1374 (100)	2138/2138 (100)	272/272 (100)	2117/2117 (100)	852/852 (100)	1158/1158 (100)	107/107 (100)	1651/1651 (100)	517/517 (100)	970/970 (100)	164/164 (100)	885/885 (100)	211/211 (100)	502/502 (100)	172/172 (100)
Teicoplanin	2007/2014 (99.7)	772/773 (99.9)	1034/1039 (99.5)	201/202 (99.5)	1054/1055 (99.9)	461/461 (100)	520/521 (99.8)	73/73 (100)	934/939 (99.5)	305/306 (99.7)	502/505 (99.4)	127/128 (99.2)	227/236 (96.2)	60/62 (96.8)	125/130 (96.2)	42/44 (95.5)
Erythromycin	2477/5828 (42.5)	956/2040 (46.9)	1294/3177 (40.7)	227/611 (37.2)	1870/3402 (55)	741/1289 (57.5)	963/1798 (53.6)	166/315 (52.7)	598/2393 (25)	211/739 (28.6)	329/1365 (24.1)	58/289 (20.1)	385/1966 (19.6)	92/400 (23)	204/1042 (19.6)	89/524 (17)
Tetracycline	4536/5060 (89.6)	1660/1816 (91.4)	2525/2813 (89.8)	351/431 (81.4)	2712/2911 (93.2)	1071/1144 (93.6)	1468/1567 (93.7)	173/200 (86.5)	1816/2138 (84.9)	587/669 (87.7)	1053/1242 (84.8)	176/227 (77.5)	1558/1885 (82.7)	336/401 (83.8)	812/980 (82.9)	410/504 (81.3)
Tigecycline	1534/1534 (100)	634/634 (100)	780/780 (100)	120/120 (100)	844/844 (100)	379/379 (100)	409/409 (100)	56/56 (100)	686/686 (100)	253/253 (100)	370/370 (100)	63/63 (100)	115/115 (100)	31/31 (100)	62/62 (100)	22/22 (100)

AMA	*Staphylococcus aureus*				*MSSA*				*MRSA*				*CoNS*			
	Total n=6008	**OPD n=2117**	**Ward n=3267**	**ICU n=624**	**Total n=3485**	**OPD n=1326**	**Ward n=1840**	**ICU n=319**	**Total n=2484**	**OPD n=777**	**Ward n=1410**	**ICU n=297**	**Total n=1985**	**OPD n=407**	**Ward n=1050**	**ICU n=528**
	(S %)	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**
Ciprofloxacin	1025/5573 (18.4)	374/2019 (18.5)	572/3034 (18.9)	79/520 (15.2)	828/3217 (25.7)	309/1267 (24.4)	455/1693 (26.9)	64/257 (24.9)	189/2319 (8.2)	62/739 (8.4)	114/1324 (8.6)	13/256 (5.1)	549/1564 (35.1)	157/370 (42.4)	282/842 (33.5)	110/352 (31.3)
Clindamycin	4454/5817 (76.6)	1667/2070 (80.5)	2413/3200 (75.4)	374/547 (68.4)	2888/3381 (85.4)	1140/1303 (87.5)	1525/1803 (84.6)	223/275 (81.1)	1542/2401 (64.2)	519/754 (68.8)	877/1381 (63.5)	146/266 (54.9)	1037/1972 (52.6)	234/405 (57.8)	545/1042 (52.3)	258/525 (49.1)
Trimethoprim-sulfamethoxazole	3752/5557 (67.5)	1318/1972 (66.8)	2051/3000 (68.4)	383/585 (65.5)	2306/3182 (72.5)	853/1216 (70.1)	1235/1669 (74)	218/297 (73.4)	1431/2352 (60.8)	458/745 (61.5)	812/1325 (61.3)	161/282 (57.1)	846/1903 (44.5)	197/401 (49.1)	424/997 (42.5)	225/505 (44.6)
Linezolid	5611/5640 (99.5)	1973/1983 (99.5)	3120/3133 (99.6)	518/524 (98.9)	3201/3207 (99.8)	1224/1226 (99.8)	1736/1740 (99.8)	241/241 (100)	2386/2408 (99.1)	741/749 (98.9)	1370/1378 (99.4)	275/281 (97.9)	1927/1946 (99)	396/402 (98.5)	1024/1033 (99.1)	507/511 (99.2)
Mupirocin High Level	2392/2526 (94.7)	932/957 (97.4)	1304/1399 (93.2)	156/170 (91.8)	1452/1483 (97.9)	598/607 (98.5)	782/800 (97.8)	72/76 (94.7)	938/1041 (90.1)	334/350 (95.4)	521/598 (87.1)	83/93 (89.2)	*0/0 (-)	*0/0 (-)	*0/0 (-)	*0/0 (-)

Table 7.3: Antimicrobial Susceptibility (AMS) Percentage RC wise of *Staphylococcus aureus* from all samples except faeces and urine

RC/ Antibiotics	Cefoxitin (n=5525)	Oxacillin (n=1842)	Penicillin (n=3366)	Vancomycin (n=3784)	Teicoplanin (n=2014)	Erythromycin (n=5828)	Tetracycline (n=5060)	Tigecycline (n=1534)	Ciprofloxacin (n=5573)	Clindamycin (n=5817)	Trimethoprim-sulfamethoxazole (n=5557)	Linezolid (n=5640)	Mupirocin High Level (n=2526)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC4	780/1065 (73.2)	1/1* (-)	1/2* (-)	1063/1063 (100)	66/66 (100)	638/1059 (60.2)	977/1066 (91.7)	40/40 (100)	299/1058 (28.3)	886/1065 (83.2)	771/1066 (72.3)	1065/1065 (100)	1027/1057 (97.2)
RC5	163/230 (70.9)	162/227 (71.4)	16/230 (7)	155/155 (100)	157/157 (100)	86/211 (40.8)	198/229 (86.5)	179/179 (100)	36/230 (15.7)	208/230 (90.4)	135/230 (58.7)	230/230 (100)	-
RC10	269/390 (69)	3/5* (-)	42/384 (10.9)	108/108 (100)	105/105 (100)	171/391 (43.7)	102/105 (97.1)	1/1* (-)	81/386 (21)	291/393 (74)	177/300 (59)	105/106 (99.1)	-
RC8	26/38 (68.4)	45/62 (72.6)	4/43 (9.3)	63/63 (100)	63/63 (100)	23/61 (37.7)	57/62 (91.9)	62/62 (100)	6/62 (9.7)	55/61 (90.2)	44/64 (68.8)	63/64 (98.4)	-
RC3	213/345 (61.7)	-	-	-	-	139/311 (44.7)	189/200 (94.5)	-	-	127/186 (68.3)	269/311 (86.5)	342/345 (99.1)	-
RC14	403/655 (61.5)	406/655 (62)	-	655/655 (100)	655/655 (100)	316/635 (49.8)	618/655 (94.4)	655/655 (100)	118/655 (18)	636/653 (97.4)	554/655 (84.6)	652/655 (99.5)	-
RC18	233/389 (59.9)	-	26/389 (6.7)	-	-	118/389 (30.3)	361/389 (92.8)	-	144/389 (37)	283/389 (72.8)	243/389 (62.5)	389/389 (100)	327/389 (84.1)
RC12	62/110 (56.4)	11/19* (-)	10/79 (12.7)	54/54 (100)	27/27 (100)	38/112 (33.9)	18/23 (78.3)	19/19* (-)	7/90 (7.8)	92/115 (80)	38/74 (51.4)	111/115 (96.5)	1/2* (-)
RC17	131/241 (54.4)	159/248 (64.1)	16/238 (6.7)	233/233 (100)	247/247 (100)	130/235 (55.3)	219/245 (89.4)	177/177 (100)	32/247 (13)	225/248 (90.7)	217/246 (88.2)	247/247 (100)	-
RC15	217/412 (52.7)	-	16/411 (3.9)	412/412 (100)	-	145/412 (35.2)	320/369 (86.7)	-	26/405 (6.4)	238/412 (57.8)	323/409 (79)	405/405 (100)	-
RC1	279/530 (52.6)	-	48/528 (9.1)	221/221 (100)	-	206/530 (38.9)	465/528 (88.1)	-	120/531 (22.6)	353/530 (66.6)	309/529 (58.4)	523/528 (99.1)	445/462 (96.3)
RC19	57/109	-	3/109	85/85	-	34/109	94/109	-	14/108	67/109	59/109	109/109	4/4*

	(52.3)		(2.8)	(100)		(31.2)	(86.2)		(13)	(61.5)	(54.1)	(100)	(-)
RC9	100/196 (51)	-	12/197 (6.1)	-	-	53/197 (26.9)	184/197 (93.4)	-	22/195 (11.3)	181/196 (92.3)	172/196 (87.8)	195/195 (100)	188/194 (96.9)
RC6	94/216 (43.5)	96/217 (44.2)	8/217 (3.7)	216/216 (100)	217/217 (100)	78/209 (37.3)	181/217 (83.4)	217/217 (100)	7/217 (3.2)	131/217 (60.4)	73/217 (33.6)	211/211 (100)	-
RC13	41/97 (42.3)	-	3/84 (3.6)	16/16* (-)	-	30/93 (32.3)	53/56 (94.6)	-	9/91 (9.9)	67/96 (69.8)	63/94 (67)	86/87 (98.9)	20/20 (100)
RC16	59/158 (37.3)	-	5/128 (3.9)	28/28 (100)	-	50/157 (31.8)	86/103 (83.5)	-	40/154 (26)	131/157 (83.4)	103/150 (68.7)	151/152 (99.3)	134/145 (92.4)
RC21	45/126 (35.7)	0/2* (-)	14/118 (11.9)	52/52 (100)	47/48 (97.9)	2/126 (1.6)	70/121 (57.9)	2/2* (-)	20/126 (15.9)	21/125 (16.8)	34/121 (28.1)	115/121 (95)	77/77 (100)
RC20	47/186 (25.3)	-	4/186 (2.2)	19/19* (-)	19/19* (-)	41/186 (22)	144/161 (89.4)	-	12/186 (6.5)	97/186 (52.2)	65/182 (35.7)	184/185 (99.5)	169/176 (96)
RC7	6/26 (23.1)	55/140 (39.3)	1/23 (4.3)	139/139 (100)	140/140 (100)	68/149 (45.6)	149/166 (89.8)	117/117 (100)	13/167 (7.8)	142/167 (85)	67/153 (43.8)	161/163 (98.8)	-
RC2	3/5* (-)	153/205 (74.6)	-	209/209 (100)	201/206 (97.6)	81/197 (41.1)	-	-	14/217 (6.5)	162/218 (74.3)	-	204/204 (100)	-
RC11	1/1* (-)	30/61 (49.2)	-	56/56 (100)	63/64 (98.4)	30/59 (50.8)	51/59 (86.4)	65/65 (100)	5/59 (8.5)	61/64 (95.3)	36/62 (58.1)	63/64 (98.4)	-
Total	3229/5525 (58.4)	1121/1842 (60.9)	229/3366 (6.8)	3784/3784 (100)	2007/2014 (99.7)	2477/5828 (42.5)	4536/5060 (89.6)	1534/1534 (100)	1025/5573 (18.4)	4454/5817 (76.6)	3752/5557 (67.5)	5611/5640 (99.5)	2392/2526 (94.7)

Table 7.4 and Figure 1: depicts the comparison of the susceptibility rates of *S. aureus* in 2020 with the rates seen between 2016-19. Susceptibility to most antibiotics showed similar rates as in the previous years. However mupirocin susceptibility which was stable between 2016 and 2018, showed a decline in 2019 and 2020. Resistance to tigecycline was not seen in 2016 but it appeared in a small number of isolates in 2017, 2018 and 2019. In 2020, none of the isolates exhibited tigecycline resistance. **Table 7.5** depicts the susceptibility rates of staphylococci from blood. MRSA rate was slightly higher among blood isolates when compared to the overall rate (45.6% vs 41.4%). CoNS were more commonly isolated from blood than *S.aureus* from the different centres across India. Cefoxitin resistance was observed more commonly among CoNS than the *S.aureus* (77.4% vs 45.6%). Only 10.8 % of MSSA isolates were susceptible to penicillin. When compared to MRSA, MSSA was more susceptible to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, tetracycline, and high-level mupirocin. The anti MRSA antibiotics such as vancomycin, linezolid, teicoplanin, and tigecycline showed excellent in vitro activity ranging from 99-100%. Teicoplanin resistance was found in a few of *S.aureus* and CoNS isolates.

As seen from **Table 7.6,** around 50% of the total *S.aureus* and 16.5% of CoNS isolates were from superficial infections. MRSA rate was 41.1% which was similar to the overall rate. Susceptibility of these isolates to different antibiotics followed the same general pattern as previously mentioned. As seen from **Table 7.7,** the proportion of MRSA from deep seated infections was slightly lower than the overall rate at 38.6%. Mupirocin susceptibility was higher among isolates from deep infections when compared to those from superficial infections while the reverse was true with respect to co-trimoxazole. **Table 7.8** and figure 7.2 depict trends in antimicrobial susceptibility among MSSA isolates across the 5 years of study (2016-20). Although *S.aureus*, overall, showed increasing trends of resistance to most antibiotics over the years, no such prominent trend could be observed with MSSA isolates. There was only a marginal decrease in the susceptibility rates of co-trimoxazole and mupirocin. The unusual occurrence of teicoplanin and linezolid resistance was observed in MSSA isolates (0.1 and 0.2 %). **Table 7.9** and figure 7.3 depict trends in antimicrobial resistance in MRSA isolates across the 5 years (2016-20). Susceptibility rates across the years were similar to most antibiotics except mupirocin which showed a significant fall in susceptibility among 2019 isolates which continued into 2020. The linezolid and teicoplanin susceptibility rates were slightly increased in 2020 (0.3% and 1.2 %) when compared to 2019 rates.

Table 7.4: Year wise susceptibility trends of *Staphylococcus aureus* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=960	Total n=5708	Total n=8644	Total n=12320	Total n=6281
	(S%)	(S%)	(S%)	(S%)	
Cefoxitin	686/958 (71.6)	3805/5668 (67.1)	4863/7919 (61.4)	6272/10835 (57.9)	3394/5787 (58.6)
Oxacillin	*0/0	314/438 (71.7)	1218/2196 (55.5)	2280/3773 (60.4)	1140/1869 (61)
Penicillin	60/737 (8.1)	267/3519 (7.6)	246/4047 (6.1)	458/7008 (6.5)	251/3608 (7)
Vancomycin	565/565 (100)	2602/2602 (100)	4640/4640 (100)	6996/6996 (100)	3846/3846 (100)
Teicoplanin	877/880 (99.7)	5233/5257 (99.5)	6544/6697 (97.7)	6194/6269 (98.8)	2043/2050 (99.7)
Erythromycin	492/955 (51.5)	2755/5570 (49.5)	3593/8102 (44.3)	4803/11975 (40.1)	2594/6096 (42.6)
Tetracycline	669/738 (90.7)	3492/3860 (90.5)	6255/7050 (88.7)	9269/10329 (89.7)	4734/5284 (89.6)
Tigecycline	*0/0	433/435 (99.5)	1529/1536 (99.5)	2902/2914 (99.6)	1559/1559 (100)
Ciprofloxacin	191/838 (22.8)	1224/5260 (23.3)	1497/8094 (18.5)	1990/11200 (17.8)	1101/5845 (18.8)
Clindamycin	729/921 (79.2)	4235/5475 (77.4)	6460/8456 (76.4)	9153/11984 (76.4)	4645/6084 (76.3)
Trimethoprim-sulfamethoxazole	513/852 (60.2)	3064/4306 (71.2)	4764/7565 (63)	7927/11401 (69.5)	3926/5821 (67.4)
Linezolid	860/863 (99.7)	5424/5445 (99.6)	8054/8148 (98.8)	11461/11547 (99.3)	5846/5877 (99.5)
Mupirocin High Level	573/584 (98.1)	2971/3012 (98.6)	3656/3742 (97.7)	4624/4892 (94.5)	2563/2719 (94.3)

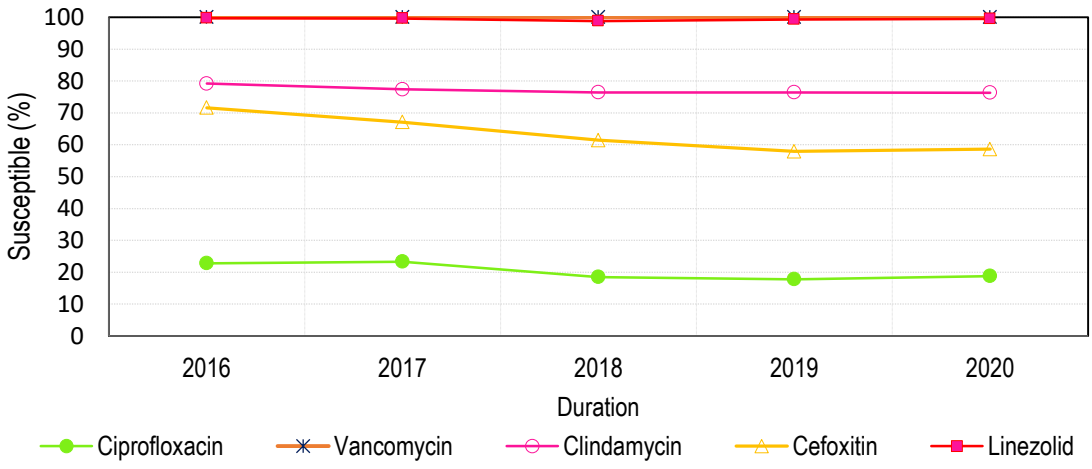


Figure 7.1: Year wise susceptibility trends of *S. aureus* from all Samples

Table 7.5 Susceptible percentages of staphylococci isolated from blood

AMA	Blood			
	S. aureus **n=1110**	**MSSA n=597**	**MRSA n=507**	**CoNS n=1501**
Cefoxitin	587/1079 (54.4)	586/586 (100)	0/493 (0)	322/1422 (22.6)
Oxacillin	195/307 (63.5)	190/190 (100)	5/117 (4.3)	*0/0 (-)
Penicillin	39/587 (6.6)	31/288 (10.8)	0/295 (0)	76/975 (7.8)
Vancomycin	617/617 (100)	307/307 (100)	307/307 (100)	641/641 (100)
Teicoplanin	394/395 (99.7)	194/194 (100)	197/198 (99.5)	174/177 (98.3)
Erythromycin	424/1087 (39)	311/587 (53)	110/495 (22.2)	285/1488 (19.2)
Tetracycline	884/1016 (87)	503/548 (91.8)	380/466 (81.5)	1179/1427 (82.6)
Tigecycline	215/215 (100)	117/117 (100)	98/98 (100)	64/64 (100)
Ciprofloxacin	159/891 (17.8)	127/463 (27.4)	31/422 (7.3)	347/1089 (31.9)
Clindamycin	788/1089 (72.4)	492/592 (83.1)	290/491 (59.1)	759/1490 (50.9)
Trimethoprim-sulfamethoxazole	709/1078 (65.8)	427/583 (73.2)	280/491 (57)	614/1436 (42.8)
Linezolid	1057/1067 (99.1)	562/565 (99.5)	490/497 (98.6)	1465/1477 (99.2)
Mupirocin High Level	370/401 (92.3)	193/200 (96.5)	177/201 (88.1)	*0/0 (-)

Table 7.6 Susceptible percentages of staphylococci isolated from Superficial Infections

AMA	Superficial Infection			
	S. aureus **n=3197**	**MSSA n=1875**	**MRSA n=1308**	**CoNS n=333**
Cefoxitin	1705/2896 (58.9)	1702/1702 (100)	0/1194 (0)	115/312 (36.9)
Oxacillin	683/1115 (61.3)	652/652 (100)	31/463 (6.7)	*2/2 (-)
Penicillin	108/1701 (6.3)	103/893 (11.5)	0/803 (0)	38/269 (14.1)
Vancomycin	2333/2333 (100)	1343/1343 (100)	982/982 (100)	162/162 (100)
Teicoplanin	1151/1156 (99.6)	640/641 (99.8)	503/507 (99.2)	31/35 (88.6)
Erythromycin	1369/3120 (43.9)	1019/1841 (55.4)	347/1266 (27.4)	70/331 (21.1)
Tetracycline	2530/2793 (90.6)	1523/1622 (93.9)	1003/1167 (85.9)	271/322 (84.2)
Tigecycline	930/930 (100)	518/518 (100)	412/412 (100)	27/27 (100)
Ciprofloxacin	552/3150 (17.5)	437/1846 (23.7)	111/1290 (8.6)	130/331 (39.3)
Clindamycin	2504/3186 (78.6)	1616/1871 (86.4)	881/1302 (67.7)	190/333 (57.1)
Trimethoprim-sulfamethoxazole	2041/2920 (69.9)	1236/1686 (73.3)	802/1229 (65.3)	159/324 (49.1)
Linezolid	3062/3073 (99.6)	1786/1788 (99.9)	1267/1276 (99.3)	319/323 (98.8)
Mupirocin High Level	1339/1416 (94.6)	837/852 (98.2)	500/562 (89)	*0/0 (-)

Table 7.7 Susceptible percentages of staphylococci isolated from Deep Infections

AMA	Deep Infection			
	S. aureus **n=720**	**MSSA n=425**	**MRSA n=281**	**CoNS n=22**
Cefoxitin	384/625 (61.4)	382/382 (100)	0/243 (0)	8/21 (38.1)
Oxacillin	103/181 (56.9)	101/101 (100)	2/80 (2.5)	*2/2 (-)
Penicillin	42/554 (7.6)	40/331 (12.1)	0/214 (0)	*4/14 (-)
Vancomycin	320/320 (100)	161/161 (100)	156/156 (100)	*16/16 (-)
Teicoplanin	219/219 (100)	95/95 (100)	118/118 (100)	*3/3 (-)
Erythromycin	302/693 (43.6)	231/417 (55.4)	68/265 (25.7)	4/22 (18.2)
Tetracycline	491/548 (89.6)	298/319 (93.4)	191/225 (84.9)	14/22 (63.6)
Tigecycline	179/179 (100)	96/96 (100)	79/79 (100)	*1/1 (-)
Ciprofloxacin	120/705 (17)	100/415 (24.1)	18/277 (6.5)	15/22 (68.2)
Clindamycin	543/711 (76.4)	354/421 (84.1)	181/278 (65.1)	14/22 (63.6)
Trimethoprim-sulfamethoxazole	385/670 (57.5)	253/387 (65.4)	124/271 (45.8)	11/22 (50)
Linezolid	580/582 (99.7)	312/312 (100)	261/262 (99.6)	20/21 (95.2)
Mupirocin High Level	272/284 (95.8)	161/166 (97)	111/118 (94.1)	*0/0 (-)

Table 7.8: Year wise susceptibility trends of MSSA from All Samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=686	Total n=3819	Total n=5135	Total n=7029	Total n=3655
	(S%)	(S%)	(S%)	(S%)	(S%)
Cefoxitin	686/686 (100)	3801/3801 (100)	4857/4857 (100)	6255/6255 (100)	3388/3388 (100)
Oxacillin	*0/0	306/306 (100)	1187/1187 (100)	2195/2195 (100)	1100/1100 (100)
Penicillin	59/557 (10.6)	248/2393 (10.4)	218/2068 (10.5)	410/3729 (11)	231/1931 (12)
Vancomycin	428/428 (100)	1935/1935 (100)	3041/3041 (100)	3986/3986 (100)	2153/2153 (100)
Teicoplanin	636/636 (100)	3509/3517 (99.8)	3642/3682 (98.9)	3391/3419 (99.2)	1074/1075 (99.9)
Erythromycin	419/684 (61.3)	2251/3739 (60.2)	2757/4841 (57)	3527/6895 (51.2)	1962/3570 (55)
Tetracycline	528/557 (94.8)	2508/2665 (94.1)	3809/4137 (92.1)	5383/5791 (93)	2838/3047 (93.1)
Tigecycline	*0/0	300/302 (99.3)	902/902 (100)	1608/1613 (99.7)	861/861 (100)
Ciprofloxacin	168/609 (27.6)	1051/3524 (29.8)	1167/4816 (24.2)	1587/6452 (24.6)	888/3386 (26.2)
Clindamycin	561/661 (84.9)	3162/3666 (86.3)	4341/5021 (86.5)	5837/6839 (85.3)	3021/3548 (85.1)
Trimethoprim-sulfamethoxazole	414/629 (65.8)	2202/2959 (74.4)	3030/4499 (67.3)	4750/6475 (73.4)	2425/3344 (72.5)
Linezolid	634/634 (100)	3630/3636 (99.8)	4775/4800 (99.5)	6433/6448 (99.8)	3343/3349 (99.8)
Mupirocin High Level	434/440 (98.6)	2119/2139 (99.1)	2414/2441 (98.9)	2775/2820 (98.4)	1564/1600 (97.8)

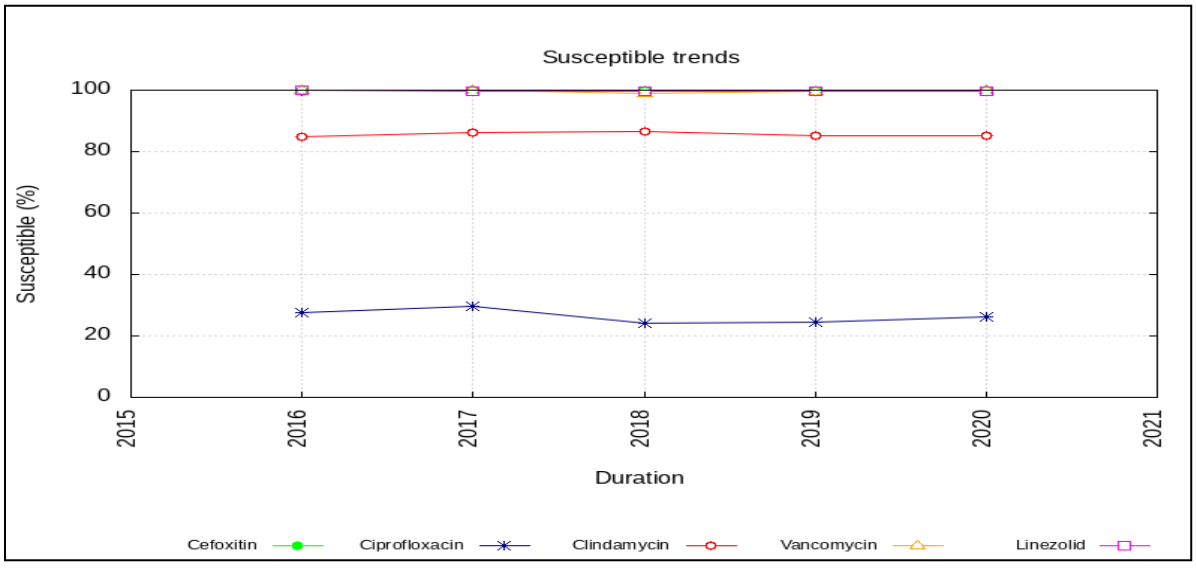


Figure 6.2: Year wise susceptibility trends of MSSA from All Samples

Table 7.9: Year wise susceptibility trends of MRSA from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=272	Total n=1870	Total n=3445	Total n=5185	Total n=2582
	(S%)	(S%)	(S%)	(S%)	(S%)
Cefoxitin	0/272 (0)	0/1867 (0)	0/3062 (0)	0/4578 (0)	0/2399 (0)
Oxacillin	*0/0	8/132 (6.1)	31/1009 (3.1)	85/1578 (5.4)	40/769 (5.2)
Penicillin	0/180 (0)	0/1111 (0)	0/1959 (0)	0/3240 (0)	0/1652 (0)
Vancomycin	137/137 (100)	667/667 (100)	1581/1581 (100)	2960/2960 (100)	1676/1676 (100)
Teicoplanin	240/242 (99.2)	1719/1735 (99.1)	2848/2956 (96.3)	2729/2775 (98.3)	948/953 (99.5)
Erythromycin	72/270 (26.7)	494/1813 (27.2)	822/3228 (25.5)	1251/4988 (25.1)	621/2490 (24.9)
Tetracycline	141/181 (77.9)	983/1193 (82.4)	2397/2859 (83.8)	3829/4473 (85.6)	1885/2223 (84.8)
Tigecycline	*0/0	133/133 (100)	627/634 (98.9)	1280/1286 (99.5)	694/694 (100)
Ciprofloxacin	23/228 (10.1)	165/1718 (9.6)	323/3222 (10)	397/4654 (8.5)	204/2417 (8.4)
Clindamycin	167/259 (64.5)	1067/1802 (59.2)	2083/3373 (61.8)	3248/5044 (64.4)	1598/2497 (64)
Trimethoprim-sulfamethoxazole	99/223 (44.4)	851/1332 (63.9)	1701/3006 (56.6)	3127/4848 (64.5)	1484/2449 (60.6)
Linezolid	225/228 (98.7)	1779/1794 (99.2)	3228/3296 (97.9)	4936/5001 (98.7)	2476/2500 (99)
Mupirocin High Level	139/144 (96.5)	852/873 (97.6)	1238/1297 (95.5)	1829/2051 (89.2)	997/1117 (89.3)

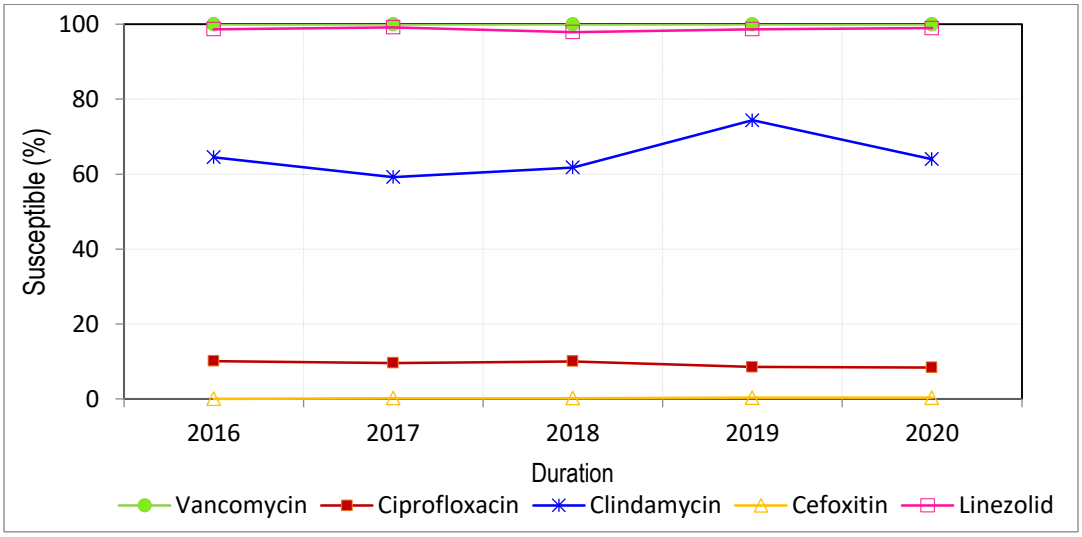


Figure 6.3: Year wise susceptibility trends of MRSA from All Samples

Coagulase negative staphylococci: Most of the CoNS isolates were obtained from blood followed by superficial infections. Only the clinically significant isolates were included for analysis. A variety of CoNS species were isolated from various centres, with the predominant species being S. haemolyticus*, S. epidermidis, S. hominis, S. lugdunensis* and *S.saprophyticus*. Cefoxitin resistance was highest in *S.haemolyticus* (87.8 %) followed by *S.hominis* (71.9%) and *S. epidermidis* (63.6%). With the exception of teicoplanin and tetracycline, *S.haemolyticus* exhibited much lower rates of susceptibility to all other antibiotics when compared to the other species. Tigecycline resistance was observed among 2.8% of *S.haemolyticus* while all other species were fully susceptible. Linezolid resistance was observed in a small number of isolates belonging to all the identified species except *S.lugdunensis* and *S. saprophyticus* **(Table 7.10). Table 7.11** and figure 7.4 depict the trends in susceptibility rates of CoNS from 2016-2019. It can be clearly observed that there is a decrease in the susceptibility rates for most of the antibiotics among CoNS except tigecycline and linezolid in 2019 and 2020 **(Table 7.11) (Figure 7.4).** For these two antibiotics susceptibility rates slightly increased in 2020 when compared to 2019. All the linezolid resistant isolates which were tested carried the *cfr* gene.

Table 7.10: Susceptibility percentages of CoNS isolated from all specimens

AMA	All Specimens					
	S. epidermidis **n=389**	***S. haemolyticus*** **n=615**	***S.hominis*** **n=301**	***S. lugdunensis*** **n=44**	***S. saprophyticus*** **n=21**	***Staphylococcus spp.*** **n=648**
Cefoxitin	129/354 (36.4%)	70/575 (12.2%)	77/274 (28.1%)	21/44 (47.7%)	*9/19 (-)	181/641 (28.2%)
Penicillin	39/329 (11.9%)	29/512 (5.7%)	39/271 (14.4%)	5/39 (12.8%)	4/20 (20%)	18/220 (8.2%)
Vancomycin	217/217 (100%)	384/385 (99.7%)	169/169 (100%)	*9/9 (-)	*8/8 (-)	102/102 (100%)
Teicoplanin	89/92 (96.7%)	80/83 (96.4%)	34/36 (94.4%)	*3/3 (-)	*2/2 (-)	21/22 (95.5%)
Erythromycin	99/386 (25.6%)	57/609 (9.4%)	64/297 (21.5%)	13/44 (29.5%)	5/21 (23.8%)	158/642 (24.6%)
Tigecycline	56/56 (100%)	35/36 (97.2%)	*16/16 (-)	*0/0	*2/2 (-)	*7/7 (-)
Tetracycline	321/364 (88.2%)	473/586 (80.7%)	227/280 (81.1%)	40/43 (93%)	14/21 (66.7%)	507/622 (81.5%)
Ciprofloxacin	191/388 (49.2%)	98/612 (16%)	130/299 (43.5%)	28/44 (63.6%)	15/21 (71.4%)	101/233 (43.3%)
Clindamycin	216/387 (55.8%)	219/613 (35.7%)	178/299 (59.5%)	28/43 (65.1%)	11/21 (52.4%)	405/642 (63.1%)
Linezolid	374/375 (99.7%)	597/608 (98.2%)	293/295 (99.3%)	43/43 (100%)	21/21 (100%)	630/636 (99.1%)
Trimethoprim-sulfamethoxazole	164/367 (44.7%)	222/583 (38.1%)	109/281 (38.8%)	28/43 (65.1%)	10/21 (47.6%)	328/640 (51.3%)

Table 7.11: Year wise susceptibility trends of CoNS from all Samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=490	Total n=2830	Total n=4016	Total n=3571	Total n=2018
	(S%)	(S%)	(S%)	(S%)	
Cefoxitin	173/490 (35.3)	930/2810 (33.1)	982/3574 (27.5)	921/3298 (27.9)	487/1907 (25.5)
Penicillin	58/224 (25.9)	223/1227 (18.2)	185/2021 (9.2)	268/2601 (10.3)	134/1391 (9.6)
Vancomycin	86/86 (100)	718/718 (100)	1619/1679 (96.4)	1681/1691 (99.4)	890/890 (100)
Teicoplanin	335/336 (99.7)	2212/2236 (98.9)	2912/3083 (94.5)	1324/1379 (96)	229/238 (96.2)
Erythromycin	148/488 (30.3)	742/2679 (27.7)	755/3459 (21.8)	815/3514 (23.2)	396/1999 (19.8)
Tigecycline	*0/1	165/167 (98.8)	434/441 (98.4)	287/292 (98.3)	116/117 (99.1)
Tetracycline	176/226 (77.9)	1177/1358 (86.7)	2236/2811 (79.5)	2658/3269 (81.3)	1582/1916 (82.6)
Ciprofloxacin	159/335 (47.5)	986/2236 (44.1)	1145/3015 (38)	1178/2798 (42.1)	563/1597 (35.3)
Clindamycin	297/488 (60.9)	1613/2782 (58)	2151/3952 (54.4)	2058/3509 (58.6)	1057/2005 (52.7)
Linezolid	375/381 (98.4)	2638/2680 (98.4)	3796/3900 (97.3)	3340/3429 (97.4)	1958/1978 (99)
Trimethoprim-sulfamethoxazole	199/379 (52.5)	923/1940 (47.6)	1579/3452 (45.7)	1687/3428 (49.2)	861/1935 (44.5)

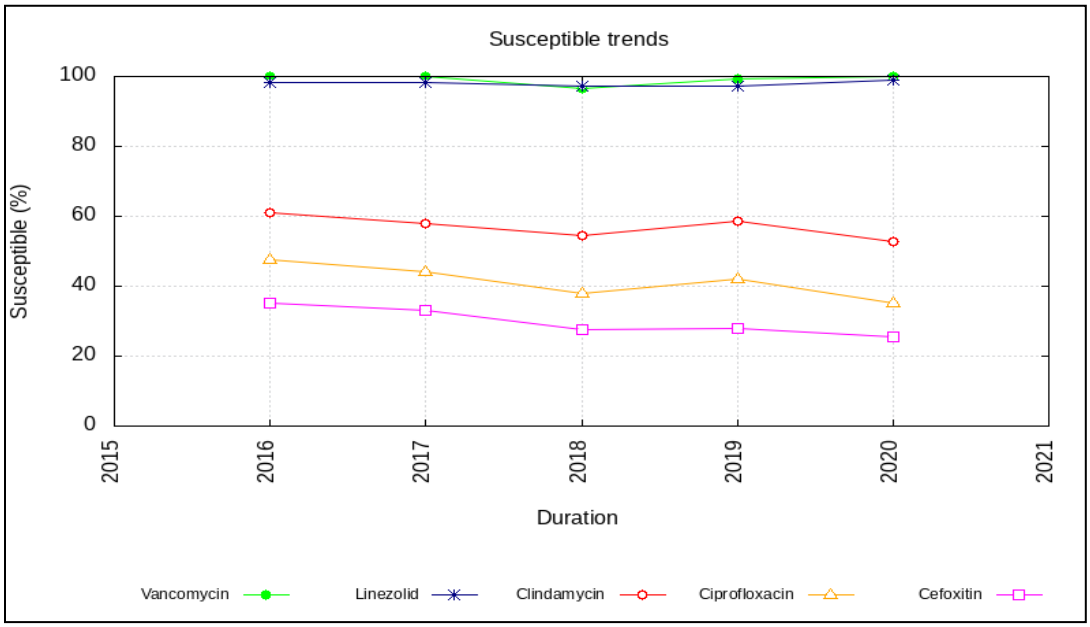


Figure 7.4: Year wise susceptibility trends of CoNS from All Samples

Enterococci

As per literature, *E. faecalis* is usually the commonest species followed by *E. faecium*. However unlike in the previous years, *E.faecium* was found to be the predominant species among the 2020 isolates in many of the centres (**Table 7.12**). The susceptibility rate in *E.faecium* to ampicillin, high level gentamicin and vancomycin was significantly lower than in *E.faecalis*. Overall vancomycin resistance in enterococci was 12.9%. However, the rate was 7 times higher in *E.faecium* compared *to E.faecalis* (22.7% vs 2.8%). Isolates from blood (both the species) appear to be more resistant when compared to isolates from superficial and deep infections (**Table 7.13**). In urinary isolates ciprofloxacin appeared to be equally ineffective against both the species while nitrofurantoin susceptibility was high in. *E. faecalis*. Fosfomycin resistance reduced from 5.2% in 2019 to 3% in 2020 (Table 7.14).

Most of the antibiotics showed lower rates of susceptibility among ICU isolates when compared to ward or OPD isolates. This difference was noted in both species (except for fosfomycin and nitrofurantoin in *E.faecalis*) (**Table 7.15**).

There were a large number of non- faecalis, non faecium spp of enterococci reported from many of the regional centres. Some of these species included *E.avium, E. raffinosus, E. gallinarum, E. hirae* and *E. casseliflavus.* **Table 7.16** and figure 6.5 depict the year wise susceptibility rates of *E.faecalis*. The susceptibility rates was slightly increased for ampicillin, high-level gentamicin , fosfomycin antibiotics in 2020 when compared to 2019 while there was a slight reduction in susceptibility to vancomycin, nitrofurantoin and linezolid. **Table 7.17** and figure 6.6 depict the trends in antibiotic susceptibility rates in *E. faecium* from 2016-2020. Unlike E. faecalis, susceptibility to all the antibiotics was lower among isolates of 2020 than 2019.

E. faecalis: The susceptibility rates of vancomycin and teicoplanin ranged from 90.2% to 100 % from most of the regional centres. Though the overall vancomycin-resistant enterococci (VRE) rate is 2.8%, there were significant differences observed between the various regional centres, the highest rate in the isolates from RC05 and RC01 (6.2% and 9.8%). The lowest VRE rates were observed from the RC10 (1.2%) and RC04 (1.3%).There were significant differences observed between the various zones of India, the highest in the North (7.9%), followed by west (4.3%), east (2.9%). Southern zone (1.4%) demonstrated much lower VRE rates, with RC04 recording the lowest rate at 1.2%. Susceptibility to linezolid was high in most of the centres ranging from 95.4% to 100%. Linezolid susceptibility was found to be the lowest (85.7 %) among RC17 isolates. Lowest susceptibility to ampicillin and high level gentamicin were recorded from RC01 (22.5%) and (25.6%), while highest susceptibility was observed in the RC 10 (97.6%) and (61.4%) (**Table 7.18).**

E. faecium: The susceptibility rates of vancomycin ranged from 65.7% to 100 % across regional centres. Though the overall VRE rate is 22.9%, there were significant differences observed between the various regional centres, the highest rate in the isolates from RC20 and RC11 (33.3 and 34.3%) The lowest VRE rates were observed from the RC18 (5.9%) while RC 19 did not report any vancomycin resistance. There were significant differences observed between the various zones of India, the highest in the North (23.9%), followed by South (23.2%), West (22.1%). Eastern zone (14.1%) demonstrated much lower VRE rates, with RC16 and RC18 recorded the lowest rate at 5.9 %. Susceptibility to linezolid was high in most of the centres ranging from 88.3% to 100%. Linezolid susceptibility was found to be the lowest (80.6 %) among RC11 isolates. Susceptibility to ampicillin and high level gentamicin was uniformly low across all centres except RC 18 (**Table 7.19).**

Table 7.12 Isolates percentages across Regional Centers of *Enterococcus faecalis, Enterococcus faecium, Enterococcus* spp. from All Specimen (Except Faeces)

Regional Center	Total Isolates	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus spp.*
	n	n(%)	n(%)	n(%)
RC4	9277	610 (6.6)	483 (5.2)	97 (1)
RC10	6547	533 (8.1)	277 (4.2)	62 (0.9)
RC1	6092	74 (1.2)	202 (3.3)	79 (1.3)
RC6	4812	98 (2)	204 (4.2)	0 (0)
RC3	4366	42 (1)	102 (2.3)	122 (2.8)
RC18	3097	113 (3.6)	137 (4.4)	0 (0)
RC20	1861	66 (3.5)	59 (3.2)	110 (5.9)
RC19	2391	128 (5.4)	51 (2.1)	24 (1)
RC17	2126	70 (3.3)	89 (4.2)	1 (0)
RC5	2709	63 (2.3)	72 (2.7)	14 (0.5)
RC16	1606	56 (3.5)	85 (5.3)	1 (0.1)
RC9	2202	104 (4.7)	40 (1.8)	0 (0)
RC12	1450	31 (2.1)	79 (5.4)	19 (1.3)
RC13	670	1 (0.1)	1 (0.1)	80 (11.9)

Regional Center	Total Isolates	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus spp.*
	n	n(%)	n(%)	n(%)
RC15	3511	3 (0.1)	14 (0.4)	53 (1.5)
RC14	3924	54 (1.4)	20 (0.5)	0 (0)
RC11	1161	7 (0.6)	41 (3.5)	0 (0)
RC21	1791	13 (0.7)	7 (0.4)	26 (1.5)
RC7	2619	22 (0.8)	7 (0.3)	7 (0.3)
RC8	598	8 (1.3)	14 (2.3)	7 (1.2)
RC2	2179	3 (0.1)	1 (0)	1 (0)
RC22	0	0 (NAN)	0 (NAN)	0 (NAN)
RC23	0	0 (NAN)	0 (NAN)	0 (NAN)
RC24	0	0 (NAN)	0 (NAN)	0 (NAN)
RC25	0	0 (NAN)	0 (NAN)	0 (NAN)
Total National	64989	2100	1958	703

Table 7.13: Susceptibility pattern of enterococci from all samples except urine

AMA	All Specimens (except urine)		Blood		Superficial Infection		Deep Infection		CSF	
	***Enterococcus faecalis* n=1189**	***Enterococcus faecium* n=1206**	***Enterococcus faecalis* n=318**	***Enterococcus faecium* n=556**	***Enterococcus faecalis* n=456**	***Enterococcus faecium* n=287**	***Enterococcus faecalis* n=203**	***Enterococcus faecium* n=104**	***E. faecalis* n=*10**	***E. faecium* n=33**
Ampicillin	945/1124 (84.1)	123/1071 (11.5)	219/284 (77.1)	40/476 (8.4)	375/439 (85.4)	48/267 (18)	187/201 (93)	11/89 (12.4)	*3/9 (-)	0/31 (0)
Vancomycin	1137/1170 (97.2)	916/1185 (77.3)	300/312 (96.2)	404/547 (73.9)	434/446 (97.3)	227/283 (80.2)	199/202 (98.5)	85/103 (82.5)	*10/10 (-)	31/33 (93.9)
Teicoplanin	1146/1170 (97.9)	952/1188 (80.1)	303/312 (97.1)	424/545 (77.8)	440/450 (97.8)	228/283 (80.6)	199/201 (99)	87/103 (84.5)	*10/10 (-)	30/33 (90.9)
Gentamicin HL	621/1084 (57.3)	345/994 (34.7)	125/252 (49.6)	117/430 (27.2)	267/445 (60)	111/263 (42.2)	116/193 (60.1)	42/91 (46.2)	*2/9 (-)	4/22 (18.2)
Linezolid	1152/1169 (98.5)	1136/1198 (94.8)	305/316 (96.5)	526/551 (95.5)	446/450 (99.1)	274/287 (95.5)	195/197 (99)	92/102 (90.2)	*10/10 (-)	32/33 (97)

Table 7.14: Susceptibility pattern of enterococci from Urine

AMA	Urine	
	Enterococcus faecalis **n=912**	***Enterococcus faecium*** **n=788**
Ampicillin	661/818 (80.8)	77/739 (10.4)
Vancomycin	881/903 (97.6)	630/781 (80.7)
Teicoplanin	855/869 (98.4)	618/759 (81.4)
Gentamicin HL	438/734 (59.7)	232/702 (33)
Ciprofloxacin	127/585 (21.7)	38/544 (7)
Nitrofurantoin	811/894 (90.7)	319/779 (40.9)
Fosfomycin	482/497 (97)	Not tested
Linezolid	722/728 (99.2)	677/698 (97)

Table 7.15: Susceptibility pattern of enterococci from all samples across OPD, Ward and ICU

AMA	***Enterococcus faecalis***				***Enterococcus faecium***			
	Total n=2100 **(S %)**	OPD n=625 **(S %)**	Ward n=1183 **(S %)**	ICU n=292 **(S %)**	Total n=1985 **(S %)**	OPD n=253 **(S %)**	Ward n=1235 **(S %)**	ICU n=497 **(S %)**
Ampicillin	1606/1942 (82.7)	520/575 (90.4)	868/1082 (80.2)	218/285 (76.5)	199/1801 (11)	59/221 (26.7)	114/1111 (10.3)	26/469 (5.5)
Vancomycin	2017/2072 (97.3)	606/616 (98.4)	1132/1168 (96.9)	279/288 (96.9)	1538/1958 (78.5)	213/245 (86.9)	963/1222 (78.8)	362/491 (73.7)
Teicoplanin	2000/2038 (98.1)	588/594 (99)	1132/1157 (97.8)	280/287 (97.6)	1561/1938 (80.5)	203/239 (84.9)	981/1207 (81.3)	377/492 (76.6)
Gentamicin HL	1058/1817 (58.2)	364/546 (66.7)	562/1006 (55.9)	132/265 (49.8)	571/1687 (33.8)	88/213 (41.3)	376/1065 (35.3)	107/409 (26.2)
Ciprofloxacin	127/586 (21.7)	52/200 (26)	72/341 (21.1)	3/45 (6.7)	38/544 (7)	17/87 (19.5)	21/368 (5.7)	0/89 (0)
Nitrofurantoin	812/895 (90.7)	329/346 (95.1)	393/448 (87.7)	90/101 (89.1)	319/779 (40.9)	70/121 (57.9)	193/495 (39)	56/163 (34.4)
Fosfomycin	483/498 (97)	144/151 (95.4)	304/312 (97.4)	35/35 (100)	*0/0 (-)	*0/0 (-)	*0/0 (-)	*0/0 (-)
Linezolid	1873/1896 (98.8)	518/523 (99)	1111/1126 (98.7)	244/247 (98.8)	1804/1887 (95.6)	231/241 (95.9)	1143/1192 (95.9)	430/454 (94.7)

Table 7.16: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

AMA	Year-2016 Total n=126 (S%)	Year-2017 Total n=1034 (S%)	Year-2018 Total n=2014 (S%)	Year-2019 Total n=2895 (S%)	Year-2020 Total n=2101 (S%)
Ampicillin	82/123 (66.7)	633/987 (64.1)	1338/1813 (73.8)	1993/2467 (80.8)	1606/1942 (82.7)
Vancomycin	123/125 (98.4)	978/1016 (96.3)	1921/2000 (96.1)	2791/2860 (97.6)	2018/2073 (97.3)
Teicoplanin	124/126 (98.4)	992/1030 (96.3)	1889/1970 (95.9)	2582/2633 (98.1)	2001/2039 (98.1)
Gentamicin HL	73/119 (61.3)	512/993 (51.6)	982/1890 (52)	1411/2458 (57.4)	1059/1818 (58.3)
Ciprofloxacin	3/40 (7.5)	41/358 (11.5)	87/641 (13.6)	162/982 (16.5)	127/586 (21.7)
Nitrofurantoin	38/40 (95)	352/375 (93.9)	710/763 (93.1)	1293/1421 (91)	812/895 (90.7)
Fosfomycin	*0/0	209/222 (94.1)	469/536 (87.5)	669/706 (94.8)	483/498 (97)
Linezolid	123/126 (97.6)	998/1011 (98.7)	1832/1863 (98.3)	2727/2753 (99.1)	1874/1897 (98.8)

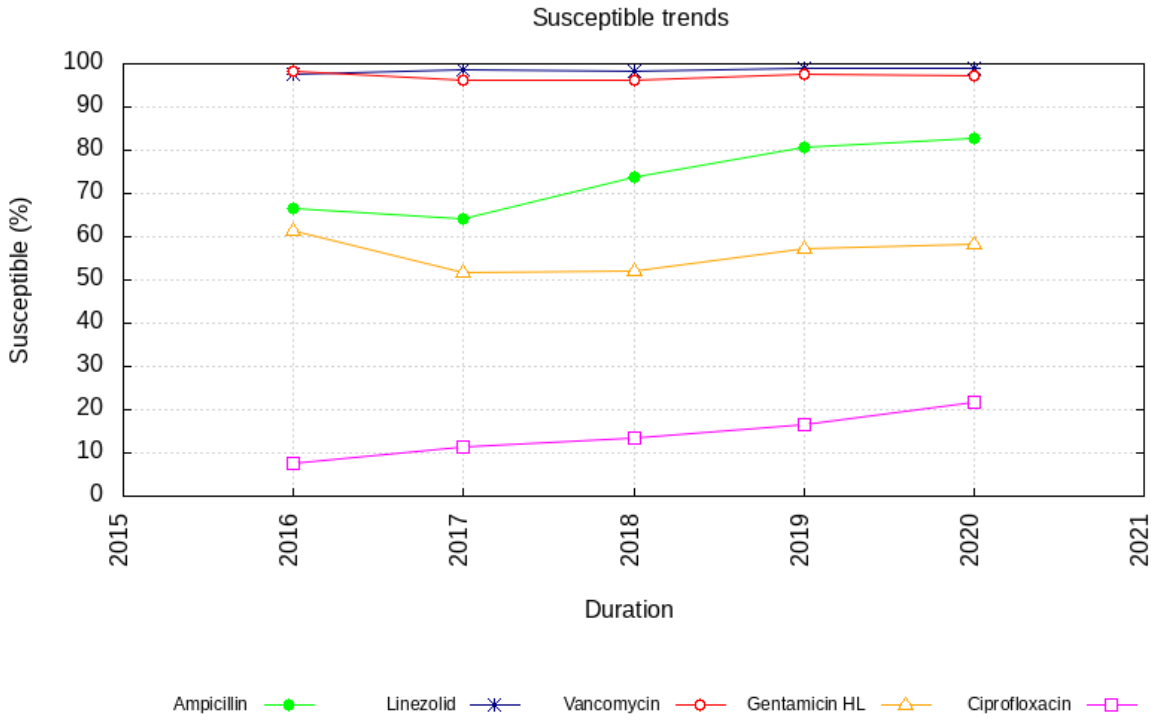


Figure 7.5: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

Table 7.17: Year wise susceptibility trends of *Enterococcus faecium* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=180	Total n=937	Total n=1476	Total n=2700	Total n=1994
	(S%)	(S%)	(S%)	(S%)	
Ampicillin	56/178 (31.5)	172/860 (20)	214/1213 (17.6)	414/2290 (18.1)	200/1810 (11)
Vancomycin	156/178 (87.6)	697/914 (76.3)	1139/1465 (77.7)	2214/2683 (82.5)	1546/1966 (78.6)
Teicoplanin	158/179 (88.3)	740/926 (79.9)	1148/1461 (78.6)	2206/2638 (83.6)	1570/1947 (80.6)
Gentamicin HL	27/102 (26.5)	208/812 (25.6)	360/1247 (28.9)	836/2392 (34.9)	577/1696 (34)
Ciprofloxacin	2/34 (5.9)	10/230 (4.3)	26/446 (5.8)	79/984 (8)	38/544 (7)
Nitrofurantoin	16/33 (48.5)	181/251 (72.1)	259/509 (50.9)	559/1221 (45.8)	319/779 (40.9)
Linezolid	170/179 (95)	860/910 (94.5)	1352/1411 (95.8)	2562/2644 (96.9)	1813/1896 (95.6)

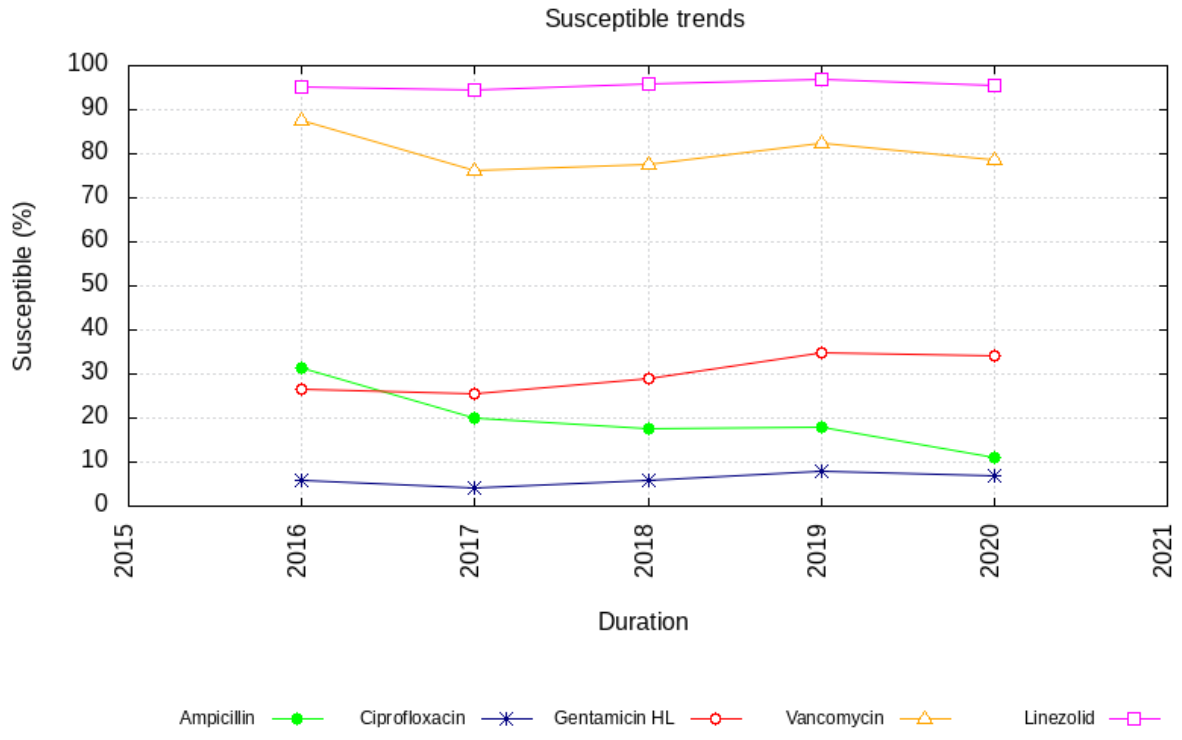


Figure 7.6: Year wise susceptibility trends of *Enterococcus faecium* from all samples

Table 7.18 Antimicrobial Susceptibilities (AMS) Percentage RC wise of *Enterococcus faecalis* from Total (Except Faeces & Urine)

RC/ Antibiotics	Ampicillin (n=1124)	Vancomycin (n=1169)	Teicoplanin (n=1169)	Gentamicin HL (n=1083)	Linezolid (n=1168)
	n(%)	n(%)	n(%)	n(%)	n(%)
RC1	9/40 (22.5)	37/41 (90.2)	37/41 (90.2)	10/39 (25.6)	40/41 (97.6)
RC2	1/3* (-)	2/3* (-)	2/2* (-)	0/3* (-)	2/3* (-)
RC3	36/42 (85.7)	39/41 (95.1)	41/42 (97.6)	-	40/42 (95.2)
RC4	420/456 (92.1)	451/457 (98.7)	447/453 (98.7)	274/454 (60.4)	457/457 (100)
RC5	51/56 (91.1)	45/48 (93.8)	52/55 (94.5)	28/56 (50)	56/56 (100)
RC6	64/72 (88.9)	70/72 (97.2)	70/72 (97.2)	28/72 (38.9)	72/72 (100)
RC7	7/9* (-)	8/9* (-)	8/9* (-)	2/8* (-)	8/9* (-)
RC8	1/1* (-)	2/2* (-)	2/2* (-)	1/1* (-)	2/2* (-)
RC9	25/41 (61)	43/43 (100)	44/44 (100)	33/47 (70.2)	46/46 (100)
RC10	249/255 (97.6)	253/256 (98.8)	252/253 (99.6)	156/254 (61.4)	236/239 (98.7)
RC11	-	6/6* (-)	6/6* (-)	-	4/6* (-)
RC12	11/11* (-)	12/12* (-)	13/13* (-)	8/10* (-)	13/13* (-)
RC13	-	-	-	-	-
RC14	-	18/18* (-)	18/18* (-)	-	18/18* (-)
RC15	2/3* (-)	2/3* (-)	2/3* (-)	2/3* (-)	3/3* (-)
RC16	7/18* (-)	18/19* (-)	17/18* (-)	15/18* (-)	16/17* (-)
RC17	1/1* (-)	24/24 (100)	27/28 (96.4)	14/27 (51.9)	24/28 (85.7)
RC18	23/39 (59)	38/39 (97.4)	39/39 (100)	22/39 (56.4)	39/39 (100)
RC19	27/50 (54)	47/49 (95.9)	48/50 (96)	9/26 (34.6)	49/50 (98)
RC20	9/19* (-)	13/19* (-)	12/13* (-)	15/18* (-)	19/19* (-)
RC21	2/8* (-)	8/8* (-)	8/8* (-)	3/8* (-)	7/8* (-)
Total	945/1124 (84.1)	1136/1169 (97.2)	1145/1169 (97.9)	620/1083 (57.2)	1151/1168 (98.5)

Table 7.19. Antimicrobial Susceptibilities (AMS) Percentage RC wise of *Enterococcus faecium* from Total (Except Faeces & Urine)

RC/ Antibiotics	Ampicillin (n=1062)	Vancomycin (n=1177)	Teicoplanin (n=1179)	Gentamicin HL (n=985)	Linezolid (n=1189)
	n(%)	n(%)	n(%)	n(%)	n(%)
RC1	12/137 (8.8)	113/138 (81.9)	114/138 (82.6)	24/105 (22.9)	134/138 (97.1)
RC2	0/1* (-)	0/1* (-)	0/1* (-)	0/1* (-)	1/1* (-)
RC3	13/102 (12.7)	67/101 (66.3)	77/98 (78.6)	-	100/102 (98)
RC4	42/334 (12.6)	263/334 (78.7)	265/331 (80.1)	128/330 (38.8)	326/332 (98.2)
RC5	0/53 (0)	33/47 (70.2)	39/53 (73.6)	19/53 (35.8)	48/53 (90.6)
RC6	2/128 (1.6)	88/128 (68.8)	89/128 (69.5)	25/128 (19.5)	113/128 (88.3)
RC7	2/3* (-)	3/3* (-)	3/3* (-)	2/3* (-)	3/3* (-)
RC8	1/5* (-)	7/14* (-)	8/14* (-)	5/9* (-)	12/14* (-)
RC9	7/15* (-)	14/14* (-)	12/12* (-)	8/16* (-)	16/16* (-)
RC10	10/125 (8)	95/126 (75.4)	96/125 (76.8)	49/125 (39.2)	114/123 (92.7)
RC11	0/2* (-)	23/35 (65.7)	26/38 (68.4)	1/4* (-)	29/36 (80.6)
RC12	7/35 (20)	35/46 (76.1)	40/47 (85.1)	12/34 (35.3)	43/47 (91.5)
RC13	-	-	-	-	-
RC14	-	9/10* (-)	9/10* (-)	-	10/10* (-)
RC15	0/12* (-)	9/12* (-)	10/12* (-)	2/12* (-)	12/12* (-)
RC16	4/17* (-)	16/17* (-)	14/15* (-)	8/17* (-)	16/17* (-)
RC17	0/1* (-)	49/58 (84.5)	55/63 (87.3)	19/62 (30.6)	58/65 (89.2)
RC18	14/34 (41.2)	32/34 (94.1)	33/34 (97.1)	20/34 (58.8)	34/34 (100)
RC19	5/32 (15.6)	32/32 (100)	31/32 (96.9)	4/25 (16)	32/32 (100)
RC20	3/21 (14.3)	14/21 (66.7)	16/19* (-)	12/21 (57.1)	20/20 (100)
RC21	0/5* (-)	6/6* (-)	6/6* (-)	1/6* (-)	6/6* (-)
Total	122/1062 (11.5)	908/1177 (77.1)	943/1179 (80)	339/985 (34.4)	1127/1189 (94.8)

Vancomycin variable Enterococcus faecium: Vancomycin variable enterococci are phenotypically susceptible to vancomycin but they carry van A gene. These have been recently reported across several countries. The worrisome feature of these isolates is that they are capable of converting to full blown resistance on exposure to vancomycin. There were 5 isolates of VVE among the phenotypically vancomycin susceptible isolates of *E. faecium*, 3 from RC05, and one each from RC04 and RC02. This is the first report of VVE from India.

Biocide resistance genes (*qac*A/B and *smr)* among MRSA and VRE isolates: 412 isolates of MRSA and 122 VRE isolates were tested for the presence of qacA/B and *smr* genes. The overall prevalence of *qac*A/B and *smr* genes in MRSA isolates was 2.6 % (11/222) and 1.7% (7/222) respectively. In *Enterococcus*, *qac*A/B was detected in 6.5 % (8/122) isolates while none had *smr* genes. Among MRSA isolates, *qac*A/B decreased from 9 % in 2019 to 2.6% in 2020 while it increased among enterococci from 2.1% in 2019 to 6.5% in 2020. Most disinfectant-resistance genes are plasmid borne and can spread between staphylococcal species.

Clinical relevance: While it is relatively easy to assign clinical significance to *S. aureus* and enterococcus species, the same is not true for CoNS. They are often dismissed as colonizers though they are being increasingly recognized as opportunistic pathogens, particularly *S.haemolyticus*. Another feature of importance is that these isolates are often multi drug resistant; the genes are carried on mobile elements which make transfer of resistance a distinct possibility.

The proportion of MRSA and VREwas found to be higher among blood isolates than from other specimens which are a cause for concern. Although vancomycin susceptibility remains very high among MRSA isolates, the occurrence of hVISA which is not usually detected in most clinical laboratories is worrisome as it may lead to therapeutic failure. Although vancomycin may continue to be used for serious MRSA infections, it is better to use alternate drugs if the MIC value is close to the breakpoint as such isolates are likely to be hVISA. As susceptibility to daptomycin continues to be close to 100% among MRSA isolates, this antimicrobial may be considered as alternative agents besides vancomycin and linezolid. This may also remove some of the selection pressure on antimicrobial resistance genes exerted by these agents. The emergence of *E.faecium* as the predominant species in 2020 across most centres of India is of concern as this species is far more drug resistant when compared to *E. faecalis*. The detection of *Enterococcus* species other than faecalis and faecium in high numbers is also significant as some of these species are intrinsically resistant to glycopeptides. This year the emergence of vancomycin variable enterococci has been reported for the first time in India.

WGS analysis

WGS of 30 hVISA isolates from different regional centres was performed and an initial analysis is being reported. Sequence types could be identified for 24 of them which revealed genetic diversity among the isolates across the country. ST772 was the most predominant clone (20%) followed by ST22, ST1482, ST239. These 3 sequence types were encountered in the previous years from RC04. A few new sequence types were identified which included ST88 and ST291. The clonal complexes, CC1, CC5, CC30 and CC8 predominated and were present in 16/25 (64%) of the isolates from various centers. In RC04 CC1 predominated, followed by CC5 and CC30. This shows that a few clones may be spreading across the country. Sequence Types were not assigned to five of the isolates and one isolate from RC13 was identified to be a novel ST (difference in aroE gene alone). Three isolates were identified to be singletons and were hence not assigned to any CC (Table 7.21).

The whole genome analysis of four linezolid resistant enterococci (3 from 2019 and one from 2020) was performed. All the isolates harboured *optr*A gene responsible for linezolid resistance. Two isolates had multiple mechanisms of linezolid resistance like *cfr*D gene, L3 ribosomal mutation and 23SrRNA mutation. Two isolates showed the presence of entire van operon except for the absence of van X-A in isolate B13743. This isolate had both tetL and tetM encoding for tetracycline resistance. All the isolates carried *erm*A and *erm*B genes along with genes coding for efflux proteins (*msr*C) responsible for macrolide resistance.

Table- 7.20: Antibiotic resistance genes among phenotypically resistant isolates of *S.aureus*, CoNS and enterococci from nodal and regional centres

S.No	Phenotypic resistance	Genes detected	Nodal center (No.positive /no tested)	Regional centers (No.positive /no tested)
1	Methicillin resistant *S.aureus* (MRSA)	*mec*A	*mec*A : 95/97 (97.9%)	*mec*A : 324/343 (94.5%)
2	Erythromycin resistance (*S.aureus*)	*erm*A, *erm* B and *erm* C	*erm*A :7/55 (12.7%) *erm* B :0/55 *erm* C :25/55 (45.4 %) Negative for *erm*A,B,C:23/55 (41.8%)	*erm*A : 11/336 (3.3%) *erm* B : 0/336 *erm* C : 193/336 (57.4%) ermA and C : 7/336 (2.1%) Negative for *erm*A,B,C: 125/336 (37.2%)
3	Mupirocin resistance (*S.aureus*)	*mup*A and *mup*B	*mup*A :25/30 (83.3%) *mup* B : 0/35	*mup*A :11/11 (100%) *mup* B :0/5
4	Linezolid resistant MRSA and MRCoNS	*cfr*	CoNS *cfr* : 2/2	*MRSA* *cfr* :1/1(MRSA)
5	Vancomycin resistant Enterococci (VRE)	*van*A, *van*B, *van*C_1/C_2	*van*A :53/53 (100%) *van*B :0/53 *van*C_1/C_2 :0/53	*van*A :71/71 (100%) *van*B : 0/71 *van*C_1/C_2 : 0/71

Fourteen Sequence types (STs) were identified for 24/30 hVISA isolates collected during the period of 2019. Of the remaining 6, sequence types were not assigned for 5 and one was a novel ST. There were 5 clonal complexes and 5 singletons.

Table- 7.21: Centerwise distribution of sequence types and clonal complexes of hVISA isolates (by WGS)

Sequence types	Clonal Complex	No of isolates	Regional centers
ST 772	CC1	5	RC02 -(1), RC04- (4)
ST 22	CC22	4	RC06-(1), RC05- (1), RC04-(1), RC15-(1)
ST 1482	CC30	2	RC04-(2)
ST 239	CC8	2	RC10-(1), RC04-(1)
ST 2689	CC5	2	RC04-(2)
ST 672	Singleton	2	RC04-(1), RC15-(1)
ST 88	Singleton	2	RC09-(1), RC04-(1)
ST 1	CC1	1	RC04- (1)
ST 291	Singleton	1	RC04 -(1)
ST 30	CC30	1	RC07-(1)
ST 368	CC8	1	RC04 -(1)
ST 6	CC5	1	RC04 -(1)
Novel	CC30	1	RC13 -(1)

Note: RC04 (Nodal center)

MLST revealed genetic diversity among the hVISA isolates across the country. ST772 was the most predominant clone (20%) followed by ST22, ST1482, ST239. These 3 sequence types were encountered in the previous years from RC04. A few sequence types were not identified in the previous years were also encountered (ST88 and ST291).

Chapter 8 *AMR profile of isolates from COVID-19 patients*

A total of 49 pathogens were isolated from culture specimens and Gram-negative bacteria were the predominant pathogen. Table 8.1 shows the frequency distribution of bacterial species isolated from secondary bacterial infections in Covid-19 patients against the bacterial isolates from all patients. Among total isolates, *Escherichia coli* was the commonly isolate (25.14%) followed by *Klebsiella pneumoniae* (18%), *Pseudomonas aeruginosa* (12%), Acinetobacter *baumannii* (10.4%) and *Staphylococcus aureus* (9.6%). In secondary bacterial infections in Covid-19 patients, *Klebsiella pneumoniae* was the commonly isolate (20.5%) follwod by *Acinetobacter baumannii* (17.8%), *E. coli* (13.8%), *Pseudomonas aeruginosa* (12.6%) and *Enterococcus faecium* (5.31%). Among *Candida* spp., *Candida tropicalis* was most common in COVID positive isolates (2%) and also in total isolates (0.76%) (Table 8.1 and Figure 8.1). As compared to overall frequency, *A. baumannii* and *E. faecium* were more frequently and *E. coli* and *S. aureus* were less frequently isolated in COVID-19 patients. The isolation rate of *Staphylococcus aureus* is 2.5 times less among COVID patients when compared with the total isolation rate. Conversely, the isolation rate of CoNS among COVID-19 patients was double that of the total. This is probably a reflection of the type of samples submitted. For eg, among COVID-19 patients, pus/wound swabs were minimal which would explain the lower isolation rate of *S. aureus*. Among *Enterococcus* species, only *E. faecium* showed a slight increase in isolation rate among COVID-19 patients.

Table 8.1- Isolation pattern of pathogens from Total isolates and COVID positive isolates

Organism	Count in COVID positive isolates	% out of COVID positive isolates (n=2054)	Count in Total isolates	% out of Total isolates (n=65561)
Klebsiella pneumoniae	421	20.50	11810	18.01
Acinetobacter baumannii	366	17.82	6851	10.45
Escherichia coli	285	13.88	16483	25.14
Pseudomonas aeruginosa	260	12.66	7843	11.96
Enterococcus faecium	109	5.31	1994	3.04
Staphylococcus spp.	81	3.94	648	0.99
Staphylococcus aureus	74	3.60	6281	9.58
Enterococcus faecalis	63	3.07	2101	3.20
Klebsiella spp.	45	2.19	401	0.61
Candida tropicalis	41	2.00	500	0.76
Stenotrophomonas maltophilia	39	1.90	360	0.55

Organism	Count in COVID positive isolates	% out of COVID positive isolates (n=2054)	Count in Total isolates	% out of Total isolates (n=65561)
Acinetobacter baumanii-calcoaceticus complex	24	1.17	85	0.13
Burkholderiacepacia	24	1.17	200	0.31
Acinetobacter spp.	21	1.02	327	0.50
Staphylococcus epidermidis	21	1.02	389	0.59
Enterobacter cloacae	18	0.88	1057	1.61
Enterococcus spp.	18	0.88	703	1.07
Proteus mirabilis	18	0.88	1236	1.89
Candida auris	15	0.73	121	0.18
Candida albicans	14	0.68	364	0.56
Enterobacter spp.	13	0.63	385	0.59
Staphylococcus hominis	12	0.58	301	0.46
Candida parapsilosis	9	0.44	189	0.29
Providencia stuartii	7	0.34	130	0.20
Staphylococcus haemolyticus	7	0.34	615	0.94
Morganella morganii	5	0.24	333	0.51
Citrobacter freundii	4	0.19	183	0.28
Aeromonas spp.	3	0.15	127	0.19
Candida glabrata	3	0.15	113	0.17
Salmonella spp.	3	0.15	163	0.25
Salmonella spp. Faecal	3	0.15	25	0.04
Acinetobacter lwoffii	2	0.10	162	0.25
Burkholderiacepacia complex	2	0.10	14	0.02
Candida rugosa	2	0.10	3	0.00
Citrobacter spp.	2	0.10	71	0.11
Proteus vulgaris	2	0.10	118	0.18
Providencia rettgeri	2	0.10	76	0.12
Salmonella Typhi	2	0.10	240	0.37
Serratia marcescens	2	0.10	313	0.48
Streptococcus pneumoniae	2	0.10	56	0.09
Vibrio cholerae	2	0.10	32	0.05
Candida famata	1	0.05	3	0.00
Candida guilliermondii	1	0.05	5	0.01
Candida krusei	1	0.05	79	0.12
Citrobacter koseri	1	0.05	445	0.68
Cryptococcus neoformans	1	0.05	7	0.01
Klebsiella oxytoca	1	0.05	223	0.34
Shigella flexneri	1	0.05	56	0.09
Shigella spp.	1	0.05	12	0.02

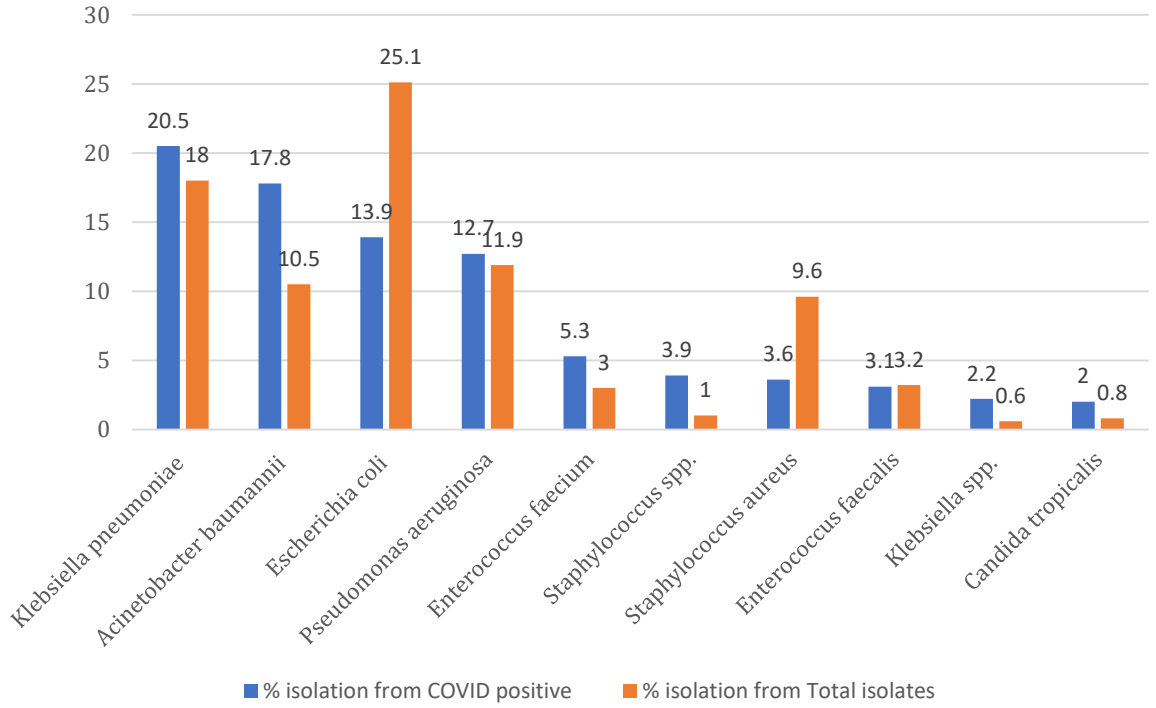


Figure 8.1- Isolation percentages of top 10 pathogens from Total isolates and COVID positive isolates

The relative distribution of the various species isolated from COVID patients in the OPD, admitted to the wards and ICUs are presented in Table 8.2 and Figures 8.2. Top 5 isolates in descending order in OPD specimen were *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter baumannii* and both *Enterococcus faecalis* and *S. aureus;* in Wards *E. coli, K. pneumoniae, Acinetobacter baumannii, P. aeruginosa* and *Enterococcus faecium*; and in ICU *K. pneumoniae, Acinetobacter baumannii*, *E. coli, P. aeruginosa* and *S. aureus.* Location wise isolation rate of *S. aureus* revealed a slightly higher rate among ward patients (4.69%) when compared to ICU (2.67%). The reverse was true with CoNS (6.43% among ICU patient's vs 4.47% among ward patients). This could again reflect the nature of samples from different locations. Among enterococci, *E. faecalis* demonstrated a slightly higher isolation rate among ward patients compared to ICU patients while no such difference was observed with *E. faecium* (Table 8.2).

Table 8.3 shows relative frequencies of different isolates in different types of specimens from Covid-19 patients. *K. pneumoniae* was more frequently isolated from lower respiratory tract infections and superficial infections. *A. baumannii* was more frequently isolated from lower respiratory tract and blood. *E. coli* was more frequent in urine and sterile body fluids and rare in lower respiratory tract specimens. *P. aeruginosa* was more frequent in lower respiratory tract and deep infections but rare in blood. Majority of *S. aureus* isolates were recovered from blood followed by LRT and superficial infections while

all the CoNS species were isolated only from blood. Both *E. faecium* and *E. faecalis* were isolated most frequently from blood followed by urine.

Table 8.2- Isolation pattern of organisms from COVID positive isolates across different locations

Organism	Total	% in Total Isolates n=2054	ICU	% in ICU Isolates n=1087	OPD	% in OPD Isolates n=100	Ward	% in Ward Isolates n=917
Klebsiella pneumoniae	421	20.50	235	21.62	8	16	178	19.41
Acinetobacter baumannii	366	17.82	229	21.07	3	6	134	14.61
Escherichia coli	285	13.88	77	7.08	18	36	190	20.72
Pseudomonas aeruginosa	260	12.66	148	13.62	5	10	107	11.67
Enterococcus faecium	109	5.31	56	5.15		0	53	5.78
Staphylococcus spp.	81	3.94	60	5.52	2	4	19	2.07
Staphylococcus aureus	74	3.60	29	2.67	2	4	43	4.69
Enterococcus faecalis	63	3.07	20	1.84	2	4	41	4.47
Klebsiella spp.	45	2.19	38	3.50		0	7	0.76
Candida tropicalis	41	2.00	34	3.13		0	7	0.76
Stenotrophomonas maltophilia	39	1.90	24	2.21		0	15	1.64
Acinetobacter baumanii-calcoaceticus complex	24	1.17	18	1.66		0	6	0.65
Burkholderia cepacia	24	1.17	15	1.38		0	9	0.98
Acinetobacter spp.	21	1.02	19	1.75		0	2	0.22
Staphylococcus epidermidis	21	1.02	8	0.74		0	13	1.42
Enterobacter cloacae	18	0.88	5	0.46	1	2	12	1.31
Enterococcus spp.	18	0.88	7	0.64	1	2	10	1.09
Proteus mirabilis	18	0.88	7	0.64	1	2	10	1.09
Candida auris	15	0.73	13	1.20		0	2	0.22
Candida albicans	14	0.68	10	0.92		0	4	0.44
Enterobacter spp.	13	0.63	4	0.37	2	4	7	0.76
Staphylococcus hominis	12	0.58	2	0.18	1	2	9	0.98

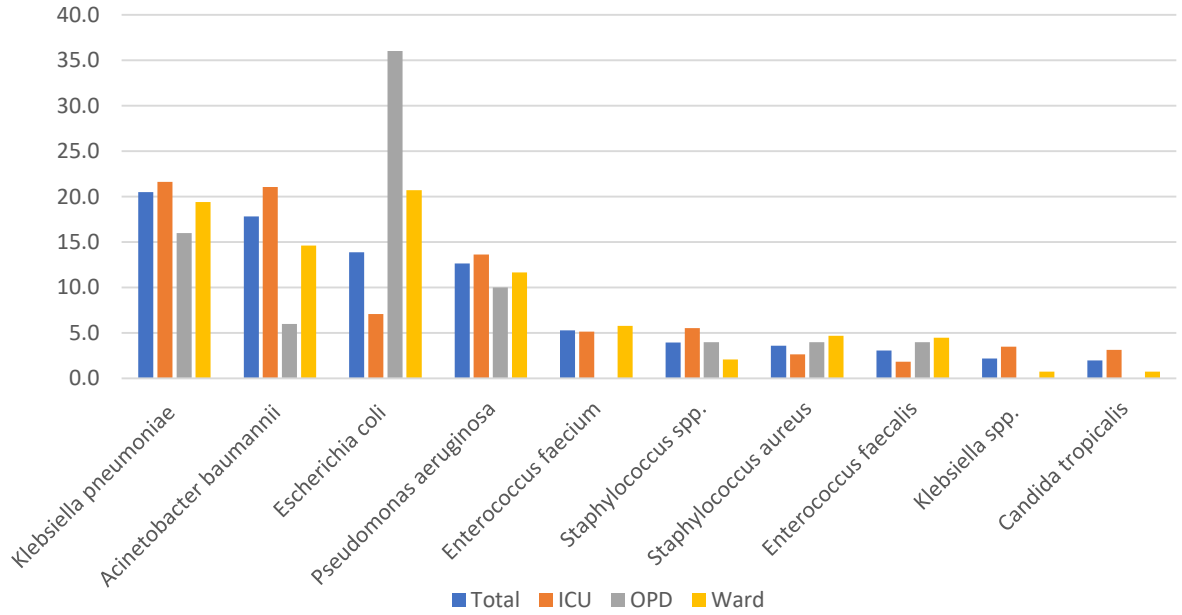


Figure 8.2- Isolation pattern of top 10 organisms from COVID positive isolates across different locations

Table 8.3- Isolation pattern of organisms from COVID positive isolates across different samples

Organism	Total n=2054	Blood n=795	Urine n=278	LRT n=697	SI n=98	DI n=44	CSF n=8*	SS n=43	Faeces n=17	Others n=74
Klebsiella pneumoniae	421 (20.5)	139 (17.5)	58 (20.9)	178 (25.5)	24 (24.5)	7 (15.9)	1 (-)	5 (11.6)	0 (0)	10 (13.5)
Acinetobacter baumannii	366 (17.8)	128 (16.1)	7 (2.5)	209 (30)	9 (9.2)	4 (9.1)	2 (-)	3 (7)	0 (0)	4 (5.4)
Escherichia coli	285 (13.9)	92 (11.6)	97 (34.9)	29 (4.2)	27 (27.6)	11 (25)	0 (-)	21 (48.8)	4 (23.5)	4 (5.4)
Pseudomonas aeruginosa	260 (12.7)	30 (3.8)	34 (12.2)	156 (22.4)	18 (18.4)	10 (22.7)	1 (-)	4 (9.3)	0 (0)	8 (10.8)
Enterococcus faecium	109 (5.3)	66 (8.3)	27 (9.7)	0 (0)	4 (4.1)	1 (2.3)	2 (-)	4 (9.3)	0 (0)	7 (9.5)
Staphylococcus spp.	81 (3.9)	81 (10.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (-)	0 (0)	0 (0)	0 (0)
Staphylococcus aureus	74 (3.6)	39 (4.9)	0 (0)	17 (2.4)	11 (11.2)	3 (6.8)	0 (-)	4 (9.3)	0 (0)	0 (0)
Enterococcus faecalis	63 (3.1)	32 (4)	16 (5.8)	0 (0)	9 (9.2)	2 (4.5)	0 (-)	1 (2.3)	0 (0)	3 (4.1)
Klebsiella spp.	45 (2.2)	15 (1.9)	0 (0)	29 (4.2)	0 (0)	0 (0)	0 (-)	1 (2.3)	0 (0)	0 (0)
Candida tropicalis	41 (2)	22 (2.8)	16 (5.8)	1 (0.1)	1 (1)	0 (0)	0 (-)	1 (2.3)	0 (0)	0 (0)

Klebsiella pneumoniae isolates from patients admitted in ICUs showed lower susceptibilities than those from patients in wards (Table 8.4). *K. pneumoniae* isolates from

Covid-19 patients showed significantly lower susceptibility to most antibiotics than those from non-Covid patients.

Acinetobacter baumannii is responsible for severe nosocomial infections, particularly in intensive care units (ICUs), where the incidence has increased over time. Bacterial co-pathogens are commonly identified in viral respiratory infections. Such scenario resulted in an increased incidence of antimicrobial resistance, which may be attributed to the excess use of antimicrobial agents during the COVID-19 pandemic. *A. baumannii* is the second most common pathogen isolated from the COVID positive group (17.8%). Table 8.5 summarises the susceptibility percentage of *A. baumannii* isolated from specimens collected during COVID-19 pandemic. The isolation rate of *A. baumannii* from ICU (62.5%) was higher compared to ward (36.6%) and the OPD which is on expected lines. Reduced susceptibility of<10% were observed against cephalosporins, βL-βLIs and carbapenems. Only minocycline showed better susceptibility of 54% among the tested antibiotics. Overall *A. baumannii* isolates showed high resistance to nearly all antibiotics tested.

Table 8.4- Susceptibility pattern of *Klebsellia pnemoniae* from all specimens

	Total n = 421	**OPD n = 8***	**Ward n= 178**	**ICU n= 235**
	S%	**S%**	**S%**	**S%**
Piperacillin-tazobactam	91/421 (21.6)	3/8 (-)	53/178 (30.1)	35/235 (14.9)
Cefotaxime	39/421 (9.2)	2/8 (-)	24/178 (13.7)	13/235 (5.6)
Ceftazidime	22/117 (18.8)	0/1 (0)	14/52 (26.9)	8/64 (12.5)
Ertapenem	108/410 (26.3)	5/8 (-)	60/167 (35.9)	43/235 (18.7)
Imipenem	113/421 (26.8)	5/8 (-)	68/178 (38.6)	40/235 (17.0)
Meropenem	93/224 (41.5)	1/1 (-)	65/129 (50.4)	27/94 (28.7)
Colistin	106/129 (82.2)	2/2 (-)	46/57 (80.7)	58/70 (82.8)
Amikacin	139/421 (33)	6/8 (-)	75/178 (42.1)	58/235 (24.8)
Ciprofloxacin	64/224 (28.6)	1/1 (-)	46/134 (34.3)	17/89 (19.1)
Levofloxacin	45/331 (13.6)	4/8 (-)	16/103 (15.5)	25/220 (11.3)

Table 8.5- Susceptibiliy pattern of *Acinetobacter baumanii* from all specimens

AMA	Total n= 366	OPD n = 3*	Ward n = 134	ICU n= 229
	S%	S%	S%	S%
Piperacillin-tazobactam	16/366 (4.4)	1/3 (-)	12/134 (8.8)	5/229 (2.2)
Cefepime	13/366 (3.5)	1/3 (-)	9/134 (6.8)	3/229 (1.3)
Ceftazidime	16/366 (4.4)	1/3 (-)	10/134 (7.5)	5/229 (2.1)
Imipenem	17/366 (4.4)	1/3 (-)	12/134 (9.1)	4/229 (1.7)
Meropenem	19/366 (5.2)	1/3 (-)	13/134 (9.7)	5/229 (2.1)
Colistin	0/0	0/0	0/0	0/0
Amikacin	63/365 (17.3)	1/2 (-)	50/134 (33.1)	12/229 (5.4)
Minocycline	166/366 (45.4)	2/3 (-)	73/134 (54.2)	91/229 (39.7)
Levofloxacin	26/366 (7.1)	1/3 (-)	19/134 (13.8)	6/229 (2.6)

Escherichia coli isolates from patients admitted in ICUs showed lower susceptibilities than those from patients in wards (Table 8.6). *Pseudomonas aeruginosa* was the third most common gram-negative pathogen isolated (12.66%), within which, 3% (*n=260*) of the total isolates were sourced from COVID-19 infected individuals, and also remained as the fourth common etiological agent in causing infections (COVID-19 group: 11.96%). The overall relative abundance of isolation rates was similar among specimens sourced from OPD, Wards and ICU settings. However, the isolation rates differed among COVID-19 infected sub-population, with ICU being the highest (13.62%), followed by wards (11.67%) and a few from OPD specimens (10%) (Table 8.7). Such a slight increase in isolation among ICU patients is an indicative of hospital-acquired infections.

Notably, *P. aeruginosa* was predominantly isolated from respiratory tract infections (22.4%); followed by urine (12.2%), skin infections (18.4%) and blood (3.8%). Among isolates from COVID-19 patients, susceptibility to anti-pseudomonals ranged from 57-65%. Isolates sourced from ICUs settings showed less susceptibility to ceftazidime, cefepime (by 3-4%), imipenem (9%), meropenem (5%), and amikacin (4%) as compared to isolates from Wards. In contrast, susceptibility to piperacillin/tazobactam (2%) and levofloxacin (2%) were slightly higher in isolates from ICU, than Wards. Together, high isolation rates and resistance to anti-pseudomonal agents in isolates of ICU settings, indicates the burden of antimicrobial resistance associated hospital-acquired infections in COVID-19 infected individuals.

Table 8.6- Susceptibiliy pattern of *Escherichia coli* from all specimens

	Total n = 285	OPD n = 18*	Ward n= 190	ICU n= 77
	S%	S%	S%	S%
Piperacillin-tazobactam	132/282 (46.8)	10/15 (-)	100/190 (52.8)	22/77 (29.2)
Cefotaxime	43/284 (15.1)	5/18 (-)	30/190 (15.7)	8/76 (10.5)
Ceftazidime	21/92 (22.8)	0/1 (0)	14/52 (26.9)	7/39 (23.4)
Ertapenem	163/248 (65.7)	12/14 (-)	117167 (70.3)	34/67 (50.7)
Imipenem	160/260 (61.5)	13/15 (-)	117/178 (67.4)	30/67 (44.7)
Meropenem	197/265 (74.3)	16/18 (-)	147/190 (77.7)	34/57 (59.6)
Colistin	106/129 (82.2)	0/0 (-)	24/24 (100)	17/17 (100)
Amikacin	234/285 (82.1)	16/18 (-)	158/190 (83.3)	60/77 (78)
Ciprofloxacin	58/201 (28.8)	8/18 (-)	46/134 (34.3)	4/49 (27.2)
Levofloxacin	22/141 (15.6)	2/13 (-)	15/84 (17.8)	5/44 (11.3)

Table 8.7- Susceptibiliy pattern of *Pseudomonas aeruginosa* from all specimens

AMA	Total n= 260	OPD n = 5*	Ward n = 107	ICU n= 148
	S%	S%	S%	S%
Piperacillin-tazobactam	147/249 (59)	2/4 (-)	56 /97 (57.7)	89/148 (60.1)
Cefepime	148/250 (59.2)	2/4 (-)	60 /98 (61.2)	86/148 (58.2)
Ceftazidime	148/251 (58.9)	2/4 (-)	61 /99 (61.6)	85/148 (57.7)
Imipenem	116/204 (56.9)	2/4 (-)	57 /92 (61.9)	57/108 (52.7)
Meropenem	140/250 (56)	2/4 (-)	58 /98 (59.1)	80/148 (54.2)
Colistin	0/0 (-)	0/0 (-)	0/0 (-)	7/7 (-)
Amikacin	163/250 (65.2)	2/4 (-)	66 /98 (67.6)	95/148 (64.2)
Levofloxacin	150/251 (59.8)	2/4 (-)	58 /99 (58.5)	90/148 (60.5)

Among *Enterococcus* species, only *E. faecium* showed a slight increase in isolation rate among COVID-19 patients. Susceptibility of *E. faecium,* isolated from Covid-19 patients was

lower to all antibiotics among ICU isolates when compared to those from the ward. The VRE rate was 31.2% overall (33.9% among ICU isolates and 28.6% among ward isolates). Linezolid resistance was 7.3%. All the isolates were resistant to ciprofloxacin regardless of the location **(Table 8.8).**

The isolation rate of *Staphylococcus aureus* is 2.5 times less among COVID patients when compared with the total isolation rate. Conversely, the isolation rate of CoNS among COVID-19 patients was double that of the total. This is probably a reflection of the type of samples collected. For eg, among COVID-19 patients, pus/wound swabs were minimal which would explain the lower isolation rate of *S. aureus* **(Table 8.9)**. MRSA rate was 59.5% overall which is significantly higher than the national average for 2020 (41.4%). As expected susceptibility to all antibiotics was lower among ICU isolates except for ciprofloxacin. No resistance was observed for vancomycin, teicoplanin, tigecycline and linezolid **(Table 8.9).**

Nosocomial fungal infections in COVID-19 patients have been reported in many studies. During the surveillance period, January 1, 2020, through December 31, 2020, we reported several fungal infections in COVID-19 patients. The prevalence of secondary fungal infections in COVID-19 patients was 4.3%. Interestingly, *C. tropicalis* ranked among the top ten isolated pathogens. *C. tropicalis* (41/ 2054, 2%) was more frequently isolated fungus followed by *C. auris* (15/2054, 0.73%), *C. albicans* (14/2054, 0.68%), *C. parapsilosis* (9/2054, 0.44%), *C.glabrata* (3/2054, 0.15%), *C. rugosa* (2/2054, 0.10%), *C. famata* (1/2054, 0.05%), *C. guilliermondii* (1/2054, 0.05%), *C. krusei* (1/2054, 0.05%), and *C. neoformans* (1/2054, 0.05%). Majority of these fungal species were isolated from ICU patientscompared to those admitted in wards (*C. tropicalis*, 43.0 *vs*. 7.0; *C. auris*, 13 *vs.*2.0; *C. albicans* 10 *vs.* 4.0). Of 41 cases of C. *tropicalis*, candidemia was the most common form (*n*, 22) of infection followed by urinary tract infections (*n*, 16). *C. tropicalis* was susceptible to anidulafungin and micafungin (100%) but showed comparatively less *in vitro* susceptibility to caspofungin (90.2%).Among azoles, a greater percentage of isolates were susceptible to voriconazole (95.2%) compared to fluconazole (92.8%) **(Table 8.10).**

In India, a multicentric study conducted across twenty seven ICUs reported *C. tropicalis* as the predominant pathogen causing candidemia. In COVID-19 patients, although a largely similar trend in species distribution was noticed, increased isolation of previously rare fungi in these patients could be a concern. The impact of COVID-19 in thischanging epidemiology may need to be monitored. Echinocandins are the front-line agents in the treatment of invasive candidiasis. Even though all isolates were susceptible to anidulafungin and micafungin, decreased *in vitro*susceptibility to caspofungin may require further evaluation. Specifically, this standalone resistance to caspofungin needs to be correlated with *FKS*1 genotype of these isolates. A substantial azole resistance was found in *C. tropicalis* with approximately 10% of the isolates resistant to fluconazole. The contribution of COVID-19 in the emergence of resistance among fungal isolates needs further evaluation.

Table 8.8- Susceptibiliy pattern of *Enterococcus faecium* from all specimens

AMA	Total n = 109	OPD n = 0*	Ward n= 53	ICU n= 56
	S%	S%	S%	S%
Ampicillin	19/109 (17.4)	0/0 (-)	18/53 (3.4)	1/56 (1.8)
Vancomycin	75/109 (68.8)	0/0 (-)	38/53 (71.4)	37/56 (66.1)
Teicoplanin	79/109 (72.5)	0/0 (-)	39/53 (74.2)	40/56 (71.4)
High Level Gentamicin	22/107 (20.5)	0/0 (-)	13/53 (25)	9/54 (16.6)
Ciprofloxacin	0/31 (0)	0/0 (-)	0/19 (0)	0/12 (0)
Nitrofurantoin	6/34 (17.6)	0/0 (-)	5/21 (23.8)	1/13 (7.7)
Fosfomycin	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)
Linezolid	101/109 (92.7)	0/0 (-)	50/53 (94.3)	51/56 (91.9)

Table 8.9- Susceptibiliy pattern of *Staphylococcus aureus* from all specimens

AMA	Total n = 74	OPD n = 2*	Ward n= 43	ICU n= 29
	S%	S%	S%	S%
Cefoxitin	30/74 (40.5)	0/2 (-)	29/43 (67.3)	1/29 (53.1)
Oxacillin	22/35 (62.9)	0/0 (-)	16/19 (84.2)	6/16 (37.5)
Vancomycin	39/39 (100)	1/1 (-)	18/18 (100)	21/21 (100)
Teicoplanin	30/30 (100)	1/1 (-)	11/11 (100)	18/18 (100)
Erythromycin	23/72 (31.9)	0/0 (-)	15/43 (34.6)	8/29 (27.6)
Tetracycline	62/72 (86.1)	1/1 (-)	39/43 (90.1)	22/28 (78.6)
Tigecycline	27/27 (100)	1/1 (-)	12/12 (100)	14/14 (100)
Ciprofloxacin	11/64 (17.2)	0/1 (-)	6/37 (16.2)	5/26 (19.2)
Clindamycin	49/70 (70)	2/2 (-)	30/43 (70.6)	17/25 (68)
Trimethoprim-sulfamethoxazole	49/72 (68)	0/0 (-)	31/43 (71.2)	18/29 (63.6)
Linezolid	74/74 (100)	2/2 (-)	43/43 (100)	29/29 (100)

Table 8.10- Susceptibiliy pattern of *Candida tropicalis* from all specimens

AMA	Total n = 41	OPD n = 0*	Ward n= 7*	ICU n= 34
	S%	S%	S%	S%
Anidulafungin	11/11 (100)	0/0 (-)	0/0 (-)	11/11 (100)
Caspofungin	37/41 (90.2)	0/0 (-)	7/8 (-)	30/33 (90.9)
Fluconazole	39/42 (92.8)	0/0 (-)	6/8 (-)	33/34 (97)
Micafungin	32/32 (100)	0/0 (-)	5/5 (-)	27/27 (100)
Voriconazole	40/42 (95.2)	0/0 (-)	7/8 (-)	33/34 (97)

Stenotrophomonas maltophilia is the third most common non-fermenting gram-negative pathogen isolated (1.9%, n=39) among COVID-19 infected individuals. Notably, 62% (n=24/39) of the isolates were from ICU, 38% (n=15/39) were from Wards and none were from OPD. This clearly highlights the hospital-acquired infections of *S.maltophilia* in COVID-19 infected hospitalized patients. Further, we observed that the susceptibility to ceftazidime was less (14.3%), while susceptibility to minocycline; levofloxacin and co-trimoxazole were higher and ranged from 81-92% (**Table 8.11).** Notably, minocycline susceptibility remained the same in isolates of both ICU and wards, in contrast to the susceptibility of levofloxacin and co-trimoxazole that differed by isolation settings of ICU and wards.

As per the **Table 1**, the isolation rate of *B.* cepacia among the COVID positive group was 1.17%. Compared to ward, the isolation rate of clinical isolates of *B. cepacia* was higher in ICU and none from OPD. The susceptibility pattern of clinical isolates of *B. cepacia* was summarized in **table 8.12**. Despite ceftazidime and Trimethoprim-sulfamethoxazole being the first line choice of drugs, decreased susceptibility of 73% and 80% were noticed, respectively. Drastic reduction in susceptibility rates to meropenem and minocycline was also observed. Such scenario clearly indicates the overuse of carbapenem and minocycline against NFGNB.

Table 8.11- Susceptible pattern of *Stenotrophomonas maltophilia* from all specimens

AMA	Total n= 39	OPD n = 0*	Ward n = 15	ICU n= 24
	S%	S%	S%	S%
Ticarcillin-clavulanic acid	1/2 (-)	0/0 (-)	0/0 (-)	1/2 (-)
Ceftazidime	2/14 (14.3)	0/0 (-)	1/8 (-)	1/6 (-)
Minocycline	36/39 (92.3)	0/0 (-)	12/13 (92.3)	24/26 (92.3)
Levofloxacin	38/43 (88.3)	0/0 (-)	14/15 (93.3)	24/28 (85.7)
Trimethoprim-sulfamethoxazole	35/43 (81.4)	0/0 (-)	13/14 (92.8)	22/29 (75.7)

Table 8.12- Susceptible pattern of *Burkholderia cepacia* from all specimens

AMA	Total n= 24	OPD n = 0*	Ward n = 9*	ICU n= 15
	S%	S%	S%	S%
Ticarcillin-clavulanic acid	1/13 (7.7)	0/0 (-)	1/3 (-)	0/12 (0)
Ceftazidime	19/24 (79.2)	0/0 (-)	8/9 (-)	11/15 (73.3)
Meropenem	19/24 (79.2)	0/0 (-)	9/9 (-)	10/15 (66.6)
Minocycline	16/23 (69.6)	0/0 (-)	8/8 (-)	8/15 (53.3)
Levofloxacin	15/23 (65.2)	0/0 (-)	6/8 (-)	9/15 (60)
Trimethoprim-sulfamethoxazole	20/23 (87)	0/0 (-)	8/8 (-)	12/15 (80)

Chapter 9 Important pathogens summaries

GROUP I

- Carbapenem Resistant Enterobacterales
- Carbapenem Resistant *A. baumannii*
- Drug resistant *Salmonella* Typhi
- *Candida auris*

GROUP II

- ESBL producing Enterobacterales
- Multidrug resistant *P. aeruginosa*
- Vancomycin-resistant enterococci
- Azole Resistant *Candida* spp

GROUP III

- Methicillin Resistant *Staphylococcus aureus*
- Azole resistant *Aspergillus fumigates*
- Amphotericin B resistant *Aspergillus flavus*
- Drug-resistant *Stenotrophomonas maltophilia*
- Colistin Resistant Enterobacterales
- Colistin resistant *Acinetobacter* spp.

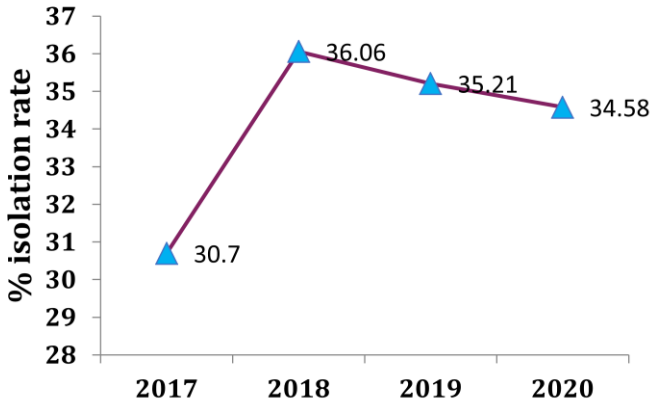
CARBAPENEM-RESISTANT Enterobacterales

GROUP -I

Why CRE is important to know

- Multiple drug resistant organisms (MDROs), predominantly bacteria, are resistant to multiple classes of antibiotics
- Carbapenem resistant *Enterobacterales* (CRE) is an important MDRO, and an emerging challenge because:
  - Prevalence is increasing
  - Treatment options are limited
  - Cause serious disease, increased length of stay, cost and mortality.
  - Demand high end antibiotics → damage normal flora → further colonization with MDROs.

Trend of CRE isolates



Containing CRE

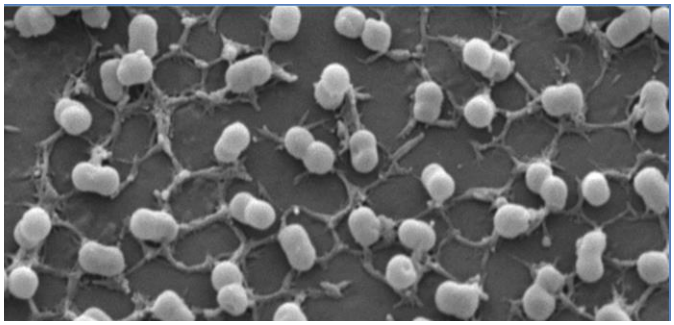
- Standard care for CRE
  - **Appropriate patient placement**. Limiting transport & movement of patients. Use of disposable & dedicated patient care equipment
  - **Personal protective equipment, gloves & gowns**
  - **Prioritized cleaning & disinfection.** HCW education in IPC principles, monitoring of contact precautions. For high risk, pre-emptive isolation/ cohorting till results available
  - **Surveillance cultures for asymptomatic CRE colonization:** Not yet a routine standard of care. Recommended in outbreaks and situation with high risk of CRE acquisition

Mechanism of resistance

- Bacteria may have multiple resistance mechanisms to carbapenems, but the most common and clinically relevant amongst *Enterobacterales*is carbapenemase enzyme production
- Carbapenemases fall under Ambler (molecular) groups A, B and D. Group A includes KPC and IMI, group B includes metallo-b-lactamases, NDM, VIM and IMP and group D includes OXA
- Different carbapenemase genes confer different phenotypic resistance to different antibiotics and susceptibility to b-lactamase inhibitors
- It is important to know the locally prevalent genes to formulate empirical treatment policies

Treatment strategy for CRE

- CRE *Rx* dependsheavily on susceptibility profile, preferably MIC based. Options include:
  - Meropenem high-dose extended-infusion (if MIC 2-8 mg/L); or meropenem + amikacin; or meropenem/imipenem + colistin
  - Tigecycline high dose with loading dose (not indicated in septicemia); minocycline alternative
  - Aztreonam for MBL producers
  - Ceftazidime-avibactam for KPC and OXA-48 producers
  - Colistin or polymyxin B



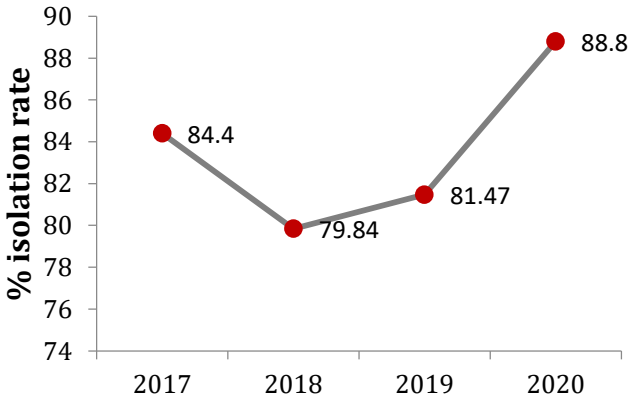
CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII(CRAB)

GROUP I

Why CRAB is important

- Carbapenem-resistant *Acinetobacter baumannii* (CRAB) cause ventilator-associated or hospital-acquired pneumonia (VAP/HAP), wound, bloodstream, and urinary tract infections especially in patients admitted in intensive care units
- CRAB produces carbapenemases which makes carbapenem antibiotics ineffective and rapidly spreads resistance via mobile genetic elements that are easily shared between bacteria

Trend of CRAB



CRAB is a threat in healthcare

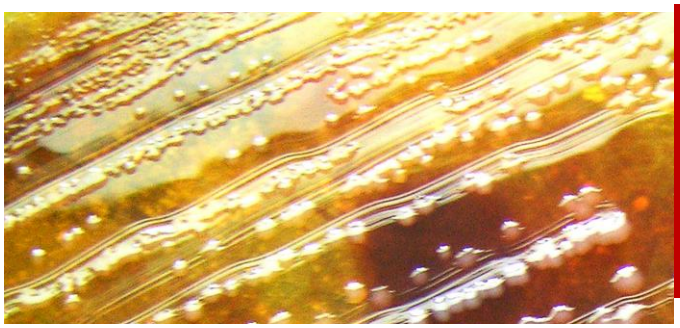
- CRAB is a challenging threat to hospitalized patients because it frequently contaminates healthcare facility surfaces and lead to outbreaks if appropriate infection control measures are not taken
- CRAB is already resistant to many antibiotics and further resistance to carbapenems reduces patient treatment options
- Biofilm forming capabilities of *A. baumannii* provides persistence nature especially on non-living and biologic surfaces (medical devices and host tissues) and allow it to resist antibiotic agents, which subsequently leads to recurrent infections
- Infections caused by CRAB are of particular concern because they are frequently difficult to treat with the available antibiotics

Mechanism of resistance

- The mechanisms of carbapenem resistance in *A. baumannii* can be typically sorted into four groups:
  - Inactivation or enzymatic degradation of carbapenems by carbapenemase enzymes like class D oxacillinases (OXA-23 like) and class B metallo-beta lactamses (NDM), usually found on plasmids and are highly transmissible
  - Membrane impermeability due to the reduced expression or mutation in outer membrane porins
  - Overexpression of efflux pumps responsible for pumping carbapenems out of the cell
  - Decreased drug affinity due to down-regulation of penicillin-binding proteins (PBPs)

Treatment strategy

- Colistin as a part of combination regimen with a second agent, such as carbapenem, tigecycline, sulbactam or rifampicin
- Minocycline, glycylcycline, tigecycline are considered additional options against CRAB infections



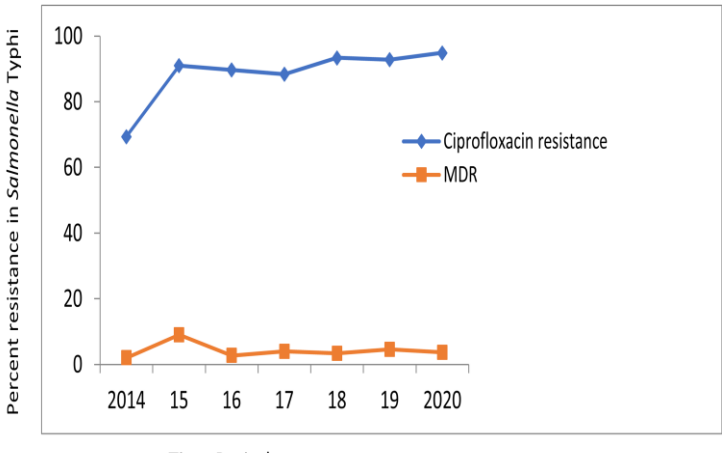
Drug Resistant *Salmonella* Typhi

GROUP I

What you need to know

- Typhoid incidence in India is reported to be 377 and 105/100,00 person years from 2 different regions, with highest incidence occurring in children between 2-4 yrs
- Antibiotic treatment is the mainstay of management. Increasing antibiotic resistance to anti-typhoidal drugs has been a challenge that keeps on increasing in its spectrum as newer drugs come in to use
- Blood culture is gold standard for diagnosis and detection is limited to hospitals with good diagnostic capacity
- Improvement in sanitation and hygiene and safe water supply can reduce the disease burden of typhoid

Resistance trends



About XDR

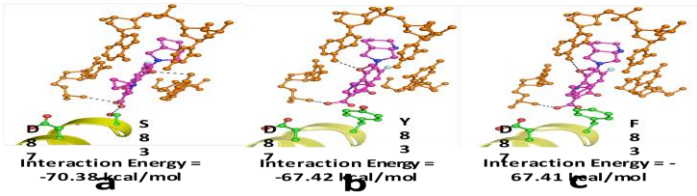
- Recently extensively drug resistant typhoid fever has been reported in some countries (XDR - MDR along with ciprofloxacin and ceftriaxone resistance)
- Maximum number of strains still falls in sensitive range of 0.125 to 0.19 µg/ml but few strains have started to show increased MIC values against ceftriaxone
- Till date very few cases of typhoid fever due to XDR *S.* Typhi have been reported from India. They remain susceptible to azithromycin
- We need to watch out for any emergence of XDR strains as ceftriaxone MIC is increasing over time and azithromycin resistance has also been reported

Mechanism of resistance

In India first outbreak of MDR *S.*Typhi harboring an IncHI1 plasmid was in 1972 followed by its spread across the country

Resistance to chloramphenicol, amoxicillin and cotrimoxazole drugs is plasmid mediated –CSSuT

Fluoroquinolone resistance is mainly associated with mutations in the chromosomal quinolone-resistance-determining regions in *gyr* A and *parC* gene and plasmid-mediated (PMQR) by acquisition of (i) *qnr* genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*) and the *aac (6′)-Ib-cr* gene, which encodes modifying enzyme that decreases FQ activity and *oqxAB* and *qepA*, encoding quinolone efflux pumps



Interactions of Ciprofloxacinin Gyrase-DNA complex (a) WT (b) S83Y and (c) S83F

Current guidelines and treatment strategy

Based on the Indian data, the first line of treatment is ceftriaxone/cefixime or azithromycin depending on the severity of illness. Ciprofloxacin can be advised based upon the susceptibility results.

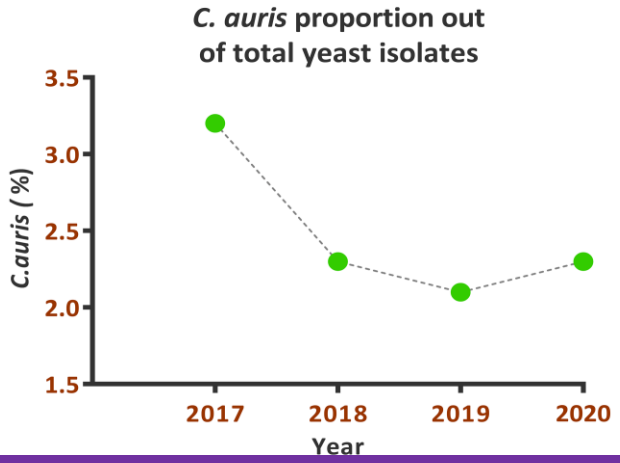
Candida auris

GROUP I

What you need to know

- *C. auris*, amultidrug resistant pathogenic yeast known for rapid transmission in hospital settings
- Causes bloodstream and other invasive infections especially in critically ill patients
- Diagnosis often misidentified by conventional methods
- MALDI-TOF and fungal genetic barcode, ITS and 28S rRNA are the only reliable methods to accurately identify this yeast
- Four major phylogeographic clades have been identified in *C. auris* (I, South Asian; II, East Asian; III, South African; and IV, South American) with a possible fifth clade in circulation worldwide

Trend of *Candida auris isolates*



Candida auris : a threat in healthcare?

- *C. auris* is the 5th leading cause of candidemia in nosocomial settings, though recently it had been reported as rank one causing candidemia in one ICU
- Readily colonizes skin and horizontally spreads through healthcare workers (HCWs) or fomites causing outbreaks
- High thermotolerance, osmotic-tolerance and capacity to form adherent biofilms help them to persist in the hospital environment and other clinical niches
- *C. auris* is resistant to most of the antifungal drugs including echinocandins, thus severely limiting the treatment options
- *C. auris* infection can adversely alter the clinical course of a patient. All-cause mortality associated with *C. auris* infection is 30-60%

Mechanism of resistance

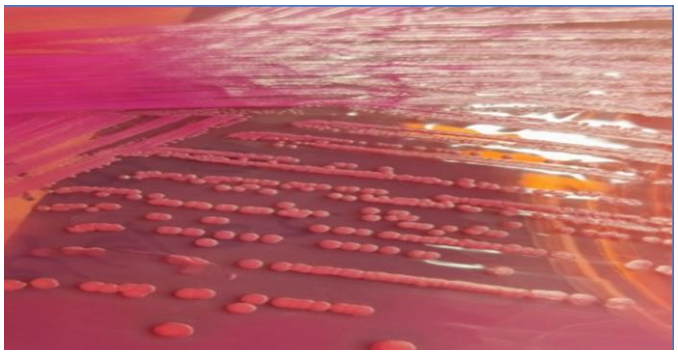
- Major mechanism of resistance is classified into four groups: target alteration, target overexpression, expulsion of drug from the cell, and biofilm-mediated resistance
- Azole resistance: Y132F (53% in clade I, 40% in clade IV) K143R (43% in clade I), and F126L (96% in clade III) in azole target gene, *ERG*11 mutations
- Copy number variations (CNVs) in *ERG*11 (clade I), and *TAC*1B gene leading to overexpression of *ERG*11 and *CDR*1 efflux transporter
- S639F, S639P, F635Y, F635L, and R1354S mutations in *fks*1 gene, that encodes catalytic subunit of β-1,3-glucan synthase, confer echinocandin resistance

Treatment strategy

- Echinocandins are recommended as initial therapy
- Liposomal amphotericin B is the usual alternative especially in brain infection
- Voriconazole may be given if the isolate is susceptible by in vitro susceptibility testing
- If possible, treatment should be guided by in-vitro susceptibility testing

Containment strategy

Isolation of the colonized or infected patient, active surveillance of hospital environment to search for the source,thorough dis-infection of the hospital fomites with phenol compounds with sufficient exposure time,compliance to hand-hygiene by the HCWs, skin decontamination with chlorhexidine batch/oral gargles with chlorhexidine mouth wash for colonized patients, terbinafine for colonized canula entry sites.



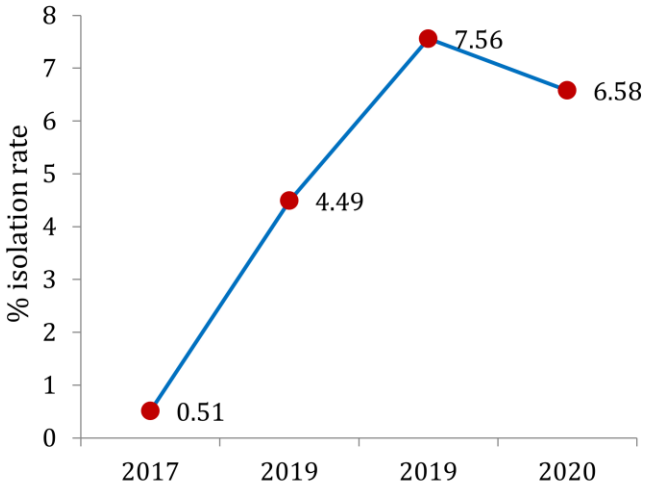
ESBL producing Enterobacterales

GROUP II

What you need to know

- Extended spectrum beta lactamases (ESBLs) are enzymes produced by Gram negative bacteria that inactivate the extended spectrum penicillins, cephalosporins and monobactams but not cephamycins and carbapenems
- ESBLs are produced by most members of Enterobacterales, most importantly by *Klebsiella pneumoniae* and *Escherichia coli*
- ESBL producing Enterobacterales are frequently resistant to other commonly used antibiotics like fluoroquinolones, aminoglycosides, and sulphonamides, and demand 'last resort' antibiotics like carbapenems for effective therapy

Trend of ESBL isolates



Where infection can happen

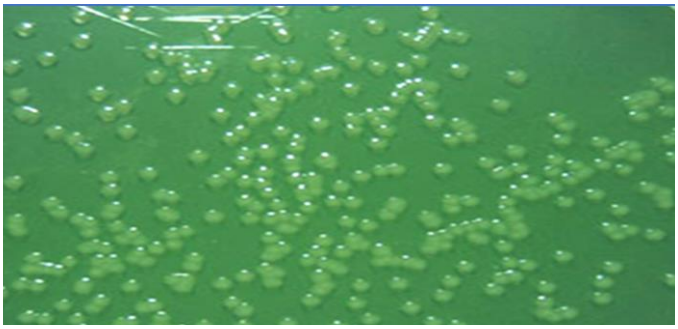
- ESBL producing Enterobacterales can cause a wide spectrum of infections including urinary tract infections, lower respiratory tract infections, intra-abdominal infections, skin and soft tissue infections and septicaemia
- They are more frequent in hospital acquired infections like catheter associated UTI, ventilator associated pneumonia, intra-abdominal sepsis and organ space post-surgical infections, though not uncommonly found in community acquired infections like UTI also
- Community based surveillance studies have shown high carriage of ESBL producing Enterobacterales in healthy individuals without history of hospitalization or recent history of antibiotic exposure

Mechanism of resistance

- ESBLs can be chromosomal or plasmid mediated, constitutional or inducible
- Most are derived from genes for TEM-1, TEM-2, or SHV-1 by mutations. Repeated mutations have led to emergence of hundreds of new β-lactamases, some with broader spectrum including carbapenems
- Ambler (molecular) classification classifies them into class A with narrow spectrum (TEM, SHV, CTX-M), class B with metallo-□-lactamases (IMP, VIM, NDM), class C (Amp-C) and class D oxacillinases (OXA)
- All class B and some of the other classes (KPC in class A, extended spectrum Amp-C in class C and carbapenemhydrolysing class D □-lactamases or CHDLs in class D) also have carbapenemase activity

Treatment & Containment strategy

- Mainstay of treatment of ESBL producing Enterobacterales includes β-lactam β -lactamase combinations like piperacillin-tazobactam and cefoperazone-sulbactam, and carbapenems. ESBLs with carbapenemase activity need to be treated like carbapenemase producing Enterobacterales
- Improved infection control and rational antimicrobial prescribing practices are recommended for containment



Multidrug resistant *Pseudomonas aeruginosa*

GROUP II

What you need to know

- *P.aeruginosa* is of the notorious nosocomial pathogen, implicated in hospital acquired infections such as ventilator associated pneumonia, bloodstream infections, urinary tract infections and surgical site infections
- Antimicrobial resistance is a major concern and is driven by multiple mechanisms, of both chromosomal and acquired determinants
- Phenotypic antimicrobial susceptibility displays discrepant profile against anti-pseudomonal agents and extrapolation of one agent's suscpetibility to the other agents within and across the class should never be done

Ceftazidime	Imipenem	Meropenem
S	R	R
S	R	S
S	S	R

S – Susceptible, R – Resistant

Mechanism of resistance

Unlike other gram negative clinical pathogens, Antimicrobial resistance mechanisms in *P. aeruginosa* is complex, with the dominance of chromosomal mediated mechanisms in addition to acquired resistance determinants

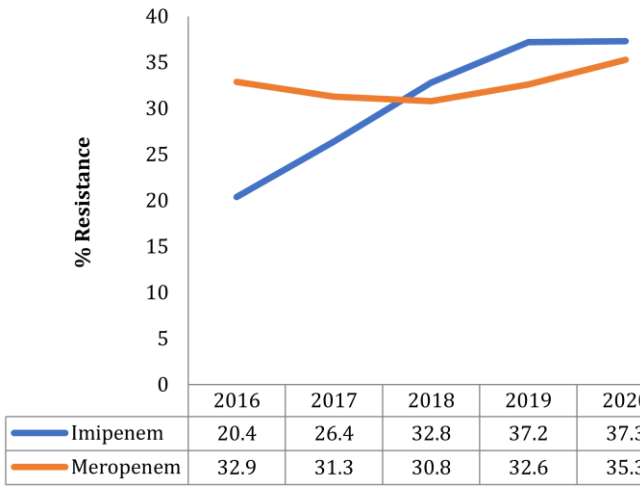
Chromosomal mediated

- Overexpression of efflux pumps (MexAB, mexCD, mexEF, mexXY)
- Pseudomonal derived cephalosporinase (PDC)
- Loss of porin (OprD)

Acquired/plasmid mediated

- ESBLs (bla_{VEB}, bla_{TEM})
- Carbapenemases (bla_{VIM}, bla_{NDM}, bla_{IMP})

Carbapenem resistance trends



	2016	2017	2018	2019	202
Imipenem	20.4	26.4	32.8	37.2	37.3
Meropenem	32.9	31.3	30.8	32.6	35.3

Treatment strategy

- Anti-pseudomonal beta lactam + aminoglycosides / fluoroquinolones
- Piperacillin/tazobactam + Aminoglycosides
- Carbapenems
- For Non-carbapenemase mediated resistance - Ceftazidime/avibactam
- *Consider adding inhaled antibiotic in VAP (Aminoglycoside - Tobramycin)

Containment strategy

- Isolate patients with pseudomonal infection to minimize the contact with other high-risk individuals
- Minimize the risk of *P.aeruginosa* contaminations by ensuring best practice advice relating to hand wash stations
- Continue to monitor drug resistant clinical isolates of *P.aeruginosa* as an alert organism, followed by compliance with appropriate guidelines to prevent HCAI
- Monitor clinically relevant isolates of *P.aeruginosa* to identify epidemic strains for implementation of appropriate infection control procedures from causing on outbreak

Rapid identification of an outbreak → Infection control assesment → Screening of paitents for colonization → Implementation of appropriate control measures → Conitnued assesment and screeening

Vancomycin resistant enterococci

GROUP II

What you need to know

- Vancomycin-resistant enterococci (VRE) are both of medical and public health importance and are associated with serious multidrug-resistant infections and persistent colonization
- Theycan cause infections in healthcare settings, including bloodstream, surgical site, intra-abdominaland urinary tract infections
- About 30% of all healthcare-associated enterococcal infections are resistant to vancomycin, reducing treatment options
- VRE is increasingly resistant to additional antibiotics, raising concern that the remaining drugs to treat VRE may become less effective
- Of the several species of enterococci isolated from human infections, *E. faecalis* is the commonest while *E. faecium* is more often associated with vancomycin resistance

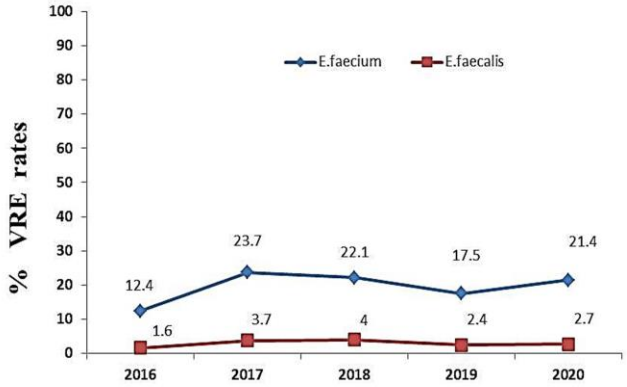
Patients at risk

Risk factors for acquisition of infection with VRE include admission to a critical care unit, severe illness, exposure to other patients with VRE, prolonged hospitalization and exposure to antimicrobials

Treatment strategy

- Vancomycin-resistant *E. faecium* isolates often have concurrent high-level resistance to beta-lactams and aminoglycosides. In contrast, vancomycin-resistant *E. faecalis* are usually susceptible to beta-lactams, as are *E. gallinarum* and *E. casseliflavus* (which are intrinsically vancomycin resistant)
- The newer agents, linezolid, daptomycin and tigecycline have activity against both vancomycin-resistant *E. faecalis* and *E.faecium*, whereas quinupristin-dalfopristin has activity only against *E. faecium* but not *E. faecalis*
- Fosfomycin is used to treat VRE infections of the urinary tract caused by E. *faecalis* but not *E. faecium*

Trends of VRE

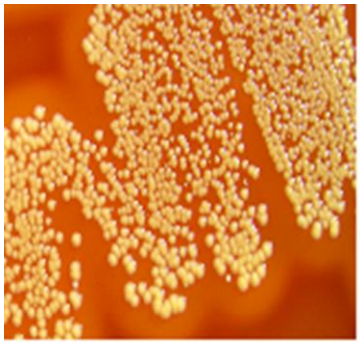


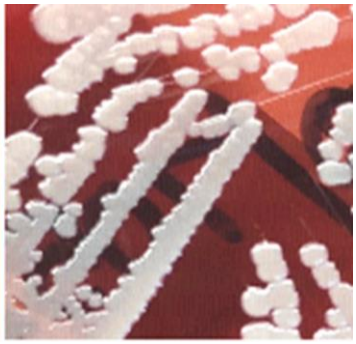
Mechanism of resistance

- Vancomycin resistance in enterococci is mediated by several *van* genes such as *van*A, *van*B, *van*Cetc of which *van*A is the most common worldwide
- VanA (encoded by the *vanA* gene) is a ligase that catalyzes the binding of D-alanine to D-lactate (the corresponding ligases in other types are VanB, VanD, VanF, and VanM), resulting in the formation of D-alanyl-D-lactate which has 1000 times less affinity for vancomycin than the original D-alanine-D-alanine
- The *vanS* and *vanR* genes encode a two-component regulatory system that is involved in the induction of expression of resistance
- Several of the *van* genes are located on transposons making them highly transmissible to susceptible strains of enterococci as well as MRSA isolates

VRE containment

- Accurately identify VRE, automated systems may be unreliable.
- Effective disinfection procedures for medical equipment
- Isolate VRE infected or colonized patients at earliest
- Since most transmission occurs via HCWs, hospital staff must be familiar with, and must follow isolation and control procedures.





Methicillin resistant *Staphylococcus aureus*

GROUP III

What you need to know

- Methicillin- resistant *Staphylococcus aureus* (MRSA) lives as a commensal on the skin and mucous membranes of man and animals and is transmitted in both health- care and community settings
- It is a leading cause of skin and soft tissue infections, bone and joint infections, bacteraemia and endocarditis
- Genetically diverse, the epidemiology of MRSA is primarily characterized by the serial emergence of epidemic strains
- MRSA still poses a formidable clinical threat, with persistently high morbidity and mortality.
- Successful treatment remains challenging with emergence of reduced susceptibility/resistance to several anti MRSA agents

MRSA infections can be prevented

The major source of MRSA in a healthcare setting is the MRSA carriers among health care workers or the patients themselves. Less important is the contaminated environment

KEY PREVENTIVE STRATEGIES

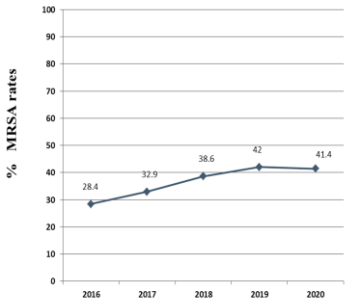
In the healthcare setting

- Strict adherence to the 5 moments of hand hygiene recommended by WHO
- If possible, isolation or at least cohorting of MRSA infected patients
- In an outbreak situation, identification and treatment of MRSA carriers among the health care workers
- Cleaning high-touch surfaces regularly

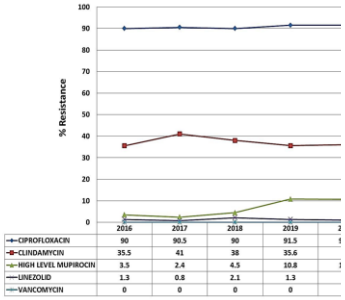
In the community setting

- Frequent hand washing with an alcohol-based hand sanitizer when soap and water are not available
- Keeping cuts and scrapes clean and bandaged until the skin heals
- Avoid touching other people's scrapes, wounds, or bandages without first washing your hands
- Avoid sharing personal items like razors, towels, or athletic equipment

Trends of MRSA



Trends of co-resistance of MRSA isolates to other antibiotics



Mechanism of resistance

- Methicillin resistance is mediated by *mecA*genepresent ona mobile geneticelement designated staphylococcal cassette chromosome*mec*(SCC*mec*)
- The gene *mecA*encodes penicillin bindingprotein 2a (PBP2a), an enzyme responsiblefor crosslinking the peptidoglycans in the bacterial cellwall
- PBP2a has a low affinity for β- lactams, resulting inresistance to this entire class of antibiotics with the exception of fifth generation of cephalosporins such asceftaroline and ceftobiprole
- Other rarer mechanisms include alternate *mec* genes such as *mec*C, *mec*B and *mec*D and occasionally hyper production of beta lactamase enzyme

Treatment strategy

- Vancomycin is the first- line therapy for MRSA bacteremia and infectiveendocarditis.
- Daptomycin is the only other FDA-approved first- line agent for MRSA bacteraemia or right- sided endocarditis.
- Linezolid, a protein synthesis inhibitor and an oral drug, has proved useful in treatment of MRSA infections of skin and soft tissue as well as bacteremia
- Other available anti MRSA agents include lipoglycopeptides such as telavancin or oritavancin and the fifth generation cephalosporins.
- The emergence of non-susceptible isolates to vancomycin (VISA and hVISA) and daptomycin as well as those resistant to linezolid currently pose a serious challenge to successful therapy of MRSA infections

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