Annual Report

Antimicrobial Resistance Surveillance and Research Network

January 2019 to December 2019

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Chapter 1 Summary of surveillance data

January 2019 to December 2019

Total number of culture positive isolates studied during the year 2019 was 107387.Of these, 17108 from blood, 30822 urine, 15571 Lower Respiratory tract, 25058 Superficial infections, 7053 Deep infections, 688 CSF, 2623 Sterile spaces, 1051 Faeces and 7413 others. Majority of the isolates were from Enterobacteriaceae (53%) followed by Non fermenting Gram-negative bacilli (NFGNB) (22%), *Staphylococci* (15%), *Enterococci* (6%), *Salmonella* (1%) and *fungi* (2%) (Table 1.1). In the distribution of major group of organisms in different specimens, member of the Enterobacteriaceae group were the commonest isolates in urine (79%), sterile body fluids (SS) (58%), others (49%), deep infections (DI) (46%), superficial infections (SI) (42%), blood and LRT (41%) and CSF (29%). Non fermenting Gram-negative bacilli (NFGNB) group were the predominant isolates in the lower respiratory tract (LRT) (51%), CSF (45%), deep infection (DI) (24%), sterile sites (SS) (23%), blood (17%) and urine (7%). *Staphylococci* constituted 28% of the superficial infections (SI) followed by blood infection (23%), deep infection (DI) (22%) and CSF (16%). *Enterococci* group constituted 11% of the isolates from urine followed by sterile body fluid (9%), CSF (8%), deep infections (7%), blood (6%) and superficial infections (5%). *Salmonella* group constituted 84% of blood infection. *Yeast* group were significant isolates in the blood infection (7%) (Table1.1 and Figure 1.1a).

The relative distribution of the various species isolated from patients in the OPD, admitted to the wards and ICUs are presented in Table 1.2 and Figures 1.2a &1.2b. Overall, *Escherichia coli* was the commonest isolate (28%) followed by the *Klebsiella pneumoniae* (17%), *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* (11%) and *Acinetobacter baumannii complex* (9%). Gram negative organisms constituted 68% of the significant top 10 isolates.Top 5 isolates in descending order in OPD specimen were *E. coli, S. aureus, K. pneumoniae, P. aeruginosa* and *Acinetobacter baumannii* complex; in Wards *E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and *Acinetobacter baumannii* complex; and in ICU *Acinetobacter baumannii* complex, *K. pneumoniae, E. coli, P. aeruginosa, S. aureus. Enterococcus faecium* was common isolate from the ICU (3%) followed by ward and OPD; whereas, *E. faecalis* was common isolate from the OPD (3%) followed by the wards and were least isolated from the ICU (1%). (Table 1.2, Figure 1.2a)

Enterobacteriaceae (except Salmonella) constituted the major group (53%) of the overall isolates (Table1.1). Out of a total of 57236 culture positive isolates, specimen percentage wise distribution of major species within family Enterobacteriaceae is shown in the Table 1.3 and Figures 1.3a. Overall, *Escherichia coli* was the commonest species (28%)

followed by *Klebsiella pneumoniae* (17%), *Proteus mirabilis* (1.8%) and *Enterobacter cloacae* (1.4%) (Table 1.3). *Escherichia coli* was the most predominant isolate from the urine (56%), sterile site (32%), others (23%), blood and superficial infection (18%) and CSF (11%). *Klebsiella pneumoniae* was the most predominant isolate in the lower respiratory tract (28%), blood and others (17%), urine (16%), deep infection (DI) and sterile sites (SS) (15%), superficial infection (SI)and CSF (13%). *Proteus mirabilis* was common in 5% of deep and 3% of superficial infections and other specimens (2%). *Enterobacter cloacae* constituted 3% of deep infections, 2% of superficial infection and blood infections. *Klebsiella species* constituted 3% of sterile site infections (SS) (Figure 1.3b). Geographic area wise distribution showed that isolates from the eastern India had higher percentage isolate rate of *Klebsiella pneumoniae* than the rest of India (Table 1.4a and Figure 1.4a).

Salmonella constituted 1% of the total isolates (1123 out of 107387). *Salmonella Typhi* isolated from the blood constituted 76% of total *Salmonella* isolates, followed by *Salmonella paratyphi A* (16%) and other *Salmonella spp* (8%) (Table 1.5a & Figure 1.5a). Geographically, the predominant total *Salmonella* isolation percentage was seen highest in central India (8.3%) followed by western India (7.9%). On the contrary, *Salmonella Typhi* isolate percentage was more in western India (5.9%) as compared to central India (5.4%). There was insignificant percentage difference in the isolation of total *Salmonella* and *Salmonella Typhi* from the northern and southern part of India with total *Salmonella* percentage around 5% and *Salmonella Typhi* around 4% (Figure 1.5b)

Non-fermenting Gram negative bacteria (NFGNB) constituted 22% of the total isolates (23684 out of 107387) (Table 1.1). Among the NFGNB, *Pseudomonas aeruginosa* was the commonest isolate (12%) followed by *Acinetobacter baumannii complex (*9%*). Stenotrophomonas maltophilia* and *Burkholderia cepacia* accounted for 0.3% and 0.2% of all isolates respectively. *Pseudomonas aeruginosa* was grossly predominant in LRT (23%), superficial infection, miscellaneous and CSF infection (15%), deep infections (14%), blood and urinary percentage isolation rates ranged 5.1 to 5.7%. *Acinetobacter baumannii complex* was the predominant isolate from LRT and CSF (25%), blood (10%), deep infections (9%), and superficial infections (8%) (Table 1.6a and Figure 1.6a). *Pseudomonas aeruginosa* was the predominant isolate of NFGNB among clinical isolates overall and in all geographical areas except central India where *Acinetobacter baumannii* was the predominant isolate (Table 1.6b and Figure 1.6b).

Staphylococci constituted overall 15%of all the isolates (Table 1.1). *Staphylococcus aureus* was the predominant species in the superficial infections (25%), deep infections (21%), miscellaneous infections (12%), sterile body fluids (7%), blood (11%) and urine (2%) (Table 1.7a). Coagulase negative Staphylococci (CoNS) were the predominant isolates in blood (12%) and CSF (9%) reflecting the high incidence of shunt infections and intra

vascular device associated infections respectively. In CSF and Blood, *Staphylococcus epidermidis* was more frequent isolate 3% and 2% respectively, reflecting the ability of the species to form biofilms and high incidence of shunt associated and dialysis associated infections. *Staphylococcus saprophyticus* was most common isolate in the urine. Predominant percentage isolation of Methicillin resistant Staphylococcus aureus (MRSA) and Methicillin sensitive Staphylococcus aureus (MSSA) was from the superficial infections (SI) i.e., 10% and 15% respectively. This was followed by isolation from deep infection (DI), 9% and 12% and from blood, 5% and 7% respectively (Figure 1.7a). Amongst the coagulase negative Staphylococci (CoNS), *S. haemolyticus* (23%) and *S. epidermidis* (20%) were the commonest species followed by *S. hominis* (12%) (Table 1.7b). There was predominance of isolation of *Staphylococcus aureus* from eastern India (14%) with MRSA percentage isolation (7%). The least percentage isolation of *Staphylococcus aureus*and MRSA was from western India i.e., 10% and 5% respectively (Table 1.7c and Figure 1.7c)

Enterococci constituted overall 6% of all the isolates (Table 1.1). Among the Enterococcus species, *E. faecalis* and *E. faecium* accounted for 84% of all the total isolates, *E. faecalis* (44%) outnumbered *E. faecium* (40%). *E. faecium* was relatively more frequent in the CSF (4.4%) and urine (4%) while *E. faecalis* was more frequent in the urine (4.8%) and deep infections (3.7%) (Table 1.8a). The relative percentage isolation of *E. faecalis* and *E. faecium* differed in the different geographical areas. *E faecium* was found to be more frequent in eastern India (4.1%) and *E. faecalis* was more frequent in southern India (4.4%) (Table 1.8b, Figure 1.8b).

Table 1.1: Specimen wise distributions of major groups of organisms

Isolate	Culture positive																			
	Total n=107387		Blood n=17108		Urine n=30822		LRT n=15571		Superficial Infection n=25058		Deep Infection n=7053		CSF n=688		SS n=2623		Faeces n=1051		Others n=7413	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
No. culture positive	107387 (100)	100	17108 (100)	15.9	30822 (100)	28.7	15571 (100)	14.5	25058 (100)	23.3	7053 (100)	6.6	688 (100)	0.6	2623 (100)	2.4	1051 (100)	1	7413 (100)	6.9
Enterobacteriaceae (except Salmonella)	57236 (53.3)	100	7055 (41.2)	12.3	24276 (78.8)	42.4	6414 (41.2)	11.2	10628 (42.4)	18.6	3266 (46.3)	5.7	196 (28.5)	0.3	1512 (57.6)	2.6	288 (27.4)	0.5	3601 (48.6)	6.3
Salmonella	1123 (1)	100	940 (5.5)	83.7	9 (0)	0.8	1 (0)	0.1	12 (0)	1.1	7 (0.1)	0.6	5 (0.7)	0.4	4 (0.2)	0.4	140 (13.3)	12.5	5 (0.1)	0.4
NFGNB	23684 (22.1)	100	2955 (17.3)	12.5	2270 (7.4)	9.6	7854 (50.4)	33.2	6086 (24.3)	25.7	1687 (23.9)	7.1	309 (44.9)	1.3	592 (22.6)	2.5	7 (0.7)	0	1924 (26)	8.1
Staphylococci	15785 (14.7)	100	3847 (22.5)	24.4	777 (2.5)	4.9	1008 (6.5)	6.4	7097 (28.3)	45	1569 (22.2)	9.9	113 (16.4)	0.7	227 (8.7)	1.4	0 (0)	0	1147 (15.5)	7.3
Enterococci	6641 (6.2)	100	961 (5.6)	14.5	3225 (10.5)	48.6	48 (0.3)	0.7	1152 (4.6)	17.3	469 (6.6)	7.1	54 (7.8)	0.8	240 (9.1)	3.6	17 (1.6)	0.3	475 (6.4)	7.2
Fungi	2155 (2)	100	1238 (7.2)	57.4	261 (0.8)	12.1	241 (1.5)	11.2	76 (0.3)	3.5	48 (0.7)	2.2	11 (1.6)	0.5	33 (1.3)	1.5	0 (0)	0	247 (3.3)	11.5
Faecal isolates	664 (0.6)	100	16 (0.1)	2.4	4 (0)	0.6	4 (0)	0.6	7 (0)	1.1	7 (0.1)	1.1	0 (0)	0	14 (0.5)	2.1	599 (57)	90.2	13 (0.2)	2

Note:

1. Figures under '%' are for respective rows.
2. Figures in parenthesis ( ) are percentages of 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. **SSI: 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.

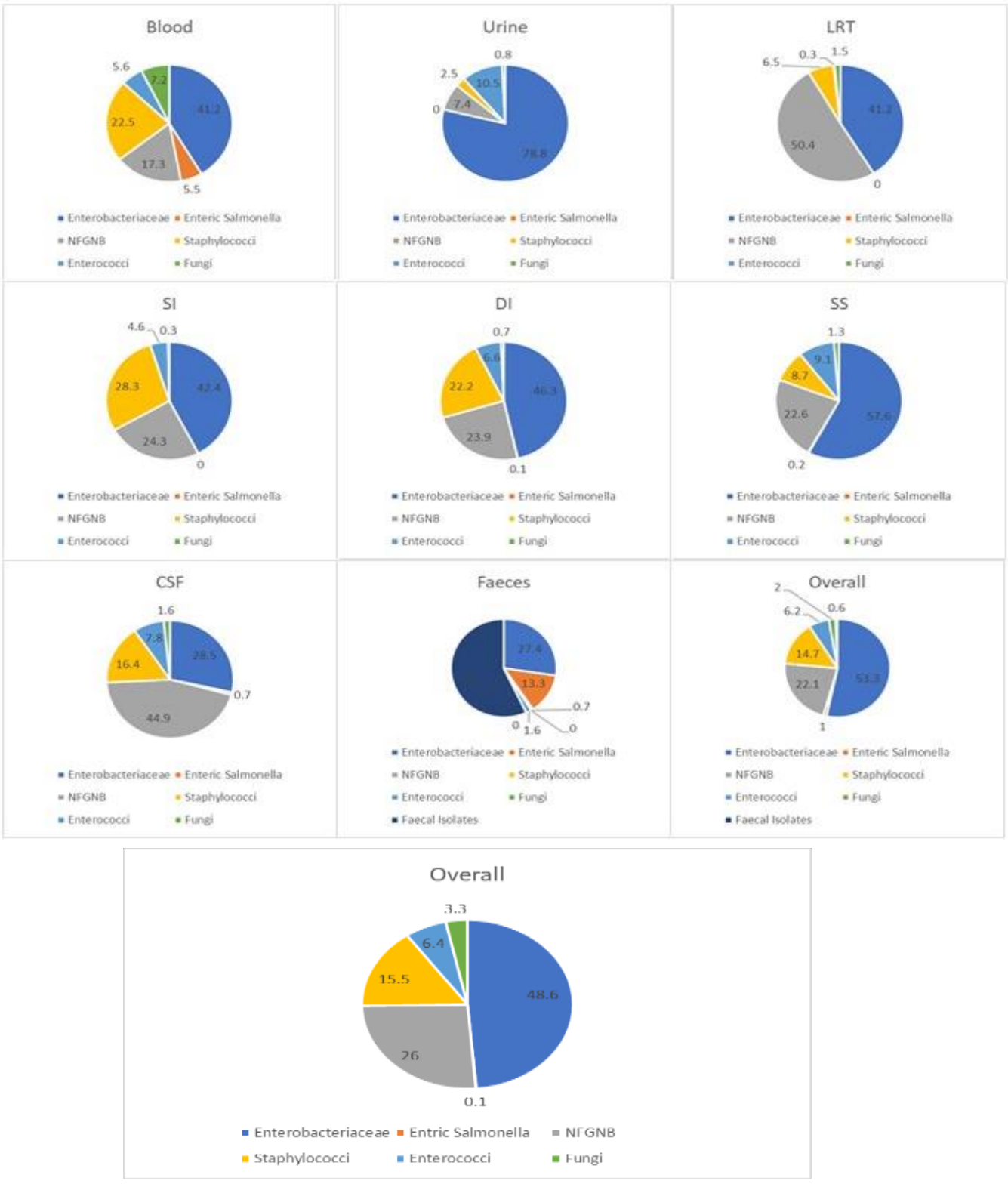


Figure 1.1a: Specimen wise distribution of major groups of organisms (number shown in percentage)

Table 1.2: Distribution of species of organisms in isolates from OPD, ward and ICU

	OPD (n=35753)	Ward (n=55782)	ICU (n=15852)	Overall (n=107387)
E. coli	12731 (35.6)	15373 (27.6)	2367 (14.9)	30471 (28.4)
K. pneumoniae	5240 (14.7)	9567 (17.2)	3483 (22)	18290 (17)
P. aeruginosa	3879 (10.8)	6742 (12.1)	1890 (11.9)	12511 (11.7)
S. aureus	5297 (14.8)	5992 (10.7)	938 (5.9)	12227 (11.4)
*A. baumannii complex**	1195 (3.3)	4594 (8.2)	3740 (23.6)	9529 (8.8)
E. faecalis	1155 (3.2)	1546 (2.8)	188 (1.2)	2889 (2.7)
E. faecium	576 (1.6)	1623 (2.9)	487 (3.1)	2686 (2.5)
Staphylococcus spp	377 (1.1)	901 (1.6)	245 (1.5)	1523 (1.4)
P. mirabilis	724 (2)	1057 (1.9)	166 (1)	1947 (1.8)
Others	4579 (12.8)	8387 (15.0)	2348 (14.8)	15314 (14.3)

**A. baumannii complex* includes *Acinetobacter baumannii* and *A. calcoaceticus*

Note: Figures in parenthesis ( ) are percentages of respective columns.

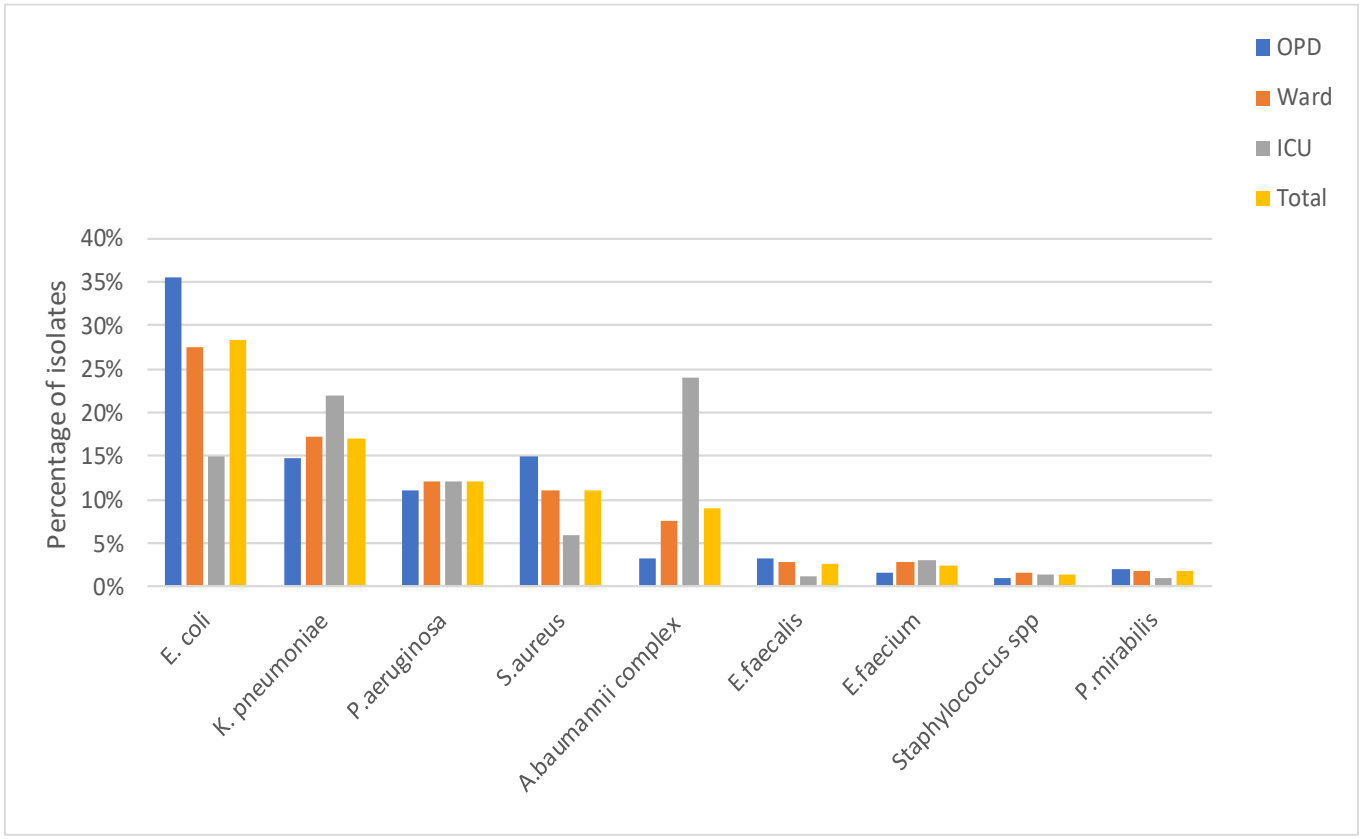


Figure 1.2a: Distribution of species of organisms in isolates of OPD,Ward and ICU

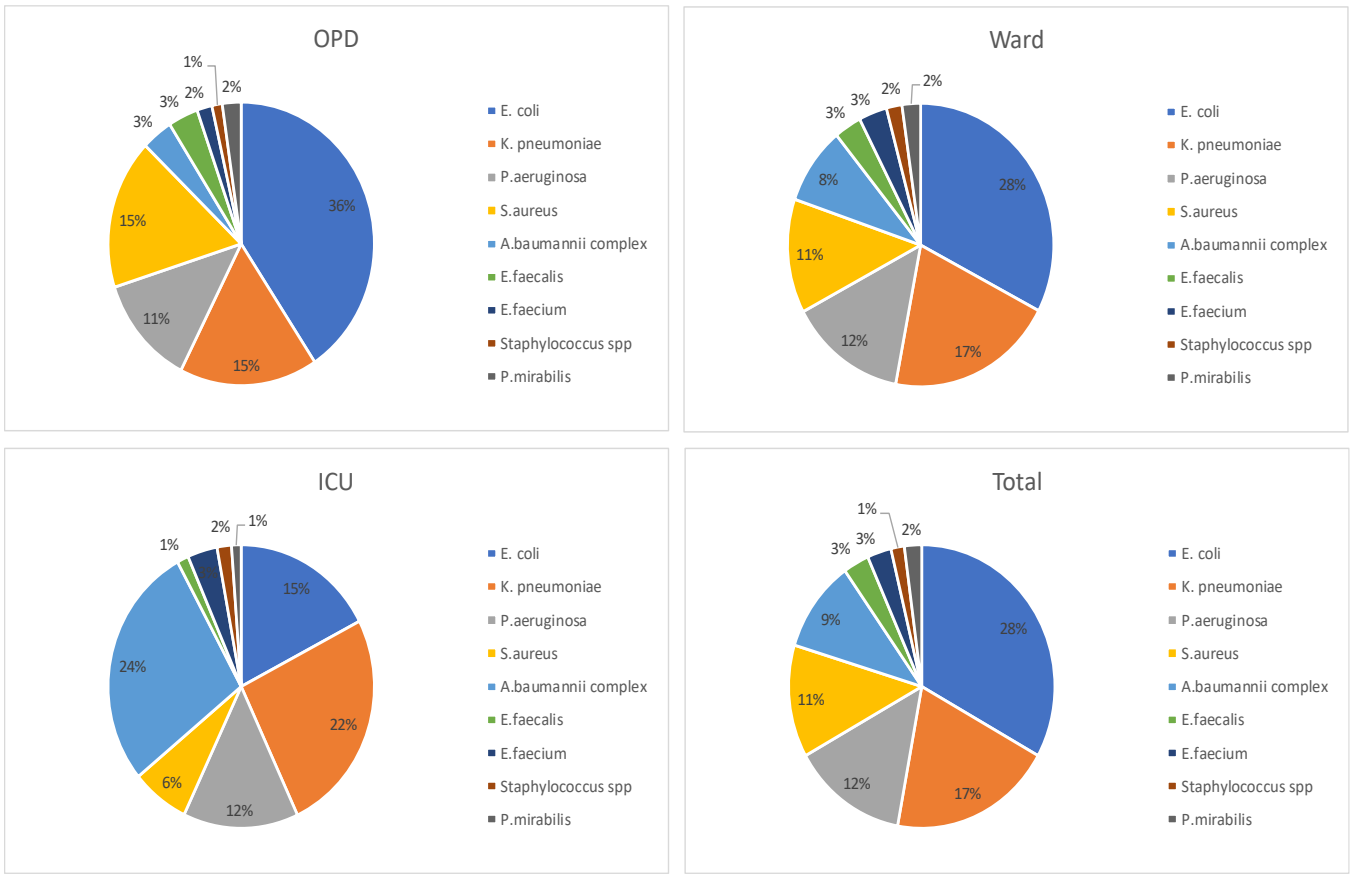


Figure 1.2b: Distribution of species of organisms in isolates of OPD, Ward and ICU

Table 1.3: Specimen wise distributions of major species of family Enterobacteriaceae

Isolate	Culture positive																			
	Total n=107387		Blood n=17108		Urine n=30822		LRT n=15571		Superficial Infection n=25058		Deep Infection n=7053		CSF n=688		SS n=2623		Faeces n=1051		Others n=7413	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Escherichia coli	30471 28.37%	100	3309 19.34%	11	17213 55.85%	57	1130 7.26%	3.7	4688 18.71%	15	1274 18.06%	4.2	73 10.61%	0	828.00 31.57%	3	222 21.12%	1	1734 23.39%	6
Klebsiella pneumonia	18290 17.03%	100	2834 16.57%	16	5003 16.23%	27	4368 28.05%	24	3279.0 13.09%	18	1036 14.69%	5.7	91 13.23%	1	402 15.33%	2	48 4.57%	0	1229 16.58%	7
Proteus mirabilis	1947 1.81%	100	80 0.47%	4.1	462 1.50%	24	106 0.68%	5.4	731 2.92%	38	341 4.83%	18	3 0.44%	0	44 1.68%	2	4 0.38%	0	176 2.37%	9
Enterobacter cloacae	1479 1.38%	100	270 1.58%	18	268 0.87%	18	118 0.76%	8	489 1.95%	33	190 2.69%	13	4 0.58%	0	37 1.41%	3	3 0.29%	0	100 1.35%	7
Klebsiella spp.	849 0.79%	100	65 0.38%	7.7	148 0.48%	17	179 1.15%	21	276 1.10%	33	16 0.23%	1.9	6 0.87%	1	83 3.16%	10	1 0.10%	0	75 1.01%	9
Citrobacter koseri	654 0.61%	100	36 0.21%	5.5	291 0.94%	45	45 0.29%	6.9	191 0.76%	29	50 0.71%	7.6	0 0.00%	0	8 0.30%	1	1 0.10%	0	32 0.43%	5
Morganella morganii	503 0.47%	100	32 0.19%	6.4	148 0.48%	29	11 0.07%	2.2	174 0.69%	35	97 1.38%	19	1 0.15%	0	9 0.34%	2	2 0.19%	0	29 0.39%	6
Serratia marcescens	419 0.39%	100	137 0.80%	33	57 0.18%	14	104 0.67%	25	53 0.21%	13	26 0.37%	6.2	2 0.29%	1	13 0.50%	3	0 0.00%	0	27 0.36%	6
Providencia rettgeri	142 0.13%	100	8 0.05%	5.6	71 0.23%	50	10 0.06%	7	25 0.10%	18	19 0.27%	13	0 0.00%	0	3 0.11%	2	0 0.00%	0	6 0.08%	4

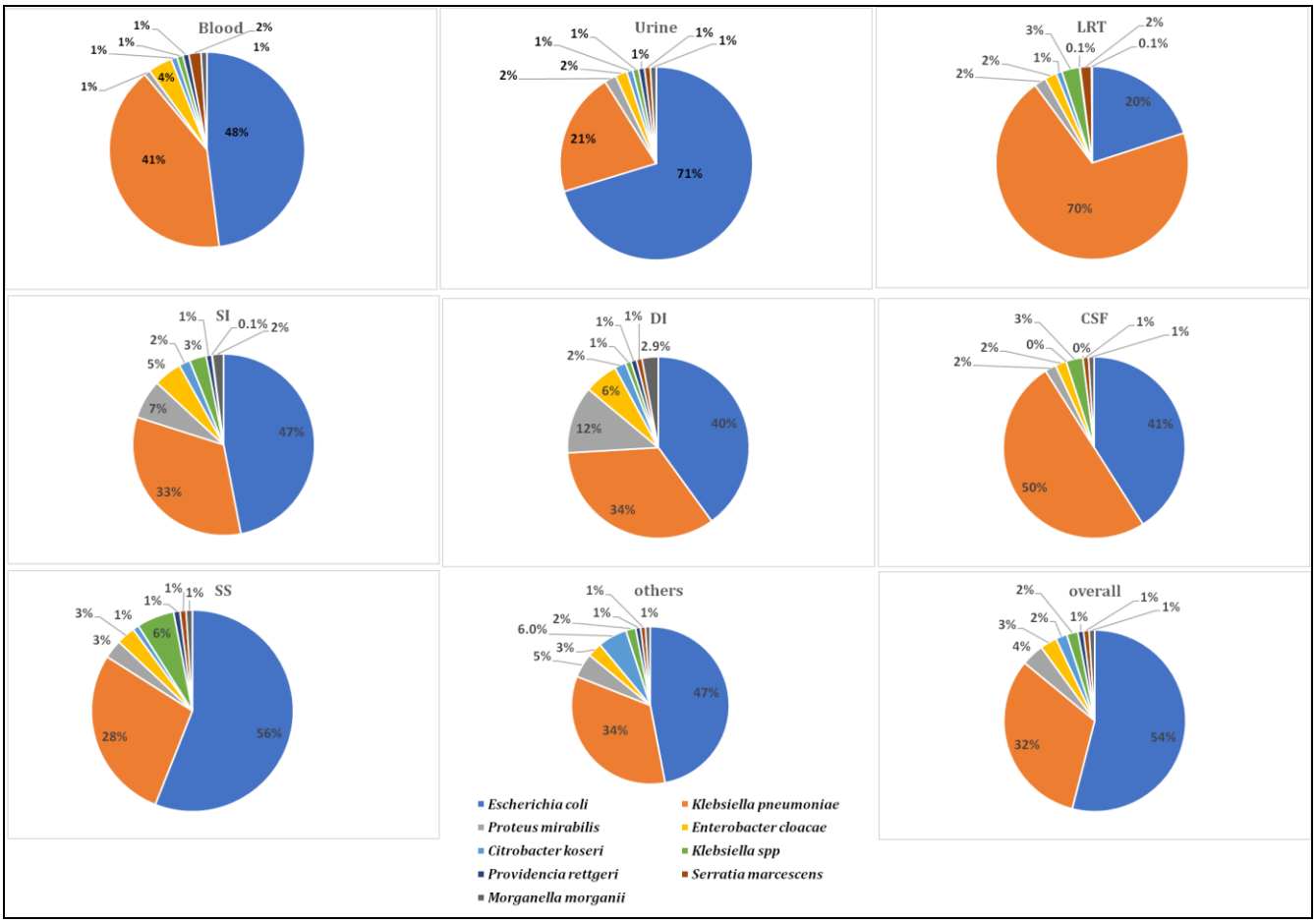


Figure 1.3a: Specimen wise distribution of major species of family Enterobacteriaceae (Percentage calculated from total of Enterobacteriaceae isolates)

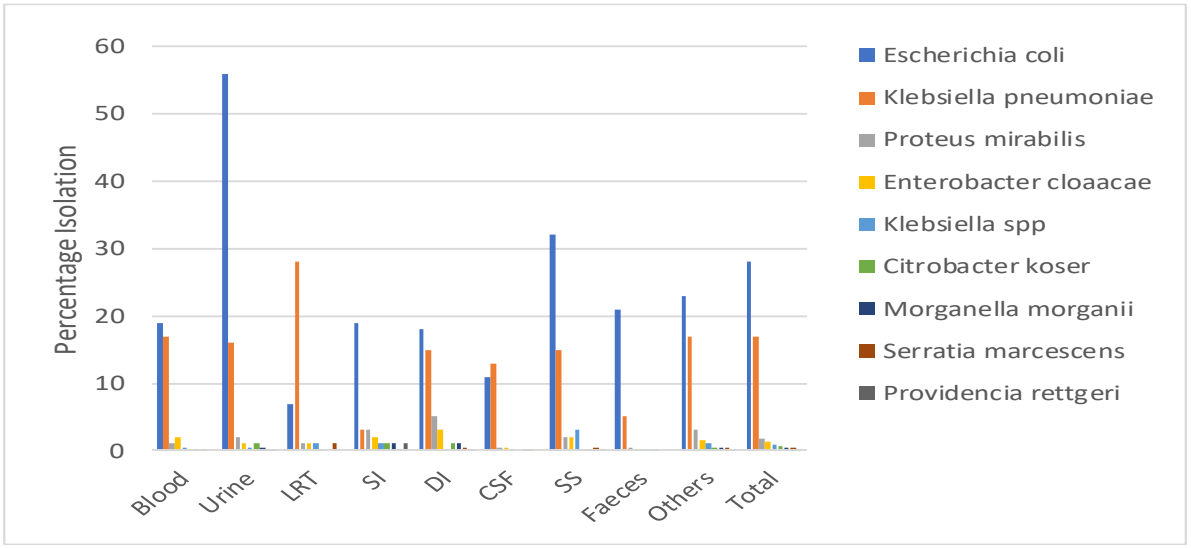


Figure 1.3b: Specimen wise distribution of major species of family Enterobacteriaceae

Table 1.4a: Geographical area wise distribution of major species of family Enterobacteriaceae in Total (except Faeces) specimen type

Organism	National (n=106336)		North (n=27954)		Central (n=4851)		East (n=12855)		West (n=21315)		South (n=39361)	
	n (%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Enterobacteriaceae	56948 (53.6)	(37.3-66.6)	13509 (48.3)	(37.3-59.4)	2592 (53.4)	(44.6-59.9)	7594 (59.1)	(51.7-62.1)	11492 (53.9)	(44.2-64.4)	21761 (55.3)	(41.4-66.6)
Escherichia coli	30249 (28.4)	(13.8-43.8)	6910 (24.7)	(17.4-32.8)	1286 (26.5)	(13.8-35.9)	3537 (27.5)	(22.3-41.3)	6250 (29.3)	(20.1-36.5)	12266 (31.2)	(21-43.8)
Klebsiella pneumoniae	18242 (17.2)	(8.8-27)	4972 (17.8)	(10.3-24.4)	761 (15.7)	(15.6-15.9)	2847 (22.1)	(12.5-27)	3116 (14.6)	(8.8-18.7)	6546 (16.6)	(12.6-19.8)
Enterobacter cloacae	1476 (1.4)	(0-5.3)	280 (1)	(0-2.3)	118 (2.4)	(0.3-5.3)	75 (0.6)	(0-2)	186 (0.9)	(0.1-2.3)	817 (2.1)	(0.4-3)
Proteus mirabilis	1943 (1.8)	(0.5-5.2)	527 (1.9)	(0.9-3.6)	145 (3)	(1.3-5.2)	184 (1.4)	(0.7-2.2)	363 (1.7)	(0.7-2.1)	724 (1.8)	(0.5-2.6)
Proteus vulgaris	206 (0.2)	(0-0.8)	45 (0.2)	(0-0.8)	9 (0.2)	(0-0.3)	31 (0.2)	(0-0.7)	86 (0.4)	(0.1-0.7)	35 (0.1)	(0-0.2)
Citrobacter koseri	653 (0.6)	(0-2.2)	86 (0.3)	(0-0.4)	21 (0.4)	(0-0.7)	152 (1.2)	(0.4-2.2)	150 (0.7)	(0-1.8)	244 (0.6)	(0.1-1.2)
Citrobacter freundii	341 (0.3)	(0-1.4)	78 (0.3)	(0.2-0.5)	24 (0.5)	(0.3-0.8)	85 (0.7)	(0.1-1.3)	95 (0.4)	(0-1.4)	59 (0.1)	(0.1-0.2)
Citrobacter spp.	224 (0.2)	(0-2.1)	29 (0.1)	(0-0.3)	14 (0.3)	(0-0.5)	122 (0.9)	(0-2.1)	23 (0.1)	(0-0.5)	36 (0.1)	(0-0.3)

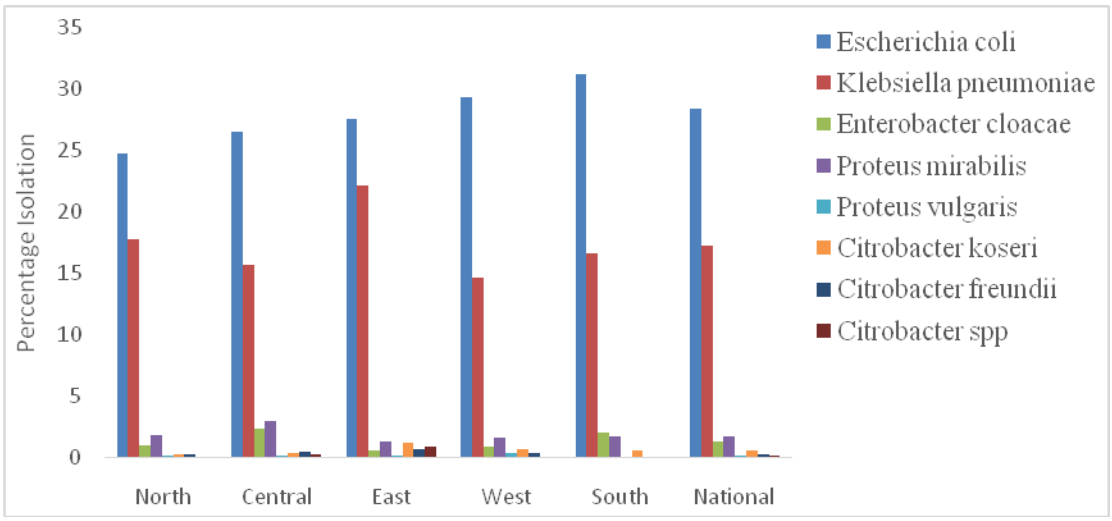


Figure 1.4a: Geographical area wise distribution of major species of family Enterobacteriaceae

Table 1.5a: Species wise distribution of Salmonella species

Isolate	Culture positive					
	Blood n=17108		Faeces n=1051		Others n=89228	
	n	%	n	%	n	%
Salmonella Paratyphi A	147	0.9	1	0.1	10	0
Salmonella Typhi	710	4.2	25	2.4	17	0
Salmonella spp.	83	0.5	114	10.8	16	0
Total Salmonella	940	5.5	140	13.3	43	0

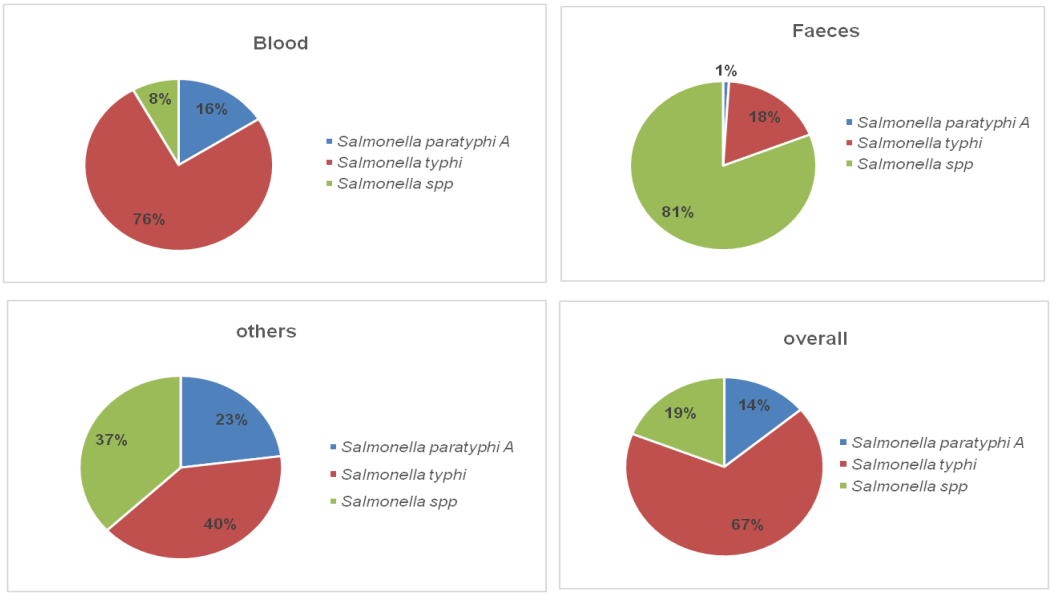


Figure 1.5a Species wise distribution of Salmonella species

Table 1.5b Geographical area wise distribution of Salmonella species from Blood

Organism	National (n=17108)		North (n=4415)		Central (n=551)		East (n=1443)		West (n=2694)		South (n=8005)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Total Salmonella	940 (5.5)	(0-17.1)	241 (5.5)	(0-17.1)	46 (8.3)	(2.2-12.8)	7 (0.5)	(0-1.6)	214 (7.9)	(3.1-14.6)	432 (5.4)	(2.1-9.6)
Salmonella Typhi	710 (4.2)	(0-12.1)	174 (3.9)	(0-12.1)	30 (5.4)	(1.7-8.1)	4 (0.3)	(0-1.6)	160 (5.9)	(2-9.6)	342 (4.3)	(0.7-6.4)

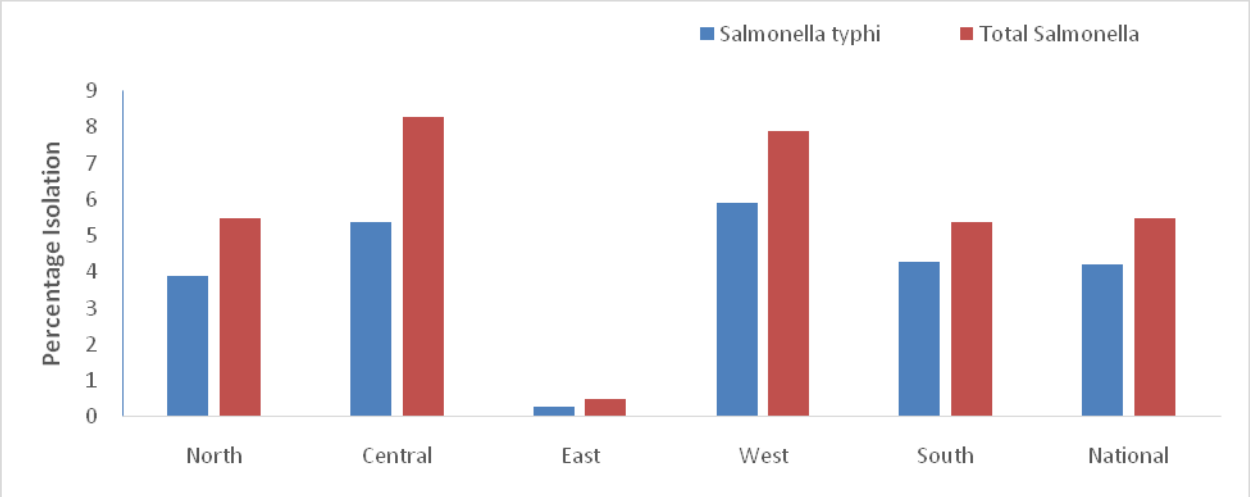


Figure 1.5b: Geographical area wise distribution of Salmonella species from Blood

Table 1.6a: Specimen wise distribution of NFGNB

Isolate	Culture positive																			
	Total n=107387		Blood n=17108		Urine n=30822		LRT n=15571		Superficial Infection n=25058		Deep Infection n=7053		CSF n=688		SS n=2623		Faeces n=1051		Others n=7413	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
NFGNB	23684 (22.1)	100	2955 (17.3)	12.5	2270 (7.4)	9.6	7854 (50.4)	33.2	6086 (24.3)	25.7	1687 (23.9)	7.1	309 (44.9)	1.3	592 (22.6)	2.5	7 (0.7)	0	1924 (26)	8.1
Pseudomonas aeruginosa	12511 (11.7)	100	868 (5.1)	6.9	1754 (5.7)	14	3549 (22.8)	28.4	3867 (15.4)	30.9	955 (13.5)	7.6	102 (14.8)	0.8	276 (10.5)	2.2	4 (0.4)	0	1136 (15.3)	9.1
Acinetobacter baumannii complex	9529 (8.9)	100	1658 (9.7)	17.3	344 (1.1)	3.6	3934 (25.2)	41.3	1900 (7.6)	20	636 (9)	7	173 (25.2)	1.8	244 (9.3)	2.6	2 (0.2)	0	638 (8.6)	6.7
Stenotrophomonas maltophilia	367 (0.3)	100	114 (0.7)	31.1	14 (0)	3.8	141 (0.9)	38.4	33 (0.1)	9	30 (0.4)	8.2	8 (1.2)	2.2	8 (0.3)	2.2	1 (0.1)	0.3	18 (0.2)	4.9
Burkholderia cepacia	180 (0.2)	100	84 (0.5)	46.7	19 (0.1)	10.6	28 (0.2)	15.6	14 (0.1)	7.8	12 (0.2)	6.7	0 (0)	0	6 (0.2)	3.3	0 (0)	0	17 (0.2)	9.4

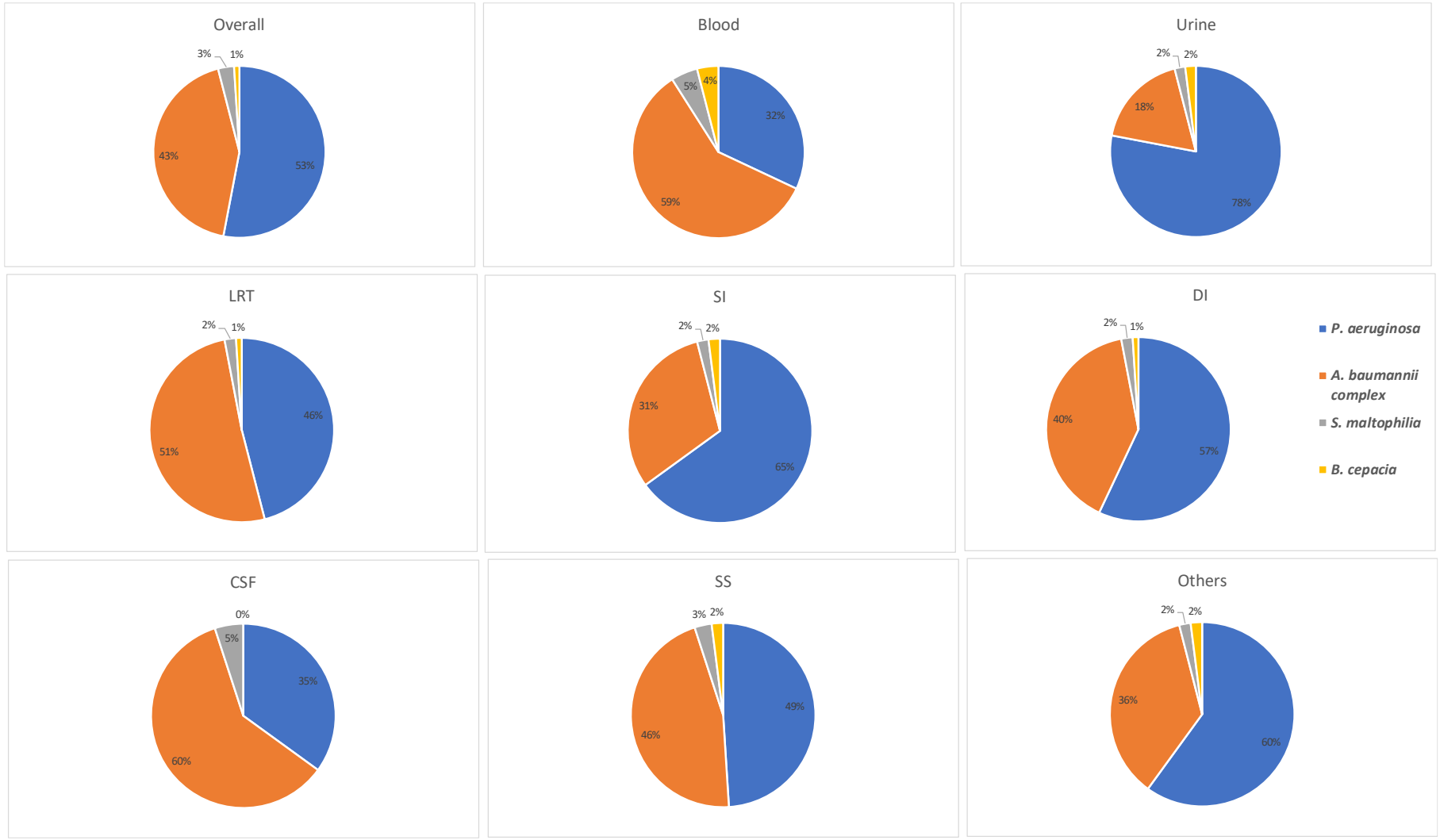


Figure 1.6a: Specimen wise distribution of NFGNB (Percentage calculated from total of NFGNB isolates)

Table 1.6b: Geographical area wise distribution of NFGNB in Total (except faeces)

Organism	National (n=106336)		North (n=27954)		Central (n=4851)		East (n=12855)		West (n=21315)		South (n=39361)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
NFGNB	23677 (22.3)	(9.6-41.5)	7527 (26.9)	(24.1-29.6)	1262 (26)	(14.6-41.5)	1948 (15.2)	(9.6-27)	5201 (24.4)	(17.7-38.4)	7739 (19.7)	(12.8-32)
Pseudomonas aeruginosa	12507 (11.8)	(5.4-19.1)	3779 (13.5)	(11.3-16.2)	500 (10.3)	(6.2-15.9)	1000 (7.8)	(5.4-12.5)	3019 (14.2)	(10.7-19.1)	4209 (10.7)	(7.6-16.8)
Acinetobacter baumannii	8460 (8)	(0-24.7)	2222 (7.9)	(0-13.8)	641 (13.2)	(4.7-24.7)	788 (6.1)	(1.7-16.1)	1675 (7.9)	(2.5-16.5)	3134 (8)	(3.7-14.5)
Acinetobacter spp.	787 (0.7)	(0-4.7)	328 (1.2)	(0-2.9)	85 (1.8)	(0-3)	40 (0.3)	(0-0.6)	270 (1.3)	(0.2-4.7)	64 (0.2)	(0-0.5)
Acinetobacter lwoffii	283 (0.3)	(0-2.6)	54 (0.2)	(0-1.1)	11 (0.2)	(0.1-0.3)	80 (0.6)	(0.4-1.1)	105 (0.5)	(0-2.6)	33 (0.1)	(0-0.2)

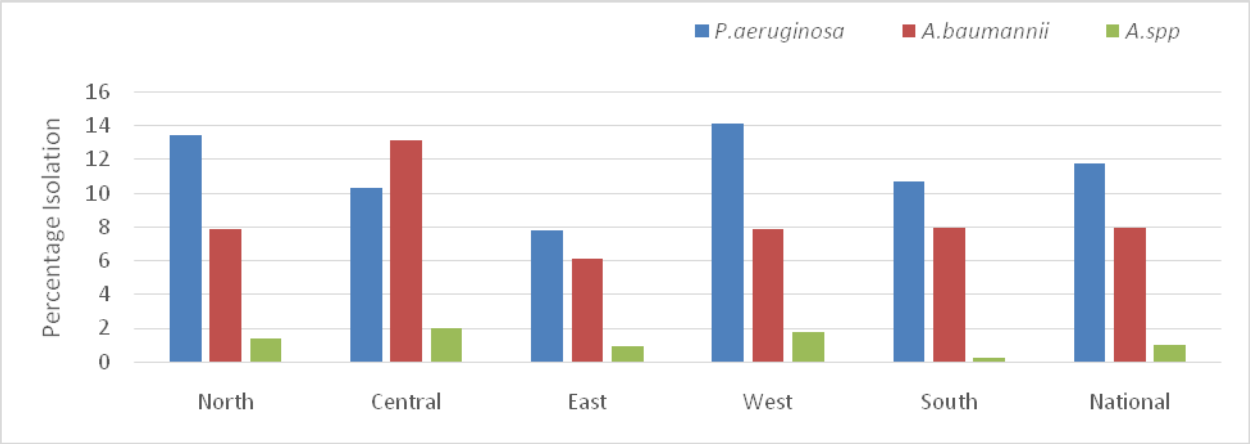


Figure 1.6b: Geographical area wise distribution of NFGNB in Total

Table 1.7a: Specimen wise relative distribution of *S. aureus* and CoNS species

Isolate	Culture positive																			
	Total n=107387		**Blood n=17108**		**Urine n=30822**		**LRT n=15571**		**Superficial Infection n=25058**		**Deep Infection n=7053**		**CSF n=688**		**SS n=2623**		**Faeces n=1051**		**Others n=7413**	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total Staphylococci	15785 (14.7)	100	3847 (22.5)	24.4	777 (2.5)	4.9	1008 (6.5)	6.4	7097 (28.3)	45	1569 (22.2)	9.9	113 (16.4)	0.7	227 (8.7)	1.4	0 (0)	0	1147 (15.5)	7.3
Staphylococcus aureus	12227 (11.4)	100	1806 (10.6)	14.8	582 (1.9)	4.8	964 (6.2)	7.9	6271 (25)	51.3	1463 (20.7)	12	51 (7.4)	0.4	173 (6.6)	1.4	0 (0)	0	917 (12.4)	7.5
MSSA	6978 (6.5)	100	986 (5.8)	14.1	320 (1)	4.6	522 (3.4)	7.5	3624 (14.5)	51.9	832 (11.8)	11.9	19 (2.8)	0.3	101 (3.9)	1.4	0 (0)	0	574 (7.7)	8.2
MRSA	5147 (4.8)	100	810 (4.7)	15.7	257 (0.8)	5	433 (2.8)	8.4	2597 (10.4)	50.5	618 (8.8)	12	30 (4.4)	0.6	69 (2.6)	1.3	0 (0)	0	333 (4.5)	6.5
CoNS	3558 (3.3)	100	2041 (11.9)	57.4	195 (0.6)	5.5	44 (0.3)	1.2	826 (3.3)	23.2	106 (1.5)	3	62 (9)	1.7	54 (2.1)	1.5	0 (0)	0	230 (3.1)	6.5
Staphylococcus haemolyticus	803 (0.7)	100	526 (3.1)	65.5	13 (0)	1.6	8 (0.1)	1	169 (0.7)	21	27 (0.4)	3.4	13 (1.9)	1.6	9 (0.3)	1.1	0 (0)	0	38 (0.5)	4.7
Staphylococcus epidermidis	701 (0.7)	100	350 (2)	49.9	35 (0.1)	5	8 (0.1)	1.1	189 (0.8)	27	32 (0.5)	4.6	21 (3.1)	3	11 (0.4)	1.6	0 (0)	0	55 (0.7)	7.8

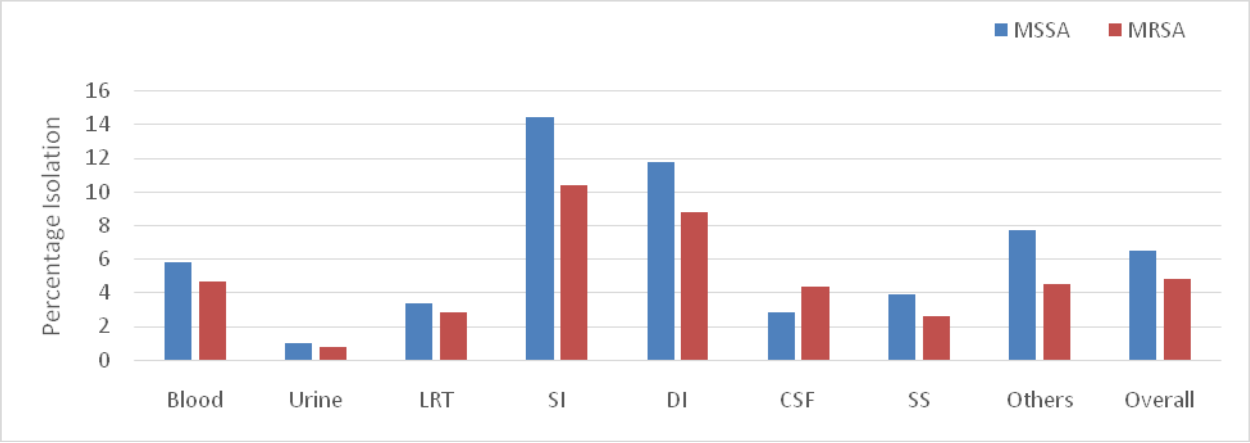


Figure1.7a: Specimen wise relative distribution of MSSA and MRSA

Table 1.7b: Specimen wise relative distribution of CoNS species

Isolate	Culture positive																			
	Total n=107387		Blood n=17108		Urine n=30822		LRT n=15571		Superficial Infection n=25058		Deep Infection n=7053		CSF n=688		SS n=2623		Faeces n=1051		Others n=7413	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
CoNS	3558 (3.3)	100	2041 (11.9)	57.4	195 (0.6)	5.5	44 (0.3)	1.2	826 (3.3)	23.2	106 (1.5)	3	62 (9)	1.7	54 (2.1)	1.5	0 (0)	0	230 (3.1)	6.5
Staphylococcus spp.	1523 (1.4)	100	762 (4.5)	50	118 (0.4)	7.7	27 (0.2)	1.8	413 (1.6)	27.1	37 (0.5)	2.4	17 (2.5)	1.1	28 (1.1)	1.8	0 (0)	0	121 (1.6)	7.9
Staphylococcus haemolyticus	803 (0.7)	100	526 (3.1)	65.5	13 (0)	1.6	8 (0.1)	1	169 (0.7)	21	27 (0.4)	3.4	13 (1.9)	1.6	9 (0.3)	1.1	0 (0)	0	38 (0.5)	4.7
Staphylococcus epidermidis	701 (0.7)	100	350 (2)	49.9	35 (0.1)	5	8 (0.1)	1.1	189 (0.8)	27	32 (0.5)	4.6	21 (3.1)	3	11 (0.4)	1.6	0 (0)	0	55 (0.7)	7.8
Staphylococcus hominis	429 (0.4)	100	365 (2.1)	85.1	2 (0)	0.5	1 (0)	0.2	26 (0.1)	6.1	9 (0.1)	2.1	11 (1.6)	2.6	4 (0.2)	0.9	0 (0)	0	11 (0.1)	2.6
Staphylococcus lugdunensis	76 (0.1)	100	34 (0.2)	44.7	7 (0)	9.2	0 (0)	0	28 (0.1)	36.8	1 (0)	1.3	0 (0)	0	1 (0)	1.3	0 (0)	0	5 (0.1)	6.6
Staphylococcus saprophyticus	26 (0)	100	4 (0)	15.4	20 (0.1)	76.9	0 (0)	0	1 (0)	3.8	0 (0)	0	0 (0)	0	1 (0)	3.8	0 (0)	0	0 (0)	0

Table 1.7c: Geographical area wise relative distribution of *S. aureus*, MSSA, MRSA and CoNS in Total (except faeces)

Organism	National (n=106336)		North (n=27954)		Central (n=4851)		East (n=12855)		West (n=21315)		South (n=39361)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Staphylococci	15785 (14.8)	(6.6-22.1)	4183 (15)	(6.6-20.6)	697 (14.4)	(9.4-18)	2164 (16.8)	(7.2-21)	3245 (15.2)	(11.3-22.1)	5496 (14)	(8.2-18.2)
Staphylococcus aureus	12227 (11.5)	(6.1-19.9)	2948 (10.5)	(6.6-18.6)	574 (11.8)	(9.2-13.8)	1803 (14)	(6.1-19.9)	2130 (10)	(9.2-11.2)	4772 (12.1)	(7.8-14.6)
MSSA	6978 (6.6)	(2.1-10.8)	1482 (5.3)	(3-10.8)	294 (6.1)	(4.2-7.5)	940 (7.3)	(2.1-10.3)	1115 (5.2)	(4.2-7.1)	3147 (8)	(5-10.3)
MRSA	5147 (4.8)	(2.5-9.6)	1423 (5.1)	(3.6-7)	271 (5.6)	(4.7-6.2)	862 (6.7)	(3.6-9.6)	990 (4.6)	(3.9-6.5)	1601 (4.1)	(2.5-5.5)
CoNS	3558 (3.3)	(0-12.7)	1235 (4.4)	(0-11.3)	123 (2.5)	(0.2-4.3)	361 (2.8)	(1-6.3)	1115 (5.2)	(0.2-12.7)	724 (1.8)	(0-7.4)

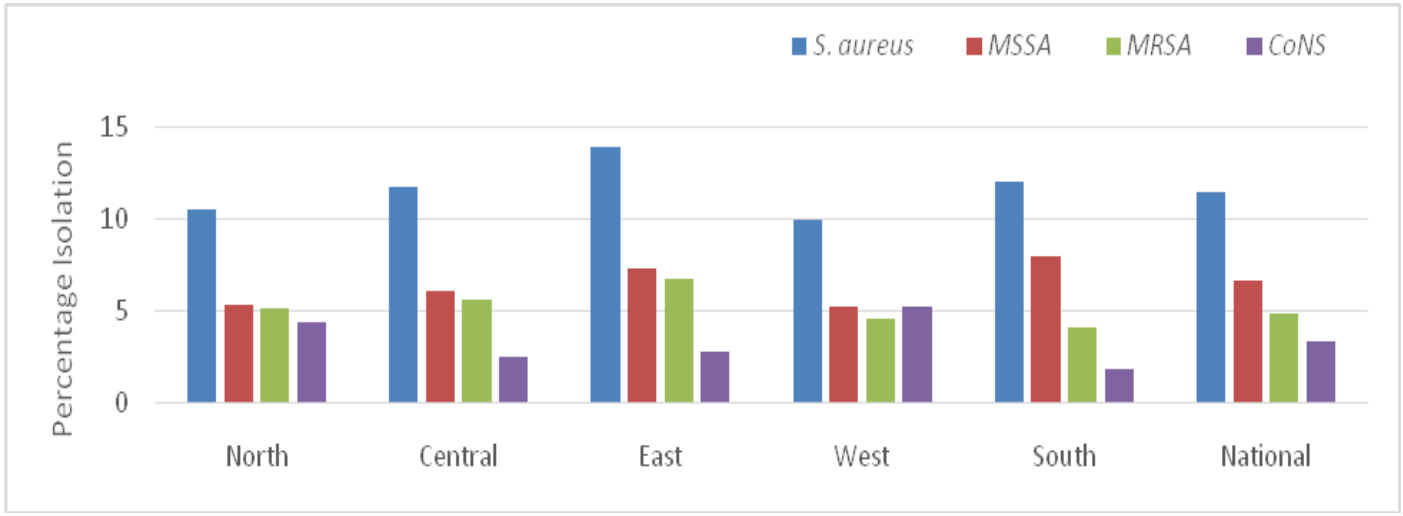


Figure 1.7c: Geographical area wise relative distribution of *S.aureus*, MSSA, MRSA and CoNS

Table 1.8a: Specimen wise distribution of Enterococcus species

Isolate	Culture positive																			
	Total n=107387		Blood n=17108		Urine n=30822		LRT n=15571		Superficial Infection n=25058		Deep Infection n=7053		CSF n=688		SS n=2623		Faeces n=1051		Others n=7413	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterococci	6641 (6.2)	100	961 (5.6)	14.5	3225 (10.5)	48.6	48 (0.3)	0.7	1152 (4.6)	17.3	469 (6.6)	7.1	54 (7.8)	0.8	240 (9.1)	3.6	17 (1.6)	0.3	475 (6.4)	7.2
Enterococcus faecalis	2889 (2.7)	100	301 (1.8)	10.4	1471 (4.8)	50.9	10 (0.1)	0.3	563 (2.2)	19.5	261 (3.7)	9	16 (2.3)	0.6	45 (1.7)	1.6	0 (0)	0	222 (3)	7.7
Enterococcus faecium	2686 (2.5)	100	540 (3.2)	20.1	1245 (4)	46.4	19 (0.1)	0.7	450 (1.8)	16.8	149 (2.1)	5.5	30 (4.4)	1.1	85 (3.2)	3.2	13 (1.2)	0.5	155 (2.1)	5.8
Enterococcus spp.	1066 (1)	100	120 (0.7)	11.3	509 (1.7)	47.7	19 (0.1)	1.8	139 (0.6)	13	59 (0.8)	5.5	8 (1.2)	0.8	110 (4.2)	10.3	4 (0.4)	0.4	98 (1.3)	9.2

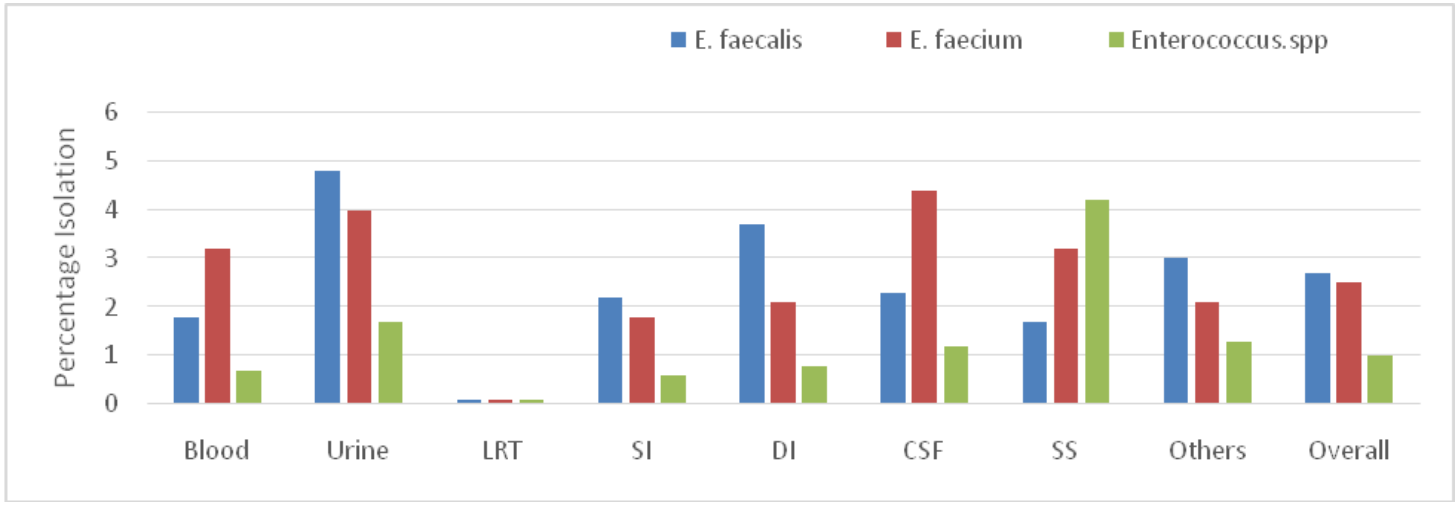
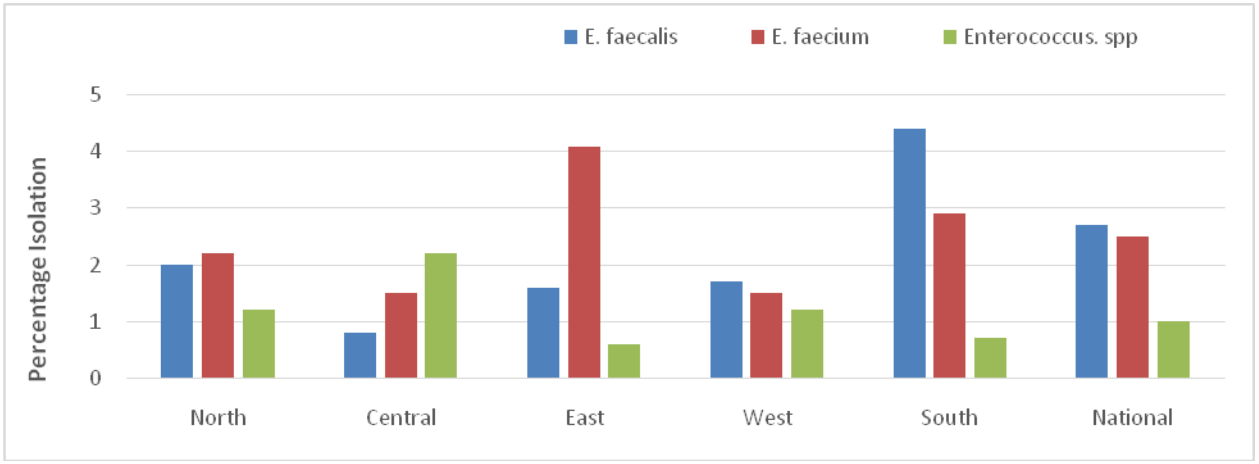


Figure 1.8a: Specimen wise distribution of Enterococcus species

Table 1.8b: Geographical area wise relative frequencies of the common species of enterococci in Total (except Faeces)

Organism	National (n=106336)		North (n=27954)		Central (n=4851)		East (n=12855)		West (n=21315)		South (n=39361)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Enterococci	6624 (6.2)	(0.1-12.6)	1521 (5.4)	(0.1-12.6)	219 (4.5)	(2.6-6)	808 (6.3)	(2.8-8.9)	911 (4.3)	(1.2-7.9)	3165 (8)	(4.5-11.1)
Enterococcus faecalis	2889 (2.7)	(0.1-7.9)	557 (2)	(0.1-4.7)	40 (0.8)	(0.7-0.9)	208 (1.6)	(1.3-2)	352 (1.7)	(0.5-2.4)	1732 (4.4)	(0.8-7.9)
Enterococcus faecium	2673 (2.5)	(0-7.3)	624 (2.2)	(0-3.6)	73 (1.5)	(1.3-1.8)	524 (4.1)	(0.4-7.3)	313 (1.5)	(0.3-2.7)	1139 (2.9)	(1.3-4.3)
Enterococcus spp.	1062 (1)	(0-6.3)	340 (1.2)	(0-5.3)	106 (2.2)	(0-3.8)	76 (0.6)	(0.3-1)	246 (1.2)	(0-6.3)	294 (0.7)	(0-2.2)



1.8b: Geographical area wise relative frequencies of the common species of enterococci

Chapter 2 Enterobacteriaceae

Species wise susceptibility of Enterobacteriaceae isolated from of all specimens except urine and faeces.

During annual reporting period ending 2019, a total of 32,672significant clinical isolates belonging to various genera and species of family *Enterobacteriaceae* from 21 participating centers were included in the analysis. The isolates belonged to various specimens including blood (7055), sterile body fluids including cerebrospinal fluid (1708), pus, wound swabs and aspirates collected from superficial and deep infections (13,894) and lower respiratory tract specimens (6414).

Significant clinical isolates from all specimens (except urine and faeces) 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 2.1 and figure 2.1. Colistin susceptibility overall was 96%;*E. coli* and *Citrobacter* species showed 100% susceptibility followed by *Klebsiella pneumoniae* and *Enterobacter* species showing 92-99% susceptibility. All 45 isolates of *Klebsiella oxytoca* were susceptible to colistin.

Out of the carbapenems, overall, imipenem and meropenem showed 55% and 65% susceptibility with ertapenem showing 60% susceptibility. *Serratia marcescens* (93%), *Proteus vulgaris* (90%), *Proteus mirabilis* (86%), *Morganella morganii*(86%), *Enterobacter cloacae* (79%) and *E. coli* (75%) showed higher susceptibility to meropenem than *Klebsiella pneumoniae* (50%), *Citrobacter* (55-74%) and *Providencia* species (65-67%). Ertapenem susceptibility reflected the same pattern though imipenem susceptibility was slightly lower than that of meropenem.

Piperacillin-tazobactam susceptibility was overall 51%. Maximum susceptibility was found in *Proteus* species (92-94%), *Morganella morganii* (84%) and *Serratia marcescens* (83%) and minimum in *Klebsiella* species (39-52%), *Citrobacter* species (49-69%) and *E. coli* (55%). Overall, only one third (32-33%) of isolates showed fluoroquinolone susceptibility. *Serratia marcescens* showed the maximum susceptibility to fluoroquinolones (81-83%) followed by *Enterobacter cloacae* (70-71%) and *Proteus vulgaris* (61-63%). *E.*

coli showed the lowest susceptibility to fluoroquinolones (19-21%). Ciprofloxacin and levofloxacin showed similar patterns of resistance for all species tested.

Third generation cephalosporins, cefotaxime and ceftazidime showed comparable susceptibility in 22% and 26% of isolates overall. *Proteus vulgaris* (58-66%) showed moderate susceptibility followed by *Serratia marcescens* (57-62%) and *Morganella morganii* (54-55%).Overall, two thirds (65%) of the isolates were susceptible to amikacin. *Morganella morganii* showed 90% susceptibility followed by *Serratia marcescens* (89%), *Proteus vulgaris* (82%), *Enterobacter cloacae* (80%) and *E. coli* (79%). *Klebsiella* species showed the lowest susceptibility (45-50%) except *K. oxytoca* (62%).

Comparison of susceptibility of isolates from OPD, ward and ICU

Overall, for all drugs tested, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* and *Citrobacter* species 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 2.2.1 to 2.2.4, figures 2.2.1 to2.2.4). The differences were more marked for *Klebsiella* species and *Enterobacter* species, and least for *Citrobacter* species.

Yearly isolation trend of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii* and *Klebsiella (Enterobacter) aerogenes*

Yearly isolation trend of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii* and *Klebsiella (Enterobacter) aerogenes* from all samples (except faeces and urine) showed a steady increase of *Klebsiella pneumoniae* from 13.9% in 2016 to 17.5% in 2019 (Table 2.3, Figure 2.3) without much change in the isolation of the other species.

Susceptibility trends of various species over time.

Over the period of study, imipenem susceptibility of *E. coli* dropped steadily from 86% in 2016 to 63% in 2019 (table 2.4.1, figure 2.4.1) and that of *Klebsiella pneumoniae* dropped steadily from 65% in 2016 to 46% in 2019 (table 2.4.2, figure 2.4.2). The drop in meropenem susceptibility was modest and inconsistent. Piperacillin-tazobactam susceptibility of *Citrobacter* species dropped from 65% in 2016 to 60% in 2019 (table 2.4.3, figure 2.4.3). There was an increase in susceptibility to amikacin from 53% in 2016 to 67% in 2019 and to ciprofloxacin from 37% in 2016 to 58% in 2019. There was an increase in susceptibility of *Enterobacter* species to ciprofloxacin from 46% in 2016 to 63% in 2019 (Table 2.4.4, Figure 2.4.4). Susceptibility to other antibiotics didn't show much change.

Clinical implications

The relative frequencies of isolation of various species and their susceptibility trends have an important role in deciding empiric antibiotic policies in hospitals. The trends of change in susceptibility indicate behavior of organisms 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 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 is may have unpredictable outcome and therefore should be highly restricted.

Carbapenem resistance was very high in *Klebsiella pneumoniae, Citrobacter species* and *Providencia* species and moderately high in *E. coli* isolates. Carbapenems have been mainstay in empiric therapy in tertiary care ICU settings. Though there was good susceptibility in *Serratia marcescens* (93%), *Proteus vulgaris* (90%) *Proteus mirabilis* (86%), *Morganella morganii* (86%), *Enterobacter cloacae* (79%) and *E. coli* (75%), the efficacy of this drug as empiric therapy protocol should depend on relative distribution of the various species in the particular set up. This also demands regular surveillance of carbapenem resistant *Enterobacteriaceae* by molecular detection of various genes.

Piperacillin-tazobactam susceptibility overall was alarmingly low as 51%. Though the drug showed good susceptibility in *Proteus* species (92-94%), *Morganella morganii* (84%) and *Serratia marcescens* (83%) it showed high resistance in commonly isolated species like *Klebsiella* species (susceptibility 39-52%), *Citrobacter* species (susceptibility 49-69%) and *E. coli* (susceptibility 55%) 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 very 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.

The slight increase (from 13.9% in 2016 to 17.5% in 2019) in the yearly prevalence of *Klebsiella pneumoniae* may reflect the higher resistance pattern of the species. Of the commonly isolated species of *Enterobacteriaceae*, *Klebsiella pneumoniae* showed more resistance than *E. coli* against carbapenems and piperacillin-tazobactam.

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 and that of *Klebsiella pneumoniae* dropping steadily from 65% in 2016 to 46% in 2019. The marginal increase in susceptibility of amikacin may reflect drop in use of the same.

Table2.1: Species wise susceptibility of Enterobacteriaceae isolated from of all specimens except urine and faeces.

	Amikacin		Cefotax		Ceftazid		Cipro		Colistin		Ertapen		Imipen		Levoflox		Meropen		Pip-taz	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
C. freundii	238	59	188	24	175	25	227	49	33	100	186	52	215	48	156	51	233	55	236	49
C. koseri	353	76	316	46	267	46	336	66	25	100	264	73	299	68	216	59	346	74	342	69
Citrobacter spp	166	58	147	23	134	25	172	55	33	100	186	52	215	48	156	51	233	55	236	49
K. oxytoca	266	62	225	25	215	29	267	47	45	100	226	53	249	59	210	49	278	64	271	52
K. pneumoniae	12880	50	11178	21	7827	25	11427	36	2352	93	9620	45	10894	46	7332	35	12026	50	12368	39
Klebsiella spp	603	45	569	18	577	22	540	36	71	99	484	48	506	46	446	39	696	51	687	44
Enterobacter cloacae	1180	80	870	46	627	46	1132	70	186	92	761	79	979	74	422	71	1132	79	1076	69
Enterobacter spp	597	68	547	22	529	32	518	55	109	98	376	73	499	56	392	59	666	73	647	64
K. (E.) aerogenes	153	48	148	29	124	22	150	41	11	91	136	51	148	68	115	37	155	63	150	47
P. mirabilis	1452	65	1116	48	989	48	1315	43			737	87	1104	49	773	39	1469	86	1347	94
P vulgaris	144	82	132	58	74	66	132	61			99	86	119	52	86	63	146	90	144	92
P. rettgeri	70	53	49	31	61	33	67	36			31	77	45	42	33	33	71	65	61	64
P. stuartii	168	53	134	31	139	31	167	38			58	76	87	48	49	41	171	67	140	54
E. coli	12453	79	10569	14	7492	20	11613	21	1595	100	9309	71	10158	63	5987	19	12072	75	12030	55
M. morganii	341	90	281	54	213	55	310	38	58	0	212	89	241	55	441	13	351	86	319	84
S. marcescens	357	89	261	57	207	62	292	81			241	91	174	93	190	83	350	93	226	83
Overall	31421	65	26730	22	19650	26	28665	33	4460	96	22926	60	25932	55	17004	32	30395	65	30280	51

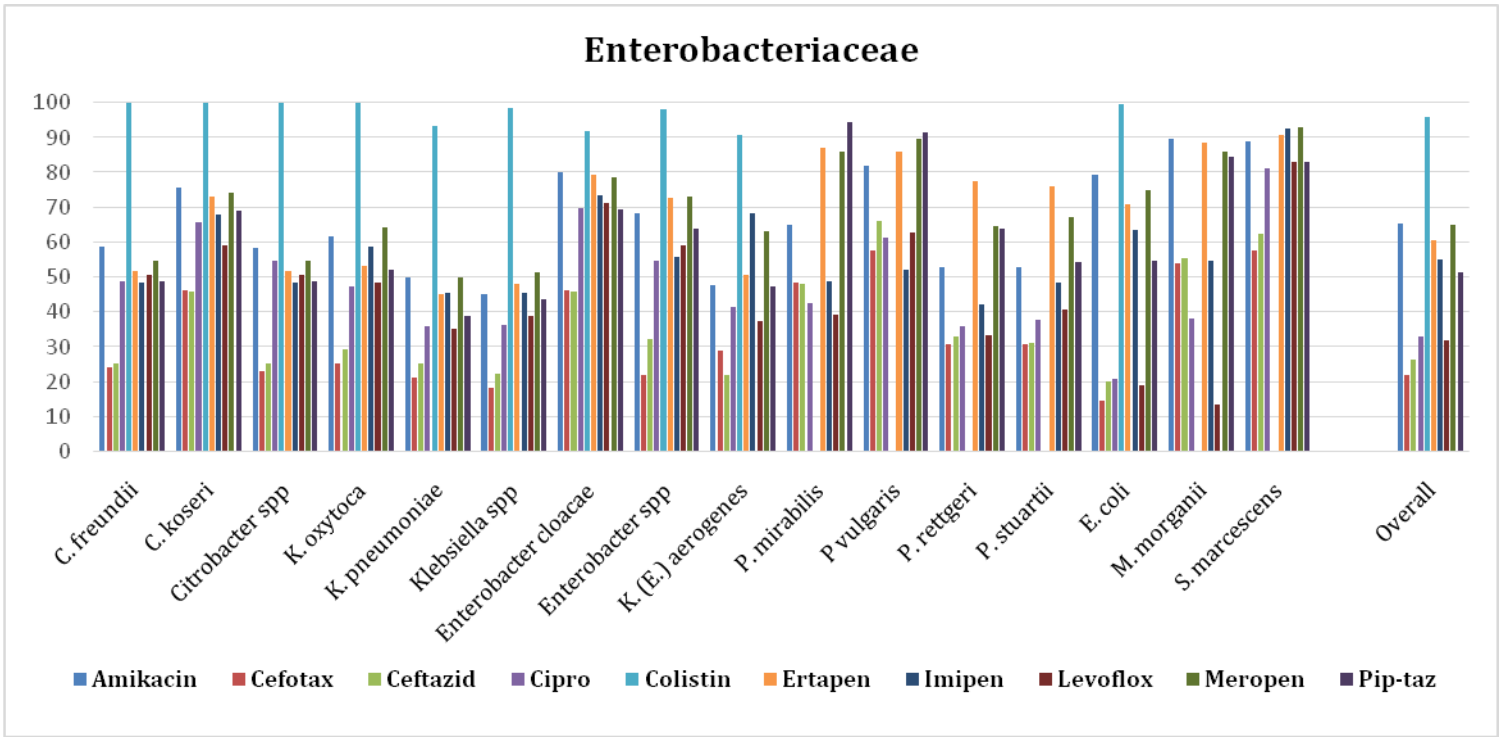


Figure2.1: Species wise susceptibility of Enterobacteriaceae isolated from of all specimens except urine and faeces.

Table2.2.1: 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	2691	84	8103	79	1659	75		12453	79
Cefotaxime	2250	21	7054	13	1265	10		10569	14
Ceftazidime	1592	30	4925	17	975	16		7492	20
Ciprofloxacin	2444	27	7753	20	1416	17		11613	21
Colistin	323	99	953	100	319	100		1595	100
Ertapenem	2058	76	6001	71	1250	62		9309	71
Imipenem	2149	73	6546	62	1463	57		10158	63
Levofloxacin	1325	26	3624	17	1038	15		5987	19
Meropenem	2517	81	8042	74	1513	67		12072	75
Pip-taz	2538	64	7869	53	1623	46		12030	55

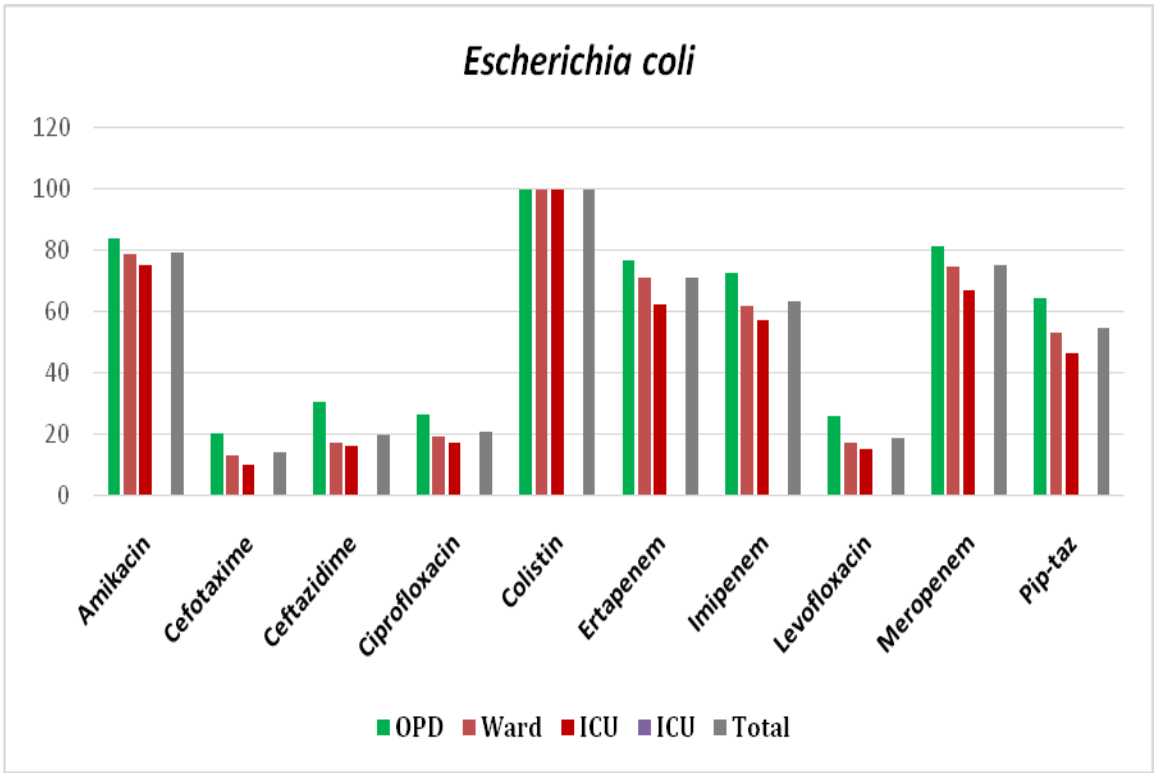


Figure 2.2.1: Comparison of susceptibility of *Escherichia coli* isolated from OPD, ward and ICU.

Table2.2.2: 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	2770	66	7072	51	3036	34	12878	50
Cefotaxime	2499	36	6155	20	2522	10	11176	21
Ceftazidime	1829	45	4313	22	1683	12	7825	25
Ciprofloxacin	2548	54	6584	34	2293	21	11425	36
Colistin	349	97	1266	94	737	91	2352	93
Ertapenem	2083	61	5103	47	2432	27	9618	45
Imipenem	2289	61	5918	47	2685	30	10892	46
Levofloxacin	1622	54	3529	35	2179	21	7330	35
Meropenem	2622	67	6825	50	2577	32	12024	50
Pip-taz	2599	56	6739	40	3028	23	12366	39

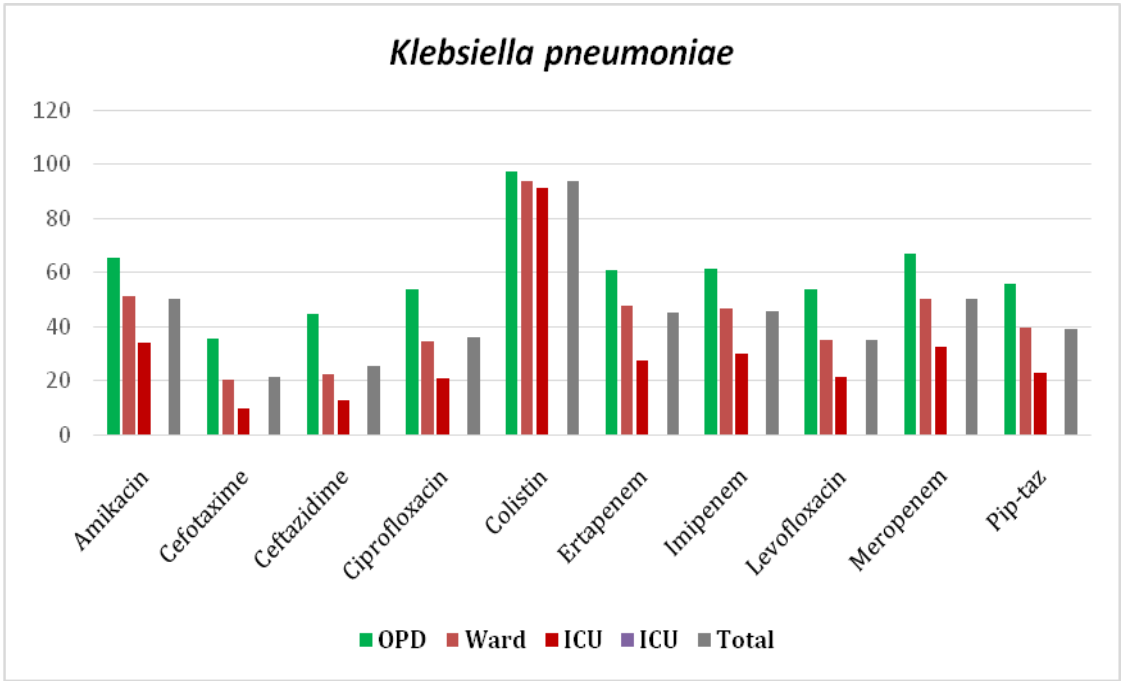


Figure2.2.2: Comparison of susceptibility of *Klebsiella pneumoniae* isolated from OPD, ward and ICU.

Table2.2.3: Comparison of susceptibility of *Citrobacter* spp isolated from OPD, ward and ICU.

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	268	79	428	60	61	57	757	67
Cefotaxime	229	51	385	26	37	19	651	35
Ceftazidime	188	55	338	24	50	32	576	35
Ciprofloxacin	242	76	433	50	60	43	735	58
Colistin	24	100	32	100	8	100	64	100
Ertapenem	209	79	343	55	42	60	594	64
Imipenem	230	73	387	52	56	50	673	59
Levofloxacin	161	74	298	51	51	43	510	58
Meropenem	253	81	442	58	64	58	759	66
Pip-taz	263	79	431	50	60	50	754	60

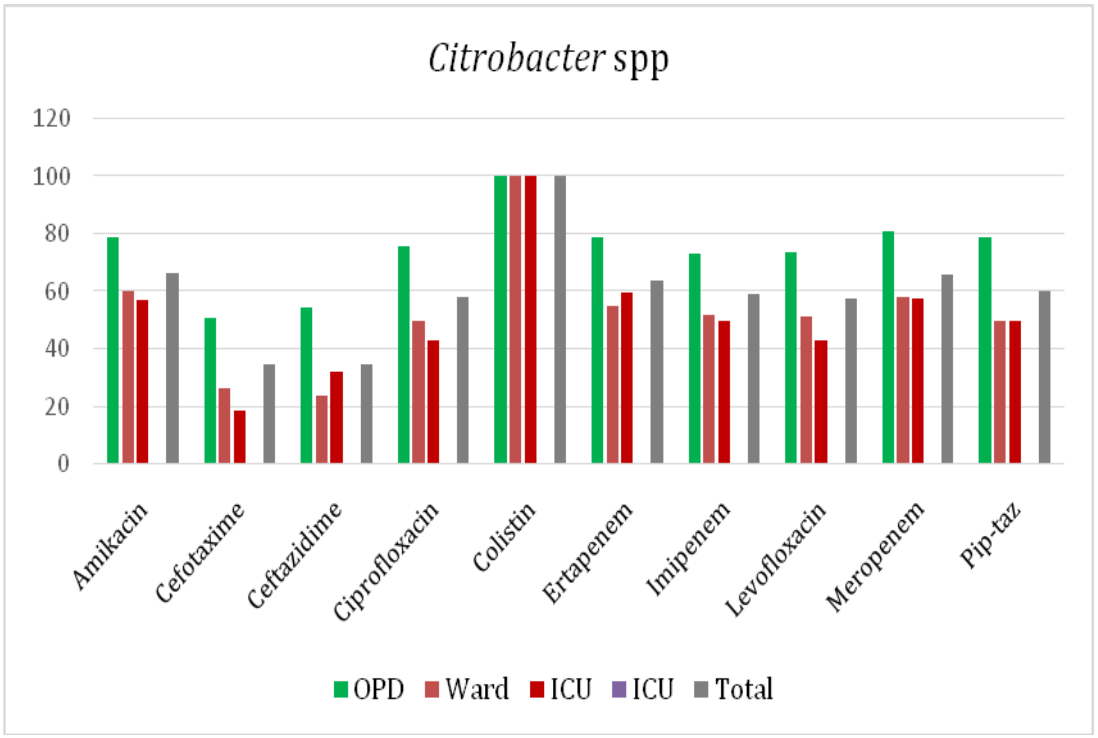


Figure2.2.3: Comparison of susceptibility of *Citrobacter* spp isolated from OPD, ward and ICU.

Table2.2.4: Comparison of susceptibility of *Enterobacter* spp isolated from OPD, ward and ICU.

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	433	88	1139	72	358	63	1930	74
Cefotaxime	349	54	929	33	287	23	1565	36
Ceftazidime	274	59	740	34	266	26	1280	38
Ciprofloxacin	400	80	1104	60	296	52	1800	63
Colistin	52	92	182	95	72	93	306	94
Ertapenem	302	86	746	73	225	62	1273	74
Imipenem	353	83	969	66	304	56	1626	68
Levofloxacin	198	81	496	62	235	46	929	62
Meropenem	431	88	1170	75	352	63	1953	76
Pip-taz	420	83	1106	63	347	54	1873	66

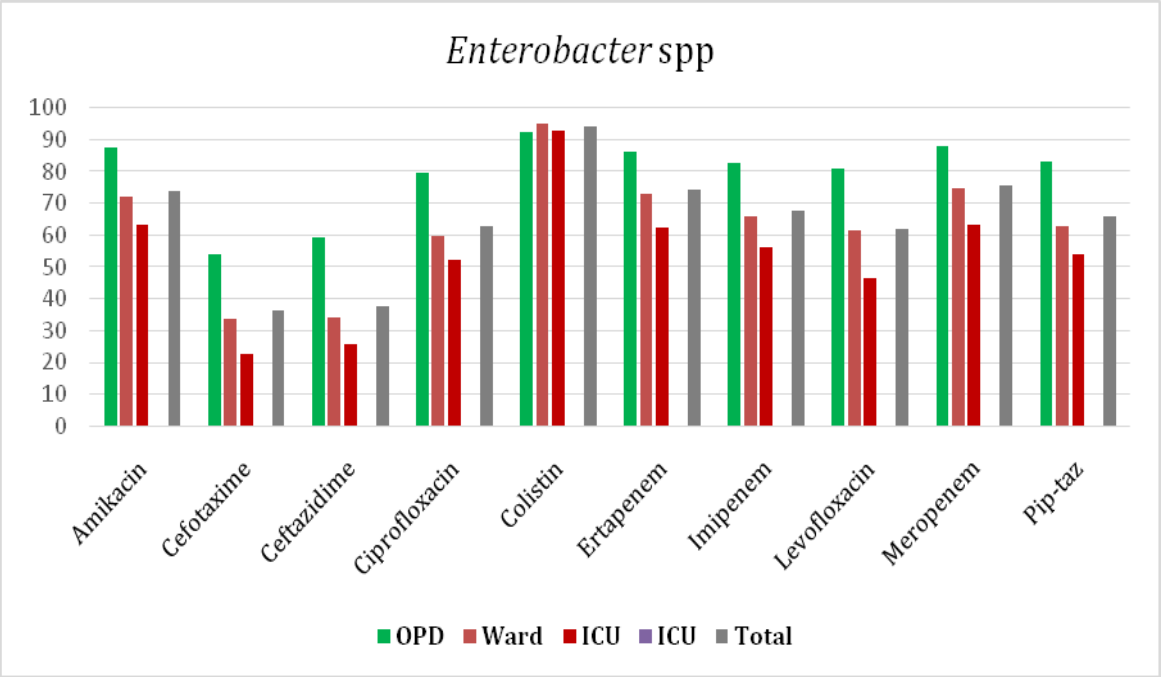


Figure 2.2.4: Comparison of susceptibility of *Enterobacter* spp isolated from OPD, ward and ICU.

Table2.3: Yearly isolation trend of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Morganella morganii* and *Klebsiella (Enterobacter) aerogenes* from all samples (except faeces and urine)

Year	**2016**	**2017**	**2018**	**2019**
Total number	6279	37054	56042	75514
Bacteria	%S	%S	%S	%S
Escherichia coli	16.2	17.0	16.4	17.3
Klebsiella pneumoniae	13.9	14.5	15.0	17.5
Proteus mirabilis	1.9	2.1	1.8	2.0
Morganella morganii	0.2	0.4	0.5	0.5
Klebsiella (Enterobacter) aerogenes	0.2	0.5	0.3	0.2

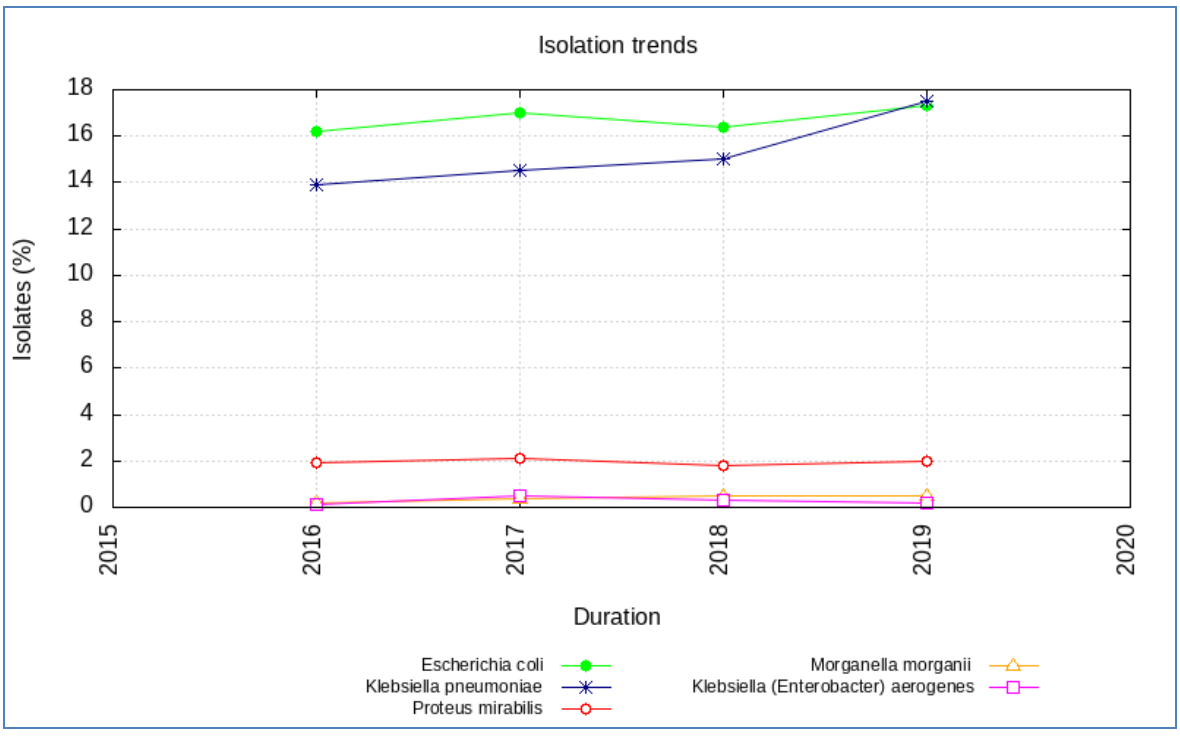


Figure 2.3: Yearly isolation trend of *E coli*, *Klebsellia pnemoniae*, *Proteus mirabilis*, *Morganella morganii* and *Klebsellia (Enterobacter) aerogenes* from all samples (except faeces and urine)

Table 2.4.1: Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%)	Year-2017 (S%)	Year-2018 (S%)	Year-2019 (S%)
Amikacin	796/961 (82.8)	4788/6048 (79.2)	7070/8911 (79.3)	9862/12453 (79.2)
Cefazolin	0/0	0/8	2/6	0/1
Cefotaxime	165/928 (17.8)	879/5747 (15.3)	1274/7817 (16.3)	1531/10569 (14.5)
Ceftazidime	244/977 (25)	1295/5513 (23.5)	1398/5956 (23.5)	1496/7492 (20)
Ciprofloxacin	151/745 (20.3)	1028/5368 (19.2)	1889/8450 (22.4)	2414/11613 (20.8)
Colistin	0/0	494/498 (99.2)	824/833 (98.9)	1589/1595 (99.6)
Ertapenem	514/705 (72.9)	3104/4605 (67.4)	4528/6877 (65.8)	6612/9309 (71)
Fosfomycin	0/0	5/7	2/3	0/0
Imipenem	699/814 (85.9)	4699/5773 (81.4)	6453/8873 (72.7)	6433/10158 (63.3)
Levofloxacin	2/4	140/889 (15.7)	600/3493 (17.2)	1138/5987 (19)
Meropenem	792/981 (80.7)	4158/5678 (73.2)	5873/8403 (69.9)	9039/12072 (74.9)
Nitrofurantoin	1/3	12/14	18/23 (78.3)	2/2
Piperacillin-tazobactam	607/1009 (60.2)	3424/6030 (56.8)	4857/8960 (54.2)	6580/12030 (54.7)
Trimethoprim-sulfamethoxazole	1/3	10/25 (40)	12/24 (50)	2/2

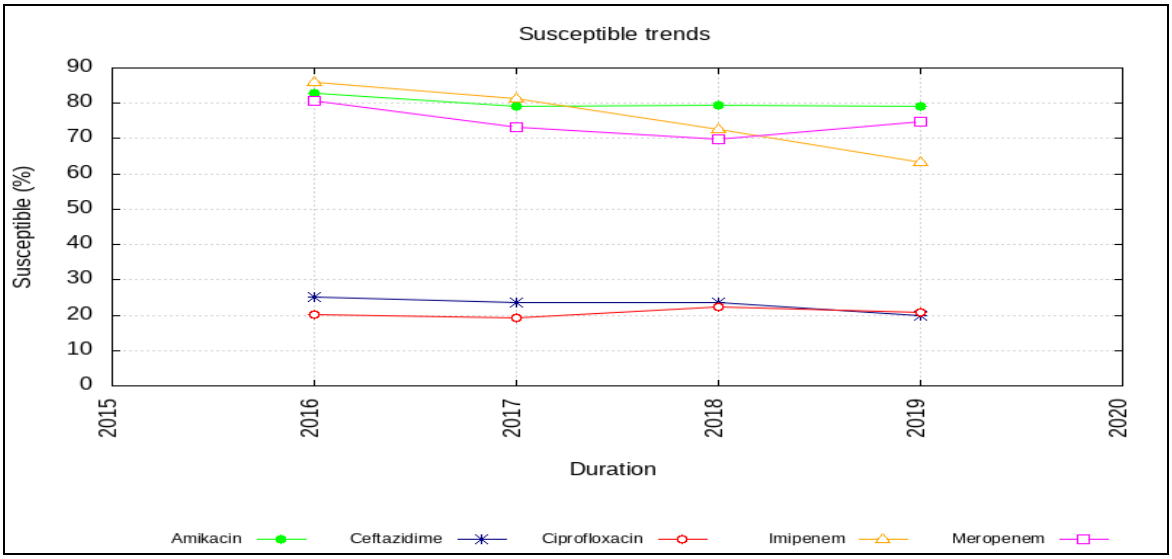


Figure 2.4.1: Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

Table 2.4.2: Yearly susceptibility trend of *Klebsellia pneumoniae* isolated from all samples (except faeces and urine).

AMA	Year-2016 (S%)	Year-2017 (S%)	Year-2018 (S%)	Year-2019 (S%)
Amikacin	396/848 (46.7)	2585/5286 (48.9)	4203/8274 (50.8)	6446/12878 (50.1)
Cefazolin	0/0	0/3	0/0	0/1
Cefotaxime	170/831 (20.5)	1109/5092 (21.8)	1577/7158 (22)	2386/11176 (21.3)
Ceftazidime	213/853 (25)	1322/4790 (27.6)	1488/5503 (27)	1979/7825 (25.3)
Ciprofloxacin	243/838 (29)	1670/5213 (32)	2766/7686 (36)	4113/11425 (36)
Colistin	0/3	452/501 (90.2)	1069/1168 (91.5)	2198/2352 (93.5)
Ertapenem	317/690 (45.9)	2022/4456 (45.4)	3189/6667 (47.8)	4348/9618 (45.2)
Fosfomycin	0/0	0/0	0/0	0/0
Imipenem	566/874 (64.8)	3136/5360 (58.5)	4256/8221 (51.8)	4962/10892 (45.6)
Levofloxacin	1/1	257/898 (28.6)	967/3333 (29)	2564/7330 (35)
Meropenem	436/847 (51.5)	2480/5147 (48.2)	3831/7589 (50.5)	6005/12024 (49.9)
Nitrofurantoin	0/4	0/5	1/6	0/1
Piperacillin-tazobactam	364/871 (41.8)	2209/5179 (42.7)	3256/8221 (39.6)	4811/12366 (38.9)
Trimethoprim-sulfamethoxazole	0/4	3/12	2/6	0/1

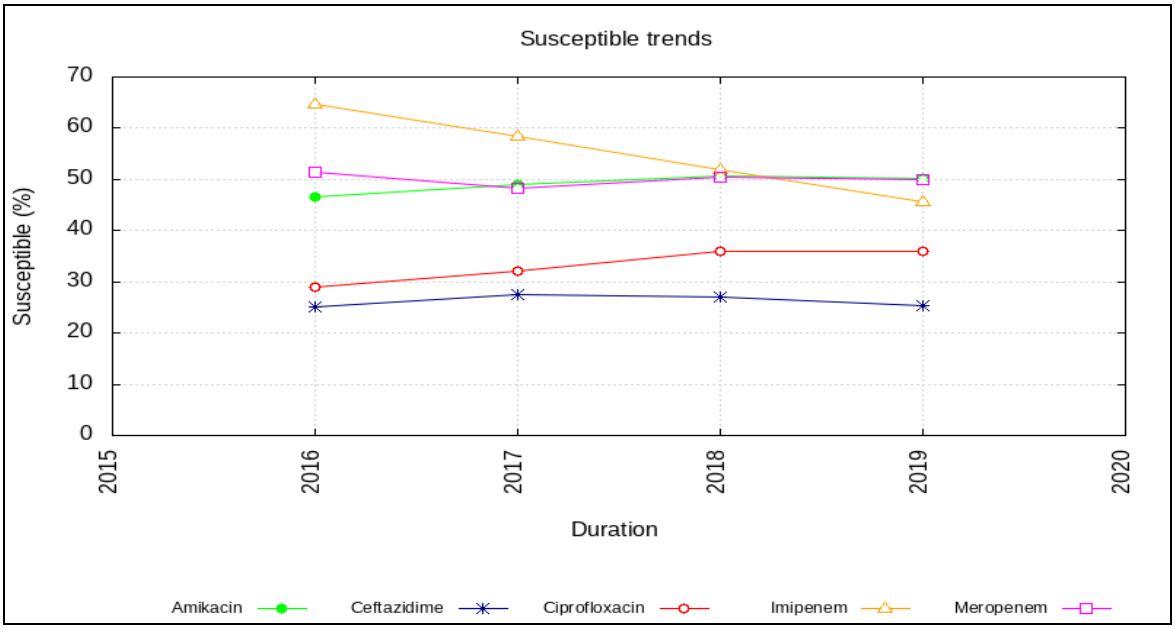


Figure2.4.2: Yearly susceptibility trend of *Klebsellia pneumoniae* isolated from all samples (except faeces and urine).

Table 2.4.3: Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine).

AMA	Year-2016 (S%)	Year-2017 (S%)	Year-2018 (S%)	Year-2019 (S%)
Amikacin	25/47 (53.2)	212/318 (66.7)	416/604 (68.9)	504/757 (66.6)
Cefazolin	0/0	0/0	0/0	0/0
Cefotaxime	5/46 (10.9)	94/306 (30.7)	193/556 (34.7)	225/651 (34.6)
Ceftazidime	13/47 (27.7)	110/285 (38.6)	168/474 (35.4)	200/576 (34.7)
Ciprofloxacin	18/49 (36.7)	138/295 (46.8)	324/599 (54.1)	426/735 (58)
Colistin	0/0	26/26 (100)	46/47 (97.9)	64/64 (100)
Ertapenem	25/46 (54.3)	161/263 (61.2)	336/522 (64.4)	379/594 (63.8)
Fosfomycin	0/0	0/0	0/0	0/0
Imipenem	39/46 (84.8)	198/303 (65.3)	369/594 (62.1)	398/673 (59.1)
Levofloxacin	0/0	44/86 (51.2)	145/319 (45.5)	294/510 (57.6)
Meropenem	33/49 (67.3)	187/284 (65.8)	396/580 (68.3)	500/759 (65.9)
Nitrofurantoin	0/0	1/3	2/2	0/0
Piperacillin-tazobactam	31/48 (64.6)	178/308 (57.8)	365/603 (60.5)	454/754 (60.2)
Trimethoprim-sulfamethoxazole	0/0	1/3	1/2	0/0

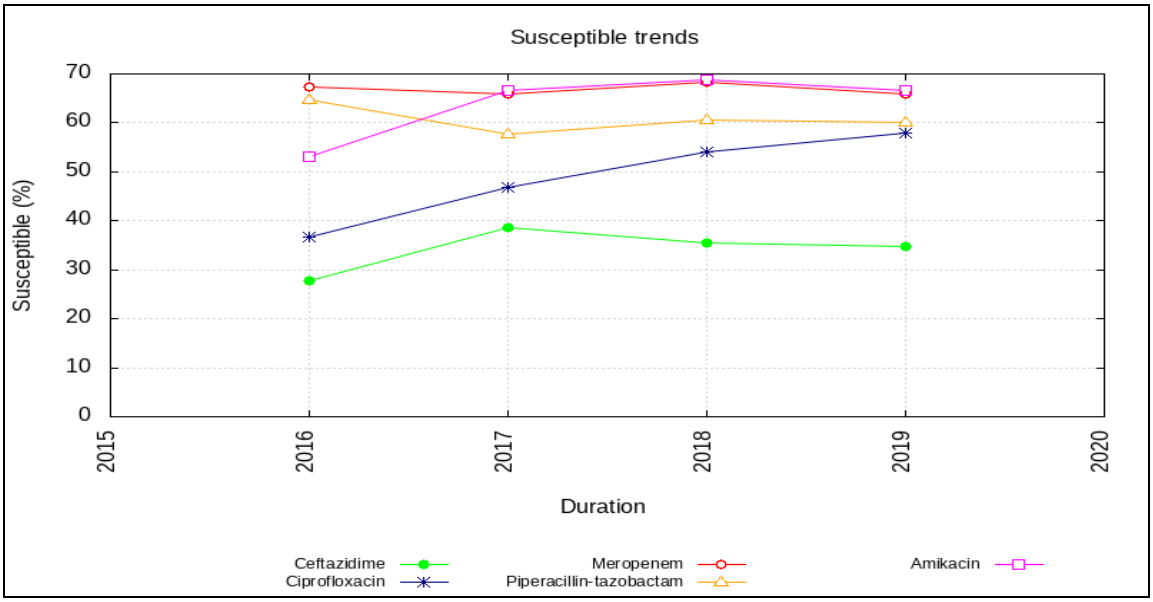


Figure2.4.3: Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine).

Table 2.4.4: Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine).

AMA	Year-2016 (S%)	Year-2017 (S%)	Year-2018 (S%)	Year-2019 (S%)
Amikacin	139/193 (72)	734/1059 (69.3)	1119/1571 (71.2)	1427/1930 (73.9)
Cefazolin	0/0	0/0	0/0	0/0
Cefotaxime	55/214 (25.7)	310/1093 (28.4)	448/1423 (31.5)	565/1565 (36.1)
Ceftazidime	71/216 (32.9)	363/1013 (35.8)	424/1158 (36.6)	484/1280 (37.8)
Ciprofloxacin	98/213 (46)	578/1088 (53.1)	837/1368 (61.2)	1133/1800 (62.9)
Colistin	0/1	77/79 (97.5)	98/109 (89.9)	288/306 (94.1)
Ertapenem	117/187 (62.6)	613/929 (66)	855/1169 (73.1)	947/1273 (74.4)
Fosfomycin	0/0	0/0	0/0	0/0
Imipenem	174/219 (79.5)	851/1133 (75.1)	1111/1574 (70.6)	1100/1626 (67.7)
Levofloxacin	0/0	93/150 (62)	289/549 (52.6)	575/929 (61.9)
Meropenem	150/215 (69.8)	735/1051 (69.9)	1068/1502 (71.1)	1475/1953 (75.5)
Nitrofurantoin	0/0	1/1	1/1	0/0
Piperacillin-tazobactam	123/216 (56.9)	682/1092 (62.5)	961/1566 (61.4)	1232/1873 (65.8)
Trimethoprim-sulfamethoxazole	0/0	0/1	1/1	0/0

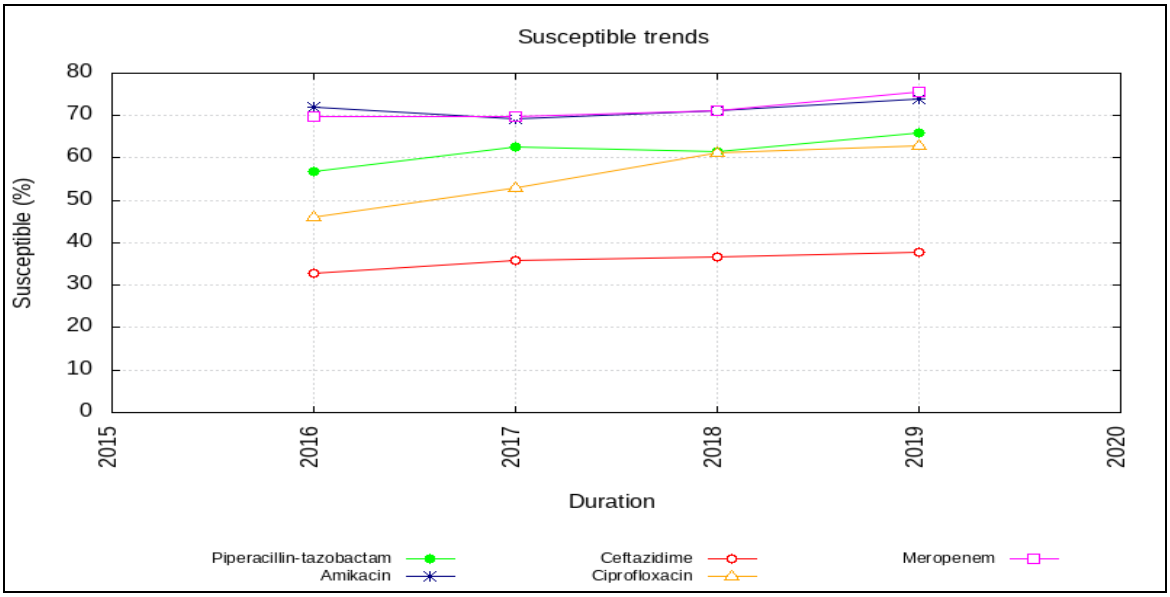


Figure2.4.4: Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine).

Molecular tests

Materials and methods

Molecular mechanism of antimicrobial resistance in clinical isolates

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

Table 2.5: 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 III ACC, FOX, MOX, DHA, CIT and EBC	AmpC beta lactamases ACC1 and 2, FOX1 to 5, MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11and CMY19 DHA-1 and DHA-2, LAT-1 to LAT-3, BIL-1, CMY-2 to CMY-7, CMY-12 to CMY-18 and CMY-21 to CMY-23, ACT-1 and MIR-1	F:CACCTCCAGCGACTTGTTAC R:GTTAGCCAGCATCACGATCC F:CTACAGTGCGGGTGGTTT R:CTATTTGCGGCCAGGTGA F:GCAACAACGACAATCCATCCT R:GGGATAGGCGTAACTCTCCCA F:TGATGGCACAGCAGGATATTC R:GCTTTGACTCTTTCGGGTATTCG F:CGAAGAGGCAATGACCAGAC R:ACGGACAGGGTTAGGTTAGGATAGY	346 162 895 997 538
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

PCR for TEM, SHV, OXA1 (Multiplex 1)

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume to 25µl. PCR was done on 386 *E. coli* and 395 *K. pneumoniae* isolates.

PCR for CTXM group 1, 2, 9 and group 8/25 (Multiplex 2)

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume to 25µl.

PCR for ACC, FOX, MOX, DHA, CIT, EBC (Multiplex 3)

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume to 25µl.

PCR for IMP, VIM, KPC (Multiplex 4)

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume to 25µl.

Monoplex PCR for CTXM15

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume to 25µl.

Monoplex PCR for NDM

25µl PCR reaction mix was prepared, in which 200 ng of DNA was amplified with 1xPCR buffer, 1U of Taq polymerase, 1.5 mM $MgCl_2$, 0.2mM of four dNTPs, 10 pm/ µl of primers and dH_2O to make the volume up to 25µl.

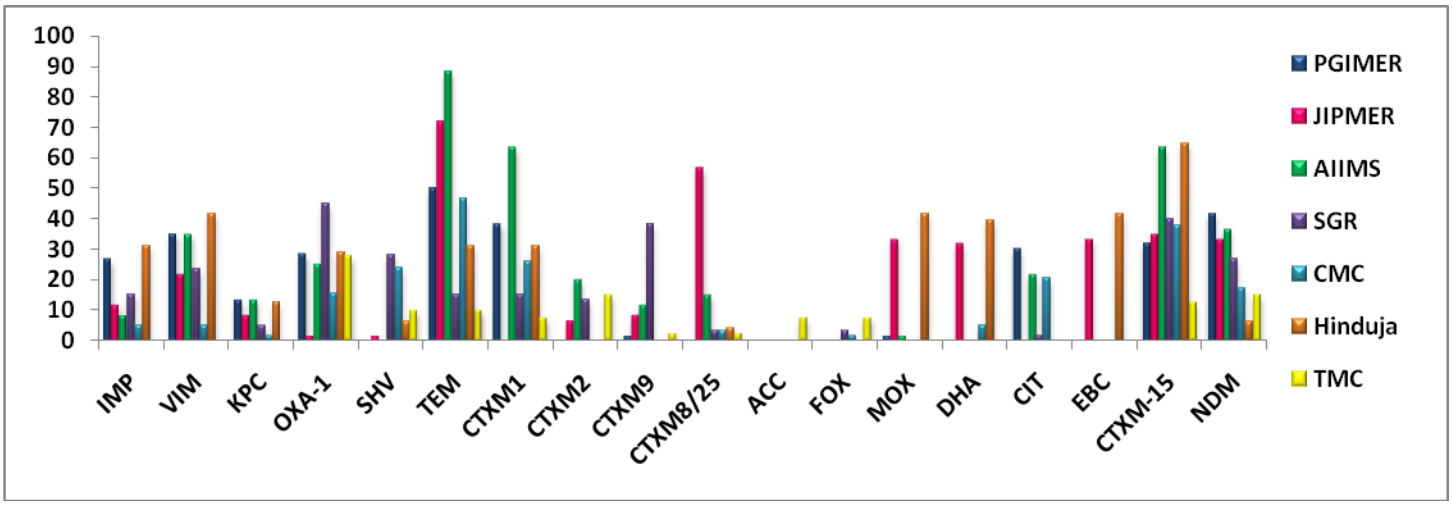
Results

E. coli

Total three hundred and eighty-six *E. coli* isolates were subjected to four multiplex PCRs and two monoplex PCRs for CTXM-15 and NDM. In PGIMER, Chandigarh, *E. coli* isolates positive for TEM were maximum (50%) followed by NDM (41.67%) and CTXM1 (38.33%) followed by CTXM15 (31.67%) andOXA-1 (28.33%). In CMC Vellore, isolates showed higher positivity for TEM (46.55%) while CTXM15 positive were 37.93%, CTXM1- 25.86% and SHV were24.14%. In JIPMER, TEM (71.66%) positive isolates were higher followed by CTXM8/25 (56.66%), CTXM-15 (35%), MOX, EBC and NDM were 33.33%. AIIMS isolates showed TEM (88.33%) followed by CTXM1 and CTXM-15 63.33% each, NDM (36.66%) followed by CIT (21.66%). In Sir Ganga Ram hospital isolates CTXM9 (38.33%) was highest followed by SHV (28.33%), NDM (26.67%), IMP (15%), CTXM1 (15%), and TEM (15%). In Hinduja hospital isolates CTXM-15 (64.58%) was most frequent followed by 41.67% for EBC, MOX and VIM. For TMC highest positivity was observed for OXA-1(27.5%) followed by NDM (15%) and CTXM-2(15%).

Table 2.5.1: Overall positivity of various genes in *E. coli* isolates.

Overall resistance			
Gene	**Total number (n)**	**Positive**	**%**
IMP	386	66	17.09
VIM	386	92	23.83
KPC	386	31	8.03
OXA-1	386	94	24.35
SHV	386	39	10.10
TEM	386	181	46.89
CTXM1	386	103	26.68
CTXM2	386	30	7.77
CTXM9	386	37	9.58
CTXM8/25	386	50	12.95
ACC	386	3	0.78
FOX	386	6	1.55
MOX	386	42	10.88
DHA	386	41	10.62
CIT	386	44	11.39
EBC	386	40	10.36
CTXM-15	386	160	41.45
NDM	386	102	26.42



PGIMER- Postgraduate Institute of Medical Education and Research, Chandigarh; JIPMER- Jawaharlal Institute of Postgraduate Medical Education and Research; AIIMS- All India Institute of Medical Education and Research, SGR- Sir Gangaram Hospital; CMC- Christian Medical College, Vellore; Hinduja- Hinduja Hospital, Mumbai; TMC- Tata Medical Center, Kolkata.

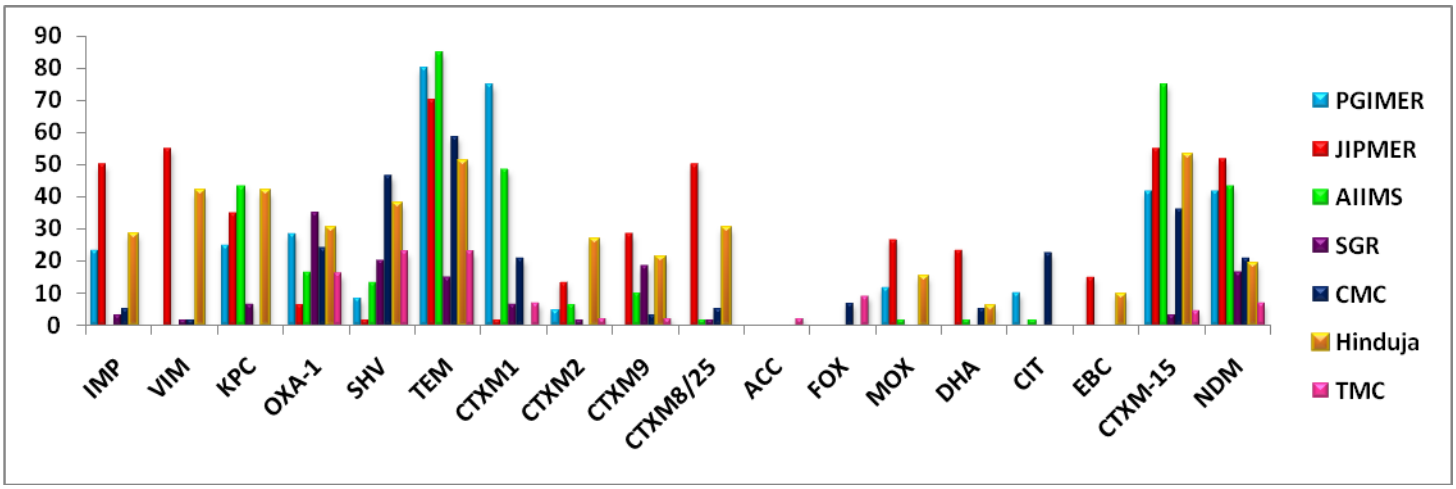
Figure 2.5.1: Overall positivity of various genes in *E. coli* isolates from various centers.

K. pneumoniae

Three hundred ninety-five *K. pneumoniae* isolates were subjected to four multiplex PCRs and two monoplex PCRs for CTXM-15 and NDM.In PGIMER, Chandigarh TEM positivity was 80%, followed by CTXM-1 (75%), CTXM15 (41.7%) and NDM (41.7%). In CMC Vellore, TEM positivity was maximum at 58.62% followed by SHV (46.6%), CTXM15 (36.2%), followed by OXA-1 (24.%) while in JIPMER, the resistance pattern was TEM (70.0%), VIM and CTXM15 (55% each), NDM (51.7%) and in AIIMS, TEM (85.0%), CTXM-15 (75%) followed by CTXM1(48.33%), KPC(43.33%), and NDM(43.33%). In Sir Ganga Ram hospital, isolates showed OXA-1 (35%) as highest positivity followed by SHV (20%), CTXM-1(18.33%), and NDM (16.67%). In Hinduja hospital highest was for CTXM-15 (52.83%), 50.94% positivity for TEM, followed by VIM and KPC 41.5% each. For TMC highest positivity was observed for SHV and TEM 22.73% each followed by OXA-1(15.91%) and FOX (9.09%).

Table 2.5.2: Overall positivity of various genes in *E. coli* isolates.

Overall			
Gene name	**Total number (n)**	**Positive**	**%**
IMP	395	64	16.20
VIM	395	57	14.43
KPC	395	88	22.28
OXA-1	395	89	22.53
SHV	395	83	21.01
TEM	395	161	40.76
CTXM1	395	94	23.79
CTXM2	395	31	7.84
CTXM9	395	48	12.15
CTXM8/25	395	51	12.91
ACC	395	1	0.25
FOX	395	8	2.02
MOX	395	32	8.10
DHA	395	21	5.32
CIT	395	20	5.06
EBC	395	14	3.54
CTXM-15	395	156	39.49
NDM	395	117	29.62



PGIMER- Postgraduate Institute of Medical Education and Research, Chandigarh; JIPMER- Jawaharlal Institute of Postgraduate Medical Education and Research; AIIMS- All India Institute of Medical Education and Research, SGR- Sir Gangaram Hospital; CMC- Christian Medical College, Vellore; Hinduja- Hinduja Hospital, Mumbai; TMC- Tata Medical Center, Kolkata.

Figure 2.5.2: Overall positivity of various genes in *K. pneumoniae* isolates from various centers.

Chapter 3 Typhoidal Salmonella

Summary of the results

During the study period of 2019, a total of 56 *Salmonella spp* were isolated at AIIMS, Delhi. Out of which, 43 were *Salmonella* Typhi,11 were*Salmonella* Paratyphi A and while two strains were *Salmonella* group C. *Salmonella* strains were received in AIIMS, Delhi nodal center from JIPMER Puducherry, PGI Chandigarh CMC Vellore and other regional centersas nodal center. Antimicrobial susceptibility was determined by standard method.Strains showing multiple resistances to ampicillin, chloramphenicol and co-trimoxazole were defined as MDR while NAR (nalidixic acid resistant) and NAS (nalidixic acid sensitive) were defined based on susceptibility to nalidixic acid. A total of 940 typhoidal *Salmonella* were reported in 2019 online. Details are shown in Fig 3.1.

There is an increase in isolation of *Salmonella* Typhi over the years e.g. total isolation was 3.6% in 2017 which increased to 4.1% in 2018 and 4.3% in 2019 from all over India. (Table 3.1 & fig 3.2). Out of a total, 94% *Salmonella* Typhi were sensitive to ampicillin, 95.4% to chloramphenicol followed by 96.4% to trimethoprim-sulfamethoxazole (Table 3.2). Among *Salmonella* Paratyphi A, 90.6% were sensitive to ampicillin, 100% to chloramphenicol and 99.3% were sensitive to trimethoprim-sulfamethoxazole. In *Salmonella* Typhi, MDR was 4% while no MDR was observed in case of *Salmonella* Paratyphi A.

We observed that the sensitivity to ciprofloxacin in *Salmonella* Typhi was 7.2% while for *S.* Paratyphi A it was only 1.2% Table 3.2. When comparing surrogate marker, Pefloxacin sensitivity was 15.3% in *S.* Typhi. 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 Himedia disks for pefloxacin. Antimicrobial susceptibility for *Salmonella* Typhi across different region of India is given in table 3.3. Three years comparative analysis of antimicrobial susceptibility in *Salmonella* Typhi was also done Table 3.4 A and Table 3.4B. Susceptibility trends of *Salmonella* Paratyphi A from 2017 to 2019 are shown in (Table 3.6 A). Yearly comparison of trends of *Salmonella* Paratyphi A from Blood is as shown in Table 3.6 B.

Clinical relevance of study:

Enteric fever is a community acquired bloodstream infection mainly caused by *Salmonella* Typhi and *Salmonella* Paratyphi A but this problem has increased by antimicrobial resistance to first line drugs and emerging resistance to third line drugs. After the increasing resistance to ciprofloxacin which has been used as first line drug for

the last two decades, ceftriaxone/cefixime are the drug of choice at present. With increasing use of ceftriaxone, MIC to ceftriaxone has now started showing increasing trend and is responsible for clinical non response[1]. Absolute resistance is also emerging in isolated cases. In 2017 WHO released a priority list of antibiotic resistant bacteria and ranked FQ resistant *Salmonella* as a high priority pathogen for the research and development of new antibiotics[2]. So to defeat the problem of increasing resistance 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*, there is a need for continuous monitoring of antimicrobial resistance in *Salmonella* Typhi and *S.* Paratyphi A to use currently available antibiotics.

To summarize, *S*. Typhi is the most common etiological agent followed by *S.* Paratyphi A in India. The ciprofloxacin susceptibility is only 7% in *S.* Typhi and 1.2% in *S.* Paratyphi A while 21.2% in other *Salmonella spp* 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 0.38µg/ml (2013) to 0.5µg/ml (2019) and MIC90 has increased from 8µg/ml (2013 – 2018) to 16µg/ml (2019) in *S.* Typhi. *Salmonella* Paratyphi A shows 100% resistance to ciprofloxacin though 91% *S*. Paratyphi A was intermediate. Overall Fluoroquinolone resistance in *S*. Paratyphi A is higher as compared to *S*. Typhibut ciprofloxacin MIC value is higher in *S.* Typhi. Fluoroquinolone resistance was mainly associated with DNA gyrase mutations. The reason for this may be the emergence of H58 MDR haplotype[3]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[3]. So, it is no longer empirical choice. MDR is decreasing from 6% in 2017 to 4% in 2019 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 0.032µg/ml to 1µg/ml MIC range.

The MICs against azithromycin in the *Salmonella* Typhi isolates were normally distributed and ranged from 0.19 to 24 µg/ ml, with MIC_{50} and MIC_{90} values of 4 and 16µg/ ml respectively.

This data reiterates the fact that at present ceftriaxone and cefixime remains the first line of drug to treat severe infections of enteric fever. Azithromycin continues to be used as drug of choice in outpatient without any associated complications[4] 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. The

increasing MIC to cephalosporins is worrisome as this may delay the therapeutic clinical response in patients, necessitating multidrug therapy, which needs further evaluation.

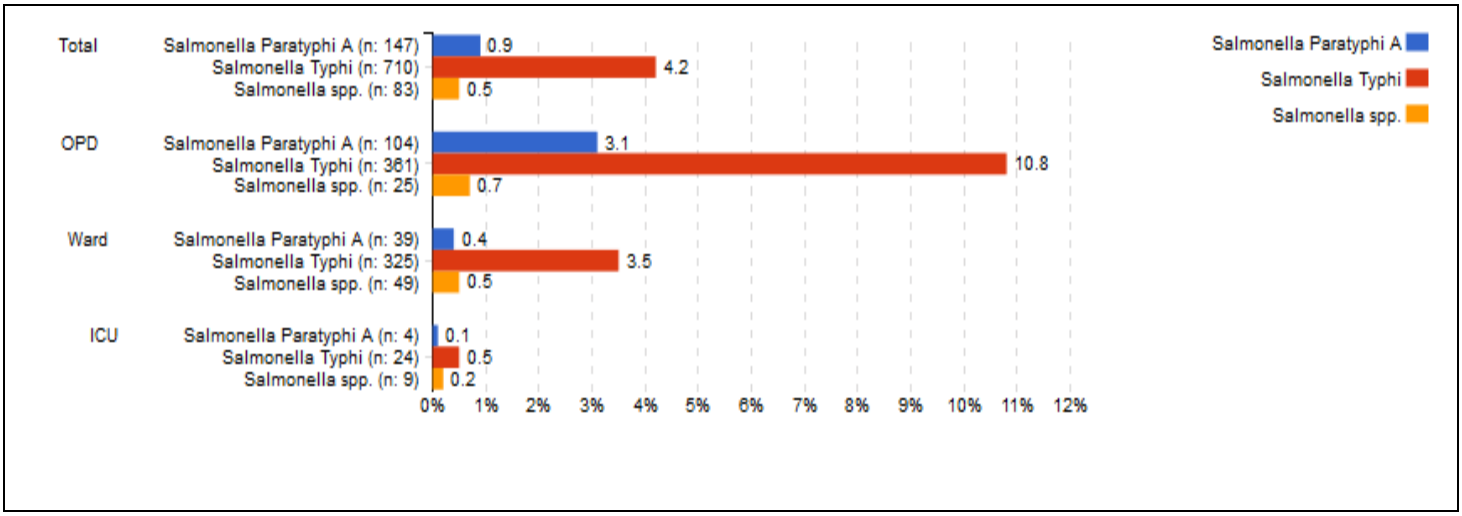


Figure 3.1: Location-wise Isolation pattern of Salmonella species isolated from Blood across OPD, Ward and ICU

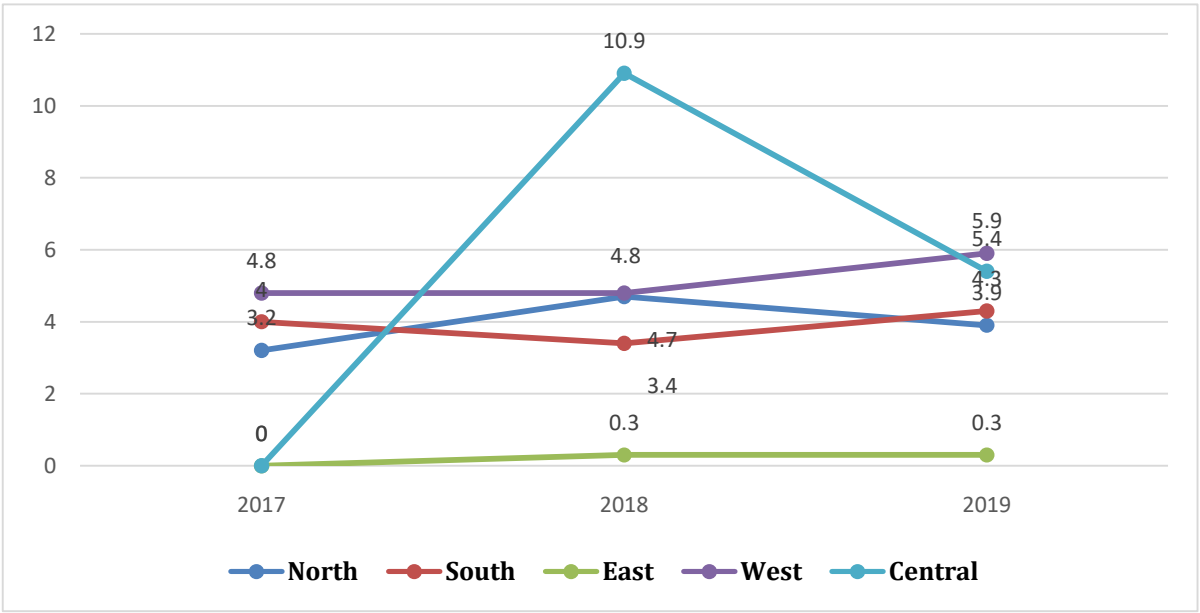


Figure 3.2: Yearly-isolation trend of Salmonella Typhi from All Samples (except Faeces)

Table 3.1: Yearly-isolation trend of Salmonella Typhi from different part of India

Years	2017	2018	2019
Total Culture	**n=9491**	**n=14091**	**n=17108**
North	138/4272 (3.2%)	246/5247 (4.7%)	174/4415 (3.9%)
Central	0/0 (-)	12/110 (10.9%)	30/551 (5.4%)
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%)
South	176/4400 (4%)	204/6012 (3.4%)	342/8005 (4.3%)
National	345/9491 (3.6%)	579/14091 (4.1%)	710/17108 (4.2%)

Table 3.2: Susceptibility pattern of Salmonella species isolated from Blood

AMA	**Blood**			
	Salmonella (non-faecal) **n=940**	***Salmonella Paratyphi A*** **n=147**	***Salmonella spp.*** **n=83**	***Salmonella Typhi*** **n=710**
Ampicillin	843/906 (93%)	125/138 (90.6%)	73/80 (91.3%)	645/688 (93.8%)
Azithromycin	536/556 (96.4%)	0/0	0/0	536/556 (96.4%)
Cefixime	582/601 (96.8%)	105/107 (98.1%)	53/56 (94.6%)	424/438 (96.8%)
Ceftriaxone	845/863 (97.9%)	139/142 (97.9%)	72/74 (97.3%)	634/647 (98%)
Chloramphenicol	771/799 (96.5%)	128/128 (100%)	77/78 (98.7%)	566/593 (95.4%)
Ciprofloxacin	50/635 (7.9%)	1/86 (1.2%)	14/66 (21.2%)	35/483 (7.2%)
Levofloxacin	3/58 (5.2%)	0/25 (0%)	0/0	3/33 (9.1%)
Ofloxacin	0/9 (-)	0/3 (-)	0/0	0/6 (-)
Pefloxacin	52/338 (15.4%)	5/31 (16.1%)	0/0	47/307 (15.3%)
Trimethoprim-sulfamethoxazole	901/927 (97.2%)	144/145 (99.3%)	82/82 (100%)	675/700 (96.4%)

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

Salmonella Typhi

Table 3.3: Susceptibility pattern of Salmonella Typhi from Blood across different regions of India

Antibiotic	National (n=710)		North (n=174)		Central (n=30)		East (n=4)		West (n=160)		South (n=342)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Ceftriaxone	634/647 (98)	88-100	174/174 (100)	100-100	26/29 (89.7)	88	3/4 (-)	-	141/145 (97.2)	98.4-100	290/295 (98.3)	97.1-100
Cefixime	424/438 (96.8)	97.5-100	172/172 (100)	100-100	11/13 (-)	-	4/4 (-)	-	57/59 (96.6)	0	180/190 (94.7)	97.5-98
Azithromycin	536/556 (96.4)	70.8-100	170/172 (98.8)	100-100	24/25 (96)	96	3/3 (-)	-	141/145 (97.2)	93.3-100	198/211 (93.8)	70.8-97.1
Trimethoprim-sulfamethoxazole	675/700 (96.4)	80.8-100	168/174 (96.6)	91.4-97.6	25/30 (83.3)	80.8	3/4 (-)	-	150/154 (97.4)	95.8-98.3	329/338 (97.3)	95.7-100
Chloramphenicol	566/593 (95.4)	91.4-98.4	163/169 (96.4)	91.4-98.4	19/22 (86.4)	0	3/4 (-)	-	134/139 (96.4)	95.8-98.4	247/259 (95.4)	94.8-95.7
Ampicillin	645/688 (93.8)	72.7-100	162/174 (93.1)	82.9-96.1	16/22 (72.7)	72.7	3/3 (-)	-	139/150 (92.7)	90.9-95.8	325/339 (95.9)	94.4-100
Pefloxacin	47/307 (15.3)	2.9-30.4	1/35 (2.9)	2.9	0/0 (-)	-	1/2 (-)	-	17/83 (20.5)	13.3-30.4	28/187 (15)	14
Levofloxacin	3/33 (9.1)	8.3	3/31 (9.7)	8.3	0/1 (-)	-	0/0 (-)	-	0/0 (-)	-	0/1 (-)	-
Ciprofloxacin	35/483 (7.2)	0-13.8	1/48 (2.1)	2.9	1/26 (3.8)	4.3	1/3 (-)	-	9/138 (6.5)	0-9.7	23/268 (8.6)	0-13.8

Table 3.4A: Yearly susceptibility trends of Salmonella Typhi from Blood

AMA	Year-2017	Year-2018	Year-2019
	Total n=345	Total n=579	Total n=710
	(S%)	(S%)	(S%)
Ampicillin	305/332 (91.9)	550/575 (95.7)	645/688 (93.8)
Azithromycin	266/278 (95.7)	497/505 (98.4)	536/556 (96.4)
Cefixime	221/223 (99.1)	343/348 (98.6)	424/438 (96.8)
Ceftriaxone	329/334 (98.5)	530/540 (98.1)	634/647 (98)
Chloramphenicol	267/278 (96)	540/559 (96.6)	566/593 (95.4)
Ciprofloxacin	35/302 (11.6)	29/439 (6.6)	35/483 (7.2)
Levofloxacin	0/3	5/18	3/33 (9.1)
Ofloxacin	0/1	0/7	0/6
Pefloxacin	36/178 (20.2)	39/199 (19.6)	47/307 (15.3)
Trimethoprim-sulfamethoxazole	322/341 (94.4)	551/574 (96)	675/700 (96.4)

Table 3.4B: Yearly susceptibility trends of Salmonella Typhi from Blood received at AIIMS, New Delhi from regional centers

AMA	Year-2017	Year-2018	Year-2019
	Total n=142	Total n=256	Total n=427
	(S%)	(S%)	(S%)
Ampicillin	135/142 (95%)	246/255 (96.4%)	310/384 (80.7%)
Azithromycin	142/142 (100%)	254/255 (99.6%)	330/332 (99.3%)
Cefixime	142/142 (100%)	256/256 (100%)	380/381 (99.7%)
Ceftriaxone	142/142 (100%)	256/256 (100%)	380/381 (99.7%)
Chloramphenicol	135/142 (95%)	246/255 (96.4%)	390/413 (94.4%)
Ciprofloxacin*	27/142 (19%)	0/256 (0)	0/375 (0)
Levofloxacin	65/134 (48.5%)	31/254 (12.2%)	28/353 (7.9%)
Ofloxacin	12/126 (9.5%)	4/256 (1.5%)	19/356 (5.3%)
Pefloxacin	27/142 (19%)	0/256 (0)	0/375 (0)
Trimethoprim-sulfamethoxazole	134/142 (94.3%)	242/256 (94.5%)	390/413 (94.4%)

*Based upon the sensitivity of strains received at AIIMS, New Delhi as Nodal Center

Salmonella Paratyphi A

Table 3.5: Susceptibility pattern of Salmonella Paratyphi A from Blood across different regions of India

Antibiotic	National (n=147)		North (n=67)		Central (n=9)		East (n=1)		West (n=34)		South (n=36)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Chloramphenicol	128/128 (100)	100-100	67/67 (100)	100	9/9 (-)	-	1/1 (-)	-	24/24 (100)	0	27/27 (100)	100
Trimethoprim-sulfamethoxazole	144/145 (99.3)	100-100	67/67 (100)	100	8/9 (-)	-	1/1 (-)	-	32/32 (100)	0	36/36 (100)	100
Cefixime	105/107 (98.1)	100	66/67 (98.5)	100	4/4 (-)	-	1/1 (-)	-	17/17 (-)	-	17/18 (-)	-
Ceftriaxone	139/142 (97.9)	100-100	67/67 (100)	100	8/8 (-)	-	1/1 (-)	-	30/32 (93.8)	0	33/34 (97.1)	100
Ampicillin	125/138 (90.6)	90.6-100	56/67 (83.6)	90.6	4/5 (-)	-	1/1 (-)	-	29/29 (100)	0	35/36 (97.2)	100
Pefloxacin	5/31 (16.1)	0	0/10 (-)	-	0/0 (-)	-	0/1 (-)	-	4/14 (-)	-	1/6 (-)	-
Ciprofloxacin	1/86 (1.2)	0	0/17 (-)	-	0/9 (-)	-	0/1 (-)	-	1/31 (3.2)	0	0/28 (0)	-

Table 3.6A: Yearly susceptibility trends of Salmonella Paratyphi A from Blood

AMA	Year-2017 Total n=41 (S%)	Year-2018 Total n=125 (S%)	Year-2019 Total n=147 (S%)
Ampicillin	38/40 (95)	122/125 (97.6)	125/138 (90.6)
Azithromycin	0/0	0/0	0/0
Cefixime	26/27 (96.3)	105/105 (100)	105/107 (98.1)
Ceftriaxone	38/40 (95)	121/124 (97.6)	139/142 (97.9)
Chloramphenicol	30/30 (100)	121/121 (100)	128/128 (100)
Ciprofloxacin	4/40 (10)	1/111 (0.9)	1/86 (1.2)
Levofloxacin	0/2	0/5	0/25 (0)
Ofloxacin	0/0	0/1	0/3
Pefloxacin	4/7	1/15 (6.6)	5/31 (16.1)
Trimethoprim-sulfamethoxazole	41/41 (100)	123/123 (100)	144/145 (99.3)

Table 3.6B: Yearly susceptibility trends of Salmonella Paratyphi A from Blood received at AIIMS, New Delhi from regional centers

AMA	Year-2017	Year-2018	Year-2019
	Total n=41 (S%)	Total n=93 (S%)	Total n=75 (S%)
Ampicillin	35/41 (85.3%)	74/93 (79.5%)	39/62 (62.9%)
Azithromycin	*0/0	*0/0	*0/0
Cefixime	41/41 (100%)	93/93 (100%)	55/55 (100%)
Ceftriaxone	41/41 (100%)	93/93 (100%)	55/55 (100%)
Chloramphenicol	41/41 (100%)	93/93 (100%)	62/62 (100%)
Ciprofloxacin	2/41 (4.8%)	0/93 (0)	0/61 (0)
Levofloxacin	0/41 (0)	1/93 (0)	0/58 (0)
Ofloxacin	0/41 (0)	0/93 (0)	0/58 (0)
Trimethoprim-sulfamethoxazole	39/41 (95%)	93/93 (100%)	62/62 (100%)

Ciprofloxacin MIC

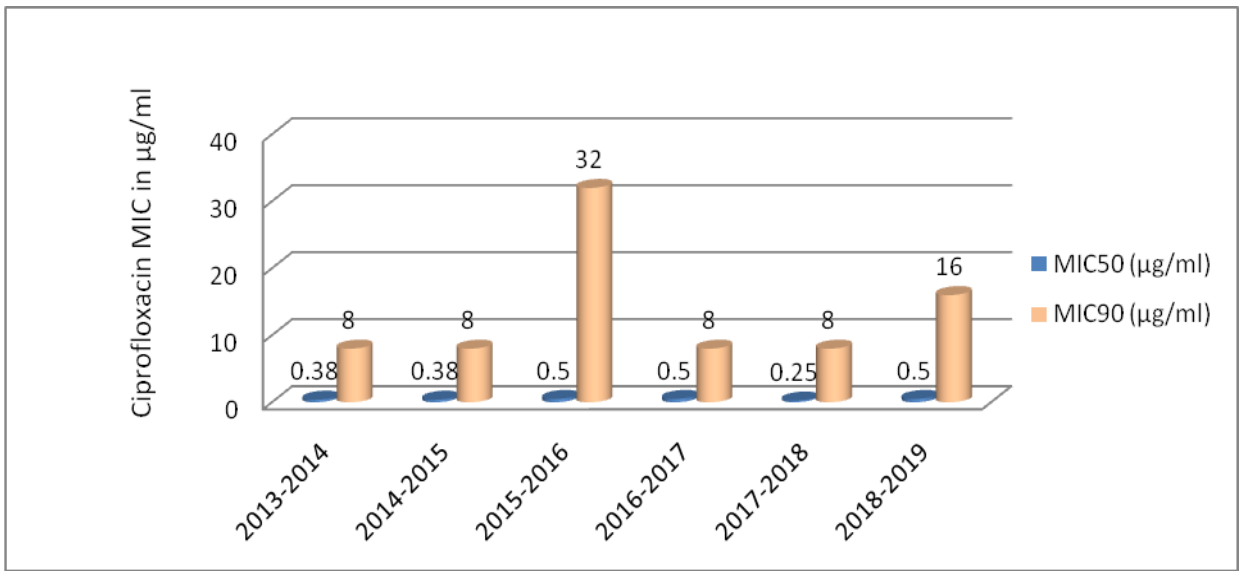
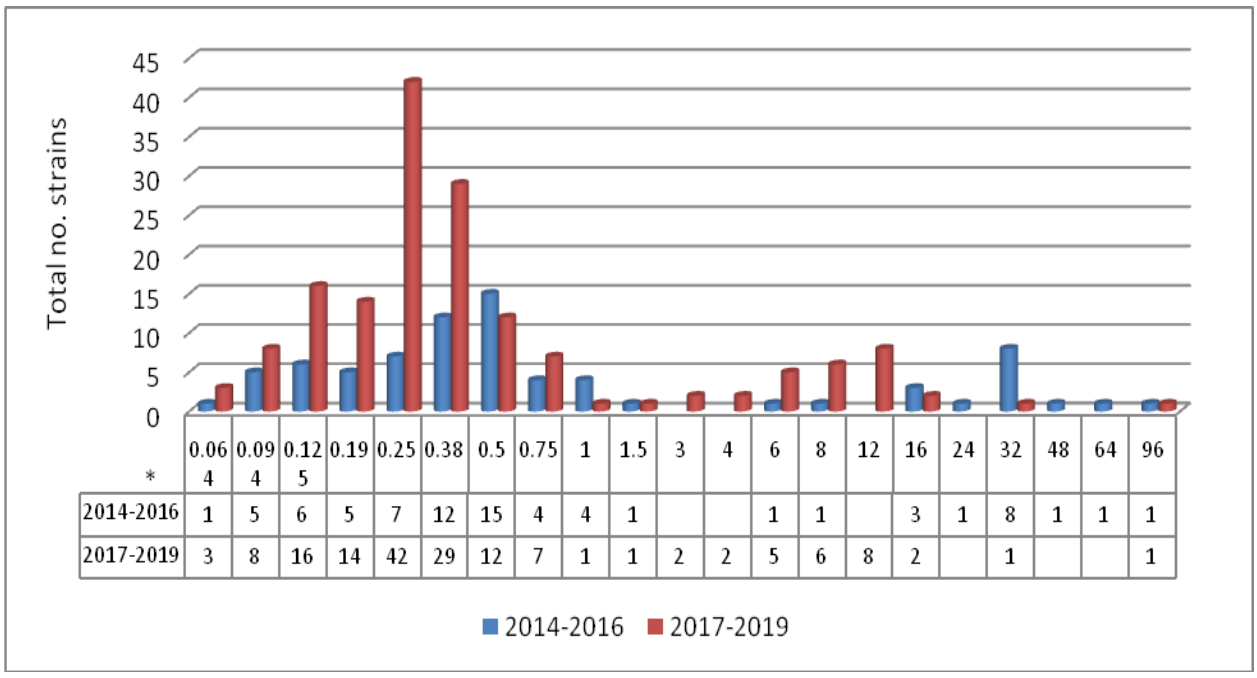


Figure 3.1: Ciprofloxacin MIC50 and MIC90 in Salmonella Typhi over a period of six years at AIIMS



*	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	3	4	6	8	12	16	24	32	48	64	96
2014-2016	1	5	6	5	7	12	15	4	4	1			1	1		3	1	8	1	1	1
2017-2019	3	8	16	14	42	29	12	7	1	1	2	2	5	6	8	2		1			1

To study ciprofloxacin MIC trend, six year time has been grouped in to two groups of three year each (2014-2016 and 2017-2019)

Figure 3.2: Ciprofloxacin MIC trends at AIIMS, New Delhi over a period of six years

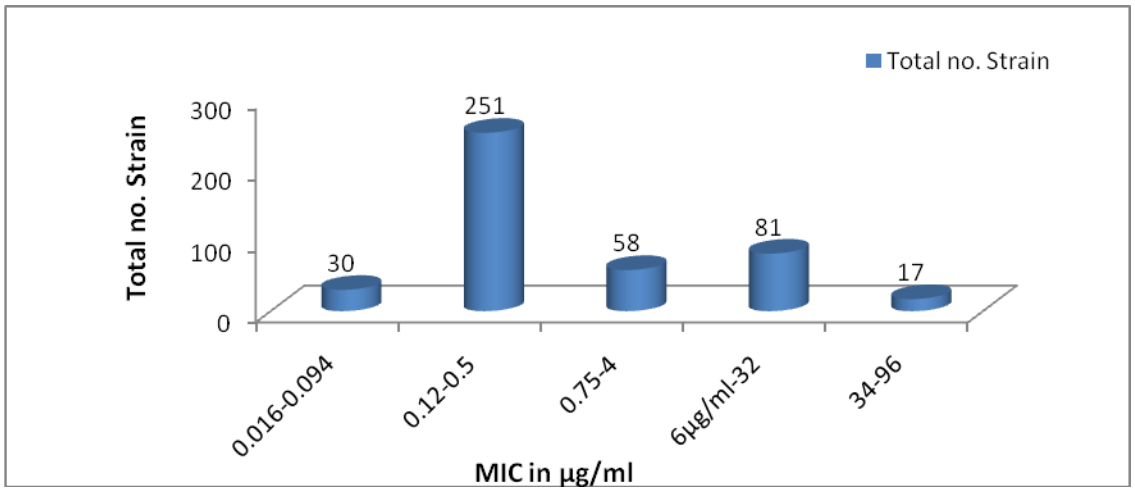


Figure 3.3: Ciprofloxacin MIC Salmonella Typhi from all centres in 2019

Table 3.4: MIC 50 and MIC 90 in Salmonella Typhi to fluoroquinolones, cephalosporin's and Macrolide from all centers received in 2019

	Min	Maxi	Median	MIC 50	MIC 90
Levo	0.016	32	0.75	0.38	12
Oflo	0.047	32	1.25	0.5	16
Cipro	0.016	125	2	0.38	64
Ceft	0.064	32	0.3	0.125	0.38
Cefixi	0.032	256	0.3	0.25	0.5
Azithro	0.19	24	3	6	12

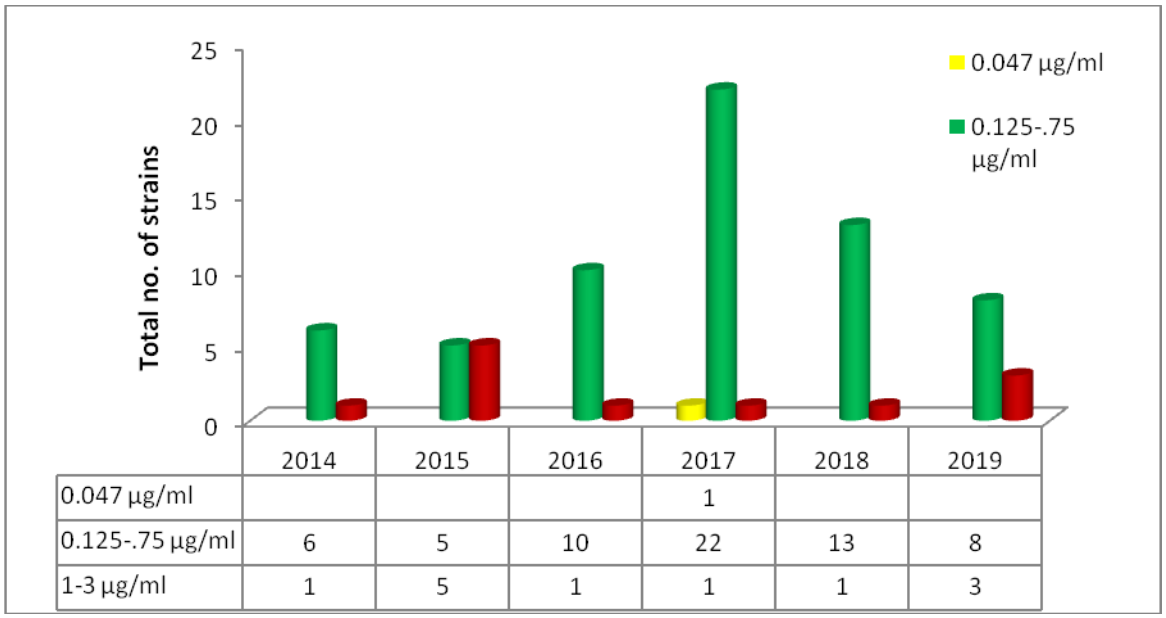


Figure 3.5: Year-wise ciprofloxacin MIC in S. Paratyphi A isolated at AIIMS, New Delhi

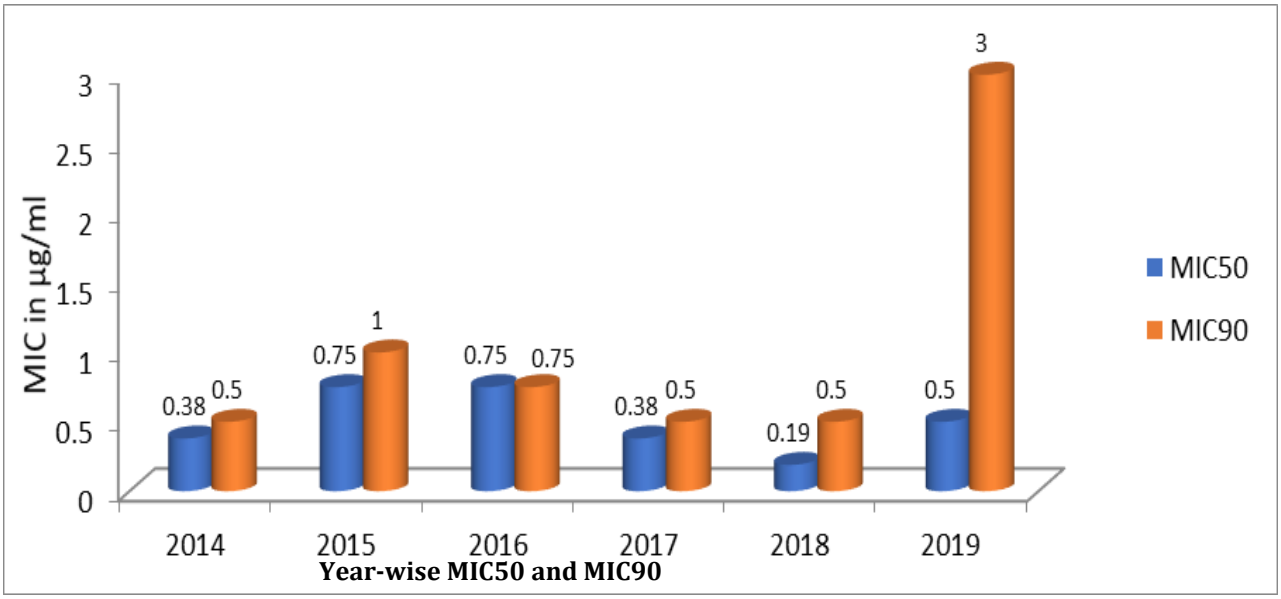
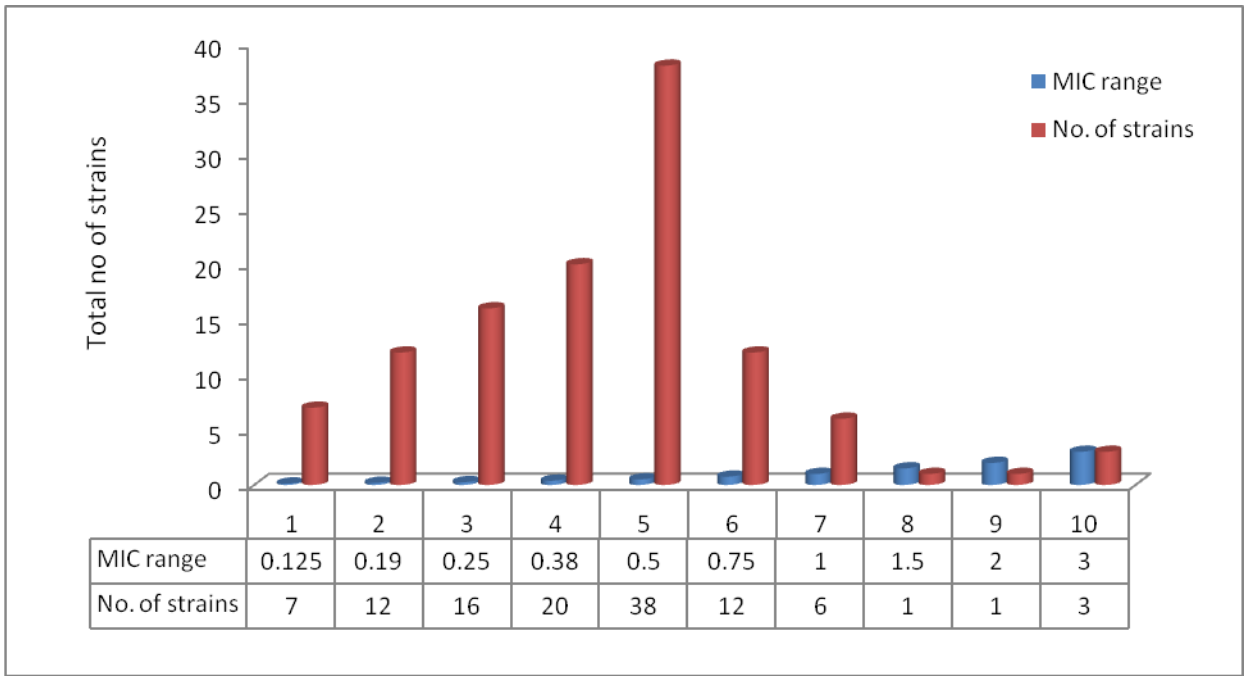


Figure 3.6: Ciprofloxacin MIC50 and MIC90 in *S.*ParatyphiA from 2014-2019 from AIIMS



	1	2	3	4	5	6	7	8	9	10
MIC range	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3
No. of strains	7	12	16	20	38	12	6	1	1	3

Figure 3.7: Ciprofloxacin MIC range in *S.* Paratyphi A from all centres received at AIIMS, New Delhi

Ceftriaxone MIC

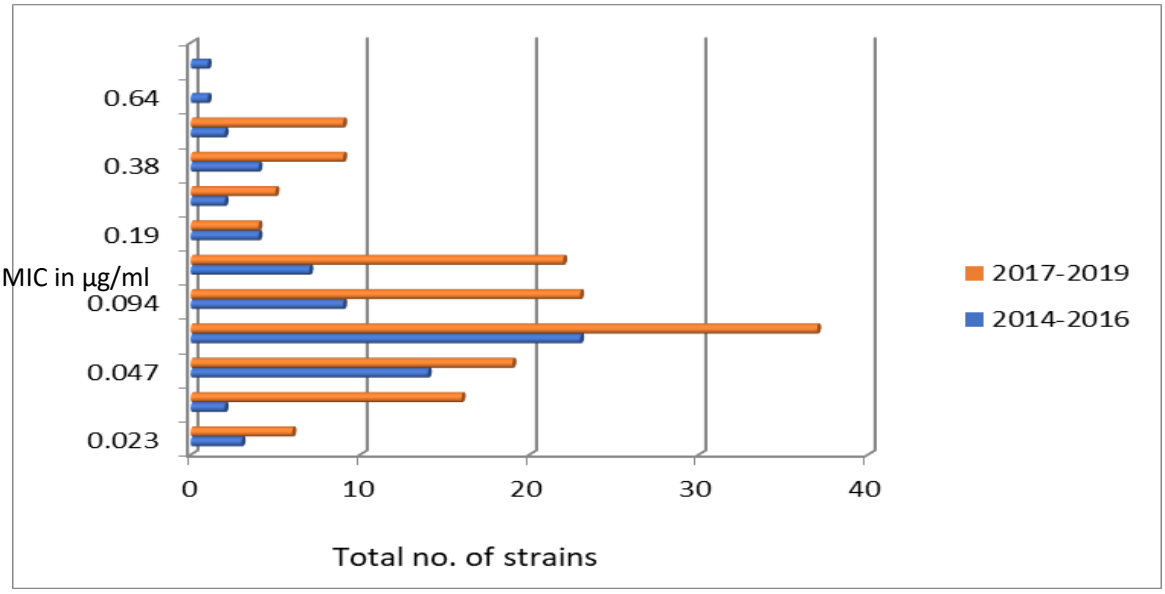


Fig 3.8: Comparison of creeping MIC for Ceftriaxone in S.Typhi over a period of six years at AIIMS, New Delhi

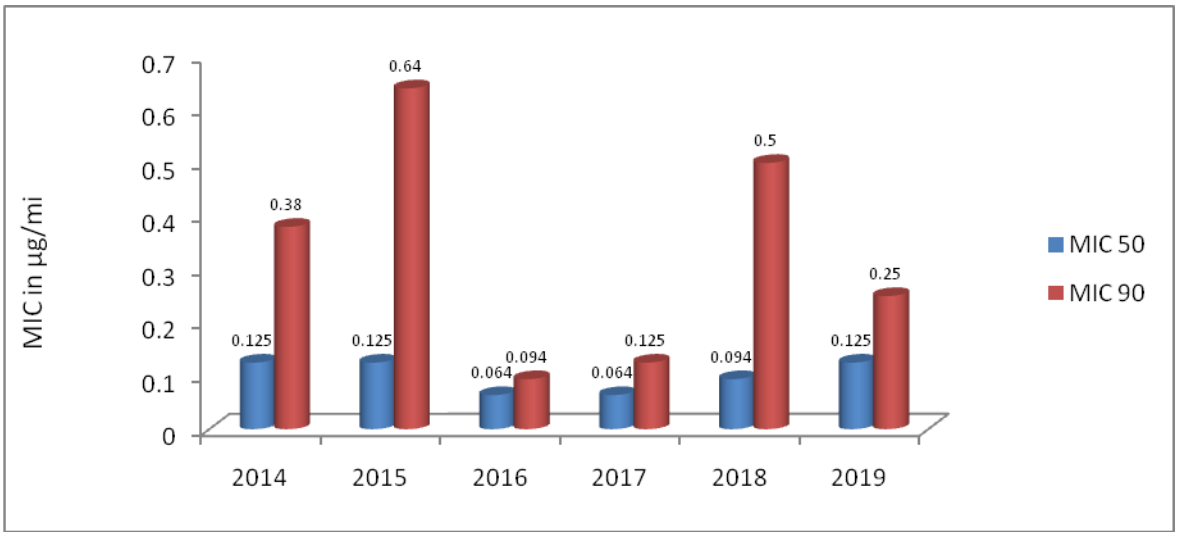


Figure 3.9: Comparison of ceftriaxone MIC50 and MIC90 for *S.*ParatyphiA at AIIMS over a period of six years

Overall ceftriaxone MIC50 and MIC90 have increased over the time.

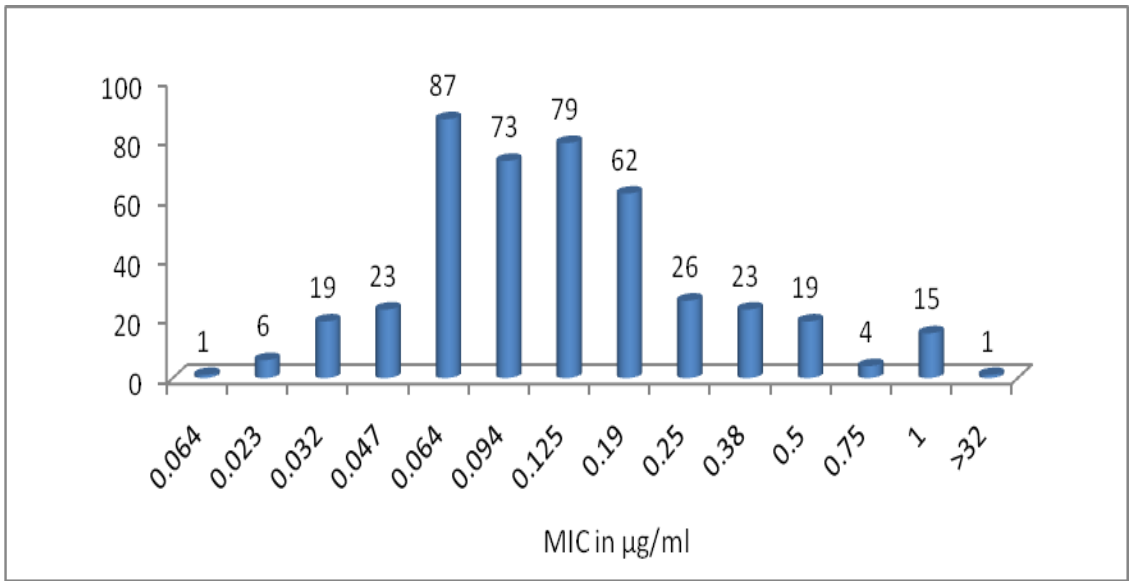
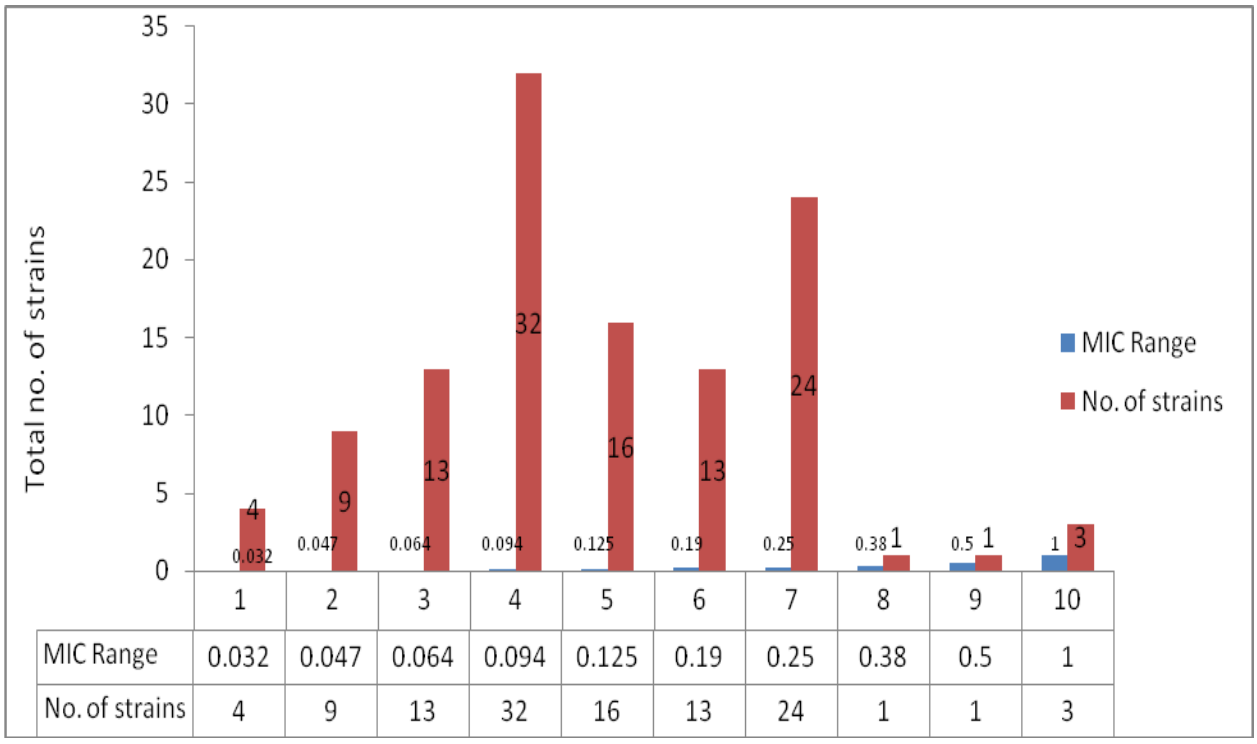


Figure 3.10: Ceftriaxone MIC in S. Typhi from all centres in 2019



	1	2	3	4	5	6	7	8	9	10
MIC Range	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	1
No. of strains	4	9	13	32	16	13	24	1	1	3

Figure 3.12: Ceftriaxone MIC in S.Paratyphi A from all centres in 2019

Table 3.11: Six year trend of ceftriaxone MIC in S. Typhi

Year	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Median (µg/ml)	Minimum (µg/ml)	Maximum (µg/ml)
2014	0.125	0.38	0.25	0.023	0.38
2015	0.125	0.064	0.125	0.032	0.094
2016	0.064	0.094	0.064	0.032	0.094
2017	0.064	0.125	0.064	0.002	0.25
2018	0.094	0.5	0.094	0.023	0.5
2019	0.125	0.25	0.125	0.094	≥32

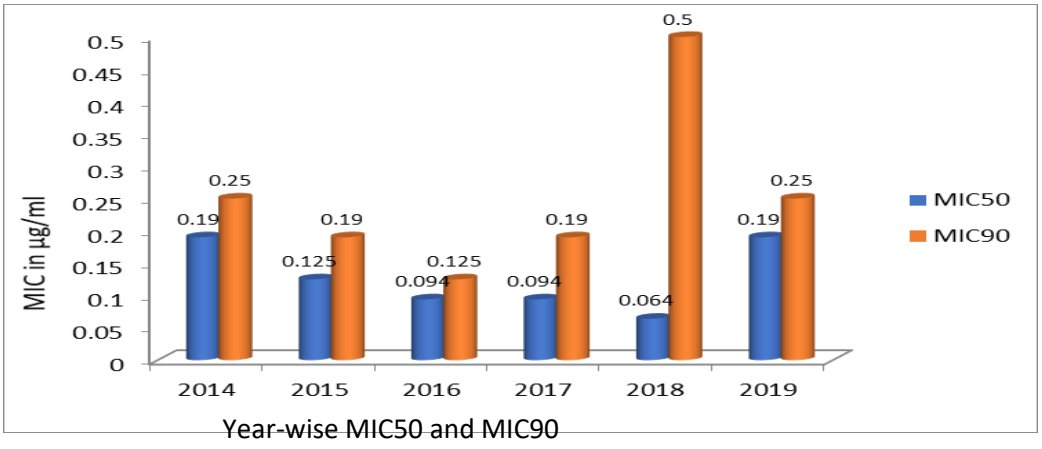
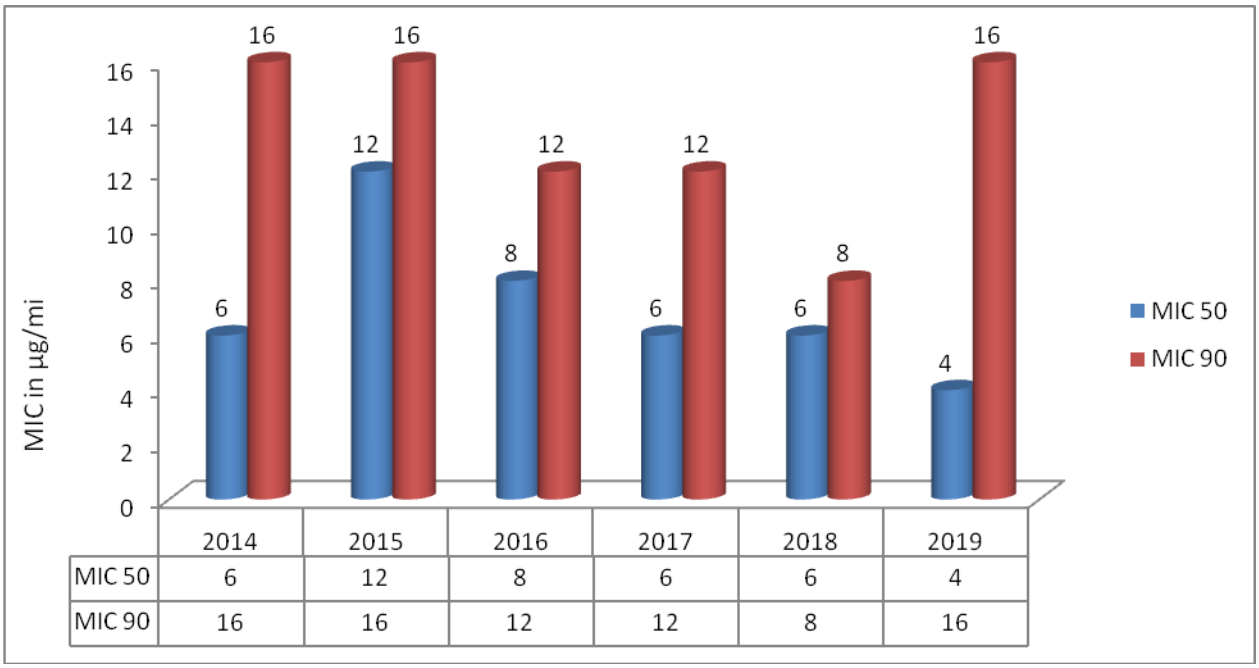


Figure 3.13: Ceftriaxone MIC in S.Paratyphi A from 2014-2019 isolated at AIIMS

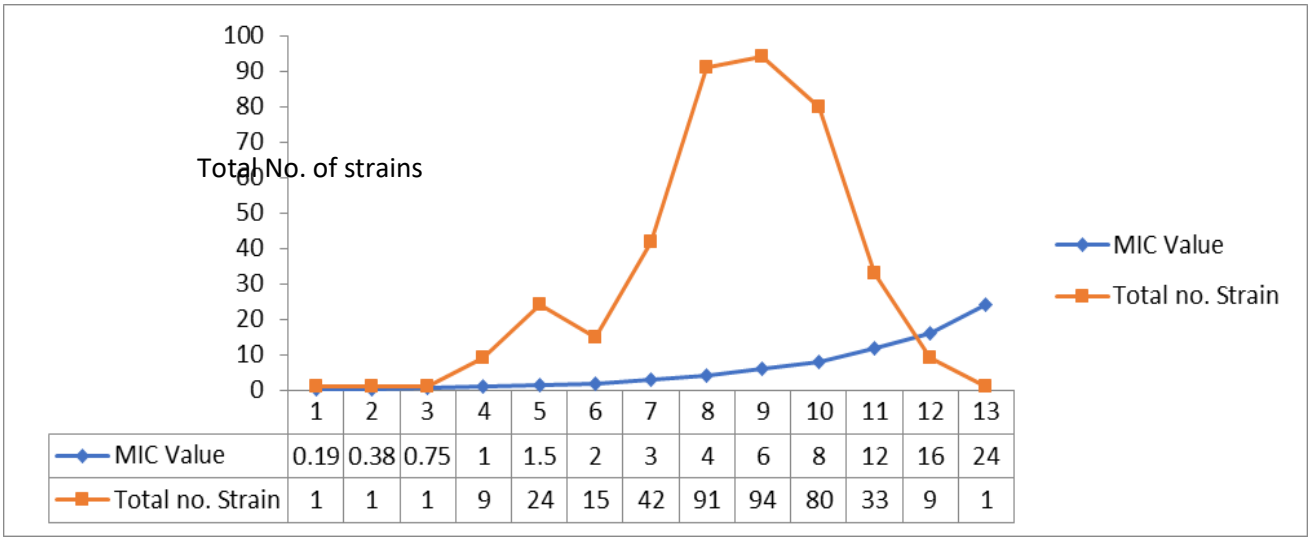
Azithromycin MIC



	2014	2015	2016	2017	2018	2019
MIC 50	6	12	8	6	6	4
MIC 90	16	16	12	12	8	16

Figure 3.14.: Comparison of Azithromycin MIC in S. Typhi over a period of six years at AIIMS

Figure 3.15: Azithromycin MIC50 and MIC90in S. Typhi over a period of six years at AIIMS



	1	2	3	4	5	6	7	8	9	10	11	12	13
MIC Value	0.19	0.38	0.75	1	1.5	2	3	4	6	8	12	16	24
Total no. Strain	1	1	1	9	24	15	42	91	94	80	33	9	1

Figure 3.16: Azithromycin MIC in S.Typhi from all centers received at AIIMS in 2019

Molecular data and its relevance: *Salmonella* Typhi &*S.* Paratyphi A

No significant change was observed in molecular mechanism and DNA gyrase mutations remain the most important cause of resistance to quinolones. Fluoroquinolone resistance at molecular level was studied in 150 typhoidal *Salmonella* isolates. The most common mutation was S83 to F/Y followed by D87 to N/G/Y (table 3.17). Par C mutation was detected in three isolates only. No mutations were detected in *gyrB* and *parE* genes. Strains with more than one mutation in *gyrA* gene had higher MIC. Efflux pump was not responsible for resistance. All strains were negative for qnr. The molecular typing using MLST shows clonal dissemination of *Salmonella* Typhi and grouped *Salmonella* Typhi in ST1 and ST2 (table 3.18-3.20) and *Salmonella* Paratyphi in ST85 and ST129[5].

As there was no ceftriaxone resistant isolate was transported or isolated at Nodal Center, so molecular typing for ESBL was not applicable.

Table3.17: Mutation present in gyrA and parC gene in Salmonella Typhi and Salmonella Paratyphi A studied at AIIMS center.

Center Name	*gyrA* mutation		*parC* Mutation S80 ↓↑	Total no. Of Strains studied
	S83F/Y→	D87N/G/y→		
AIIMS	25	8	2	40
Vellore	8	2	ND	10
Chandigarh	17	5	ND	20
Puducherry	8	1	ND	10
Hinduja	8	1	ND	10
Sir Gangaram	16	8	ND	20
Apollo	13	10	ND	20
Hinduja	10	3	ND	10
KMC, Karnataka	8	2	1	10

Table 3.18: PCR primers, conditions and product size of all the seven housekeeping genes used for MLST

S.No	Gene	PCR Primers	Annealing temperature	Product Size
1.	**AroC**	*F 5'-CCTGGCACCTCGCGCTATAC-3'* *R 5'-CCACACACGGATCGTGGCG-3'*	65 °C, 60 Sec	826 bp
2.	**HemD**	*F 5'-GAAGCGTTAGTGAGCCGTCTGCG-3'* *R 5'-ATCAGCGACCTTAATATCTTGCCA-3'*	65 °C , 60 Sec	666 bp
3.	**HisD**	*F 5'-GAAACGTTCCATTCCGCGCAGAC-3'* *R 5'-CTGAACGGTCATCCGTTTCTG-3'*	65 °C , 60 Sec	894 bp
4.	**PurE**	*F 5'-ATGTCTTCCCGCAATAATCC-3'* *R 5'-TCATAGCGTCCCCCGCGGATC-3'*	55 °C, 60 Sec	510 bp
5.	**SucA**	*F 5'-AGCACCGAAGAGAAACGCTG-3'* *R 5'-GGTTGTTGATAACGATACGTAC-3'*	55 °C, 60 Sec	643 bp
6.	**ThrA**	*F 5'-GTCACGGTGATCGATCCGGT-3'* *R 5'-CACGATATTGATATTAGCCCG-3'*	55 °C, 60 Sec	852 bp
7.	**DnaN**	*F 5'-ATGAAATTTACCGTTGAACGTGA-3'* *R 5'-AATTTCTCATTCGAGAGGATTGC-3'*	62 °C, 60 Sec	833 bp

Figure 3.19: PCR for all seven housekeeping genes used for amplification in MLST.

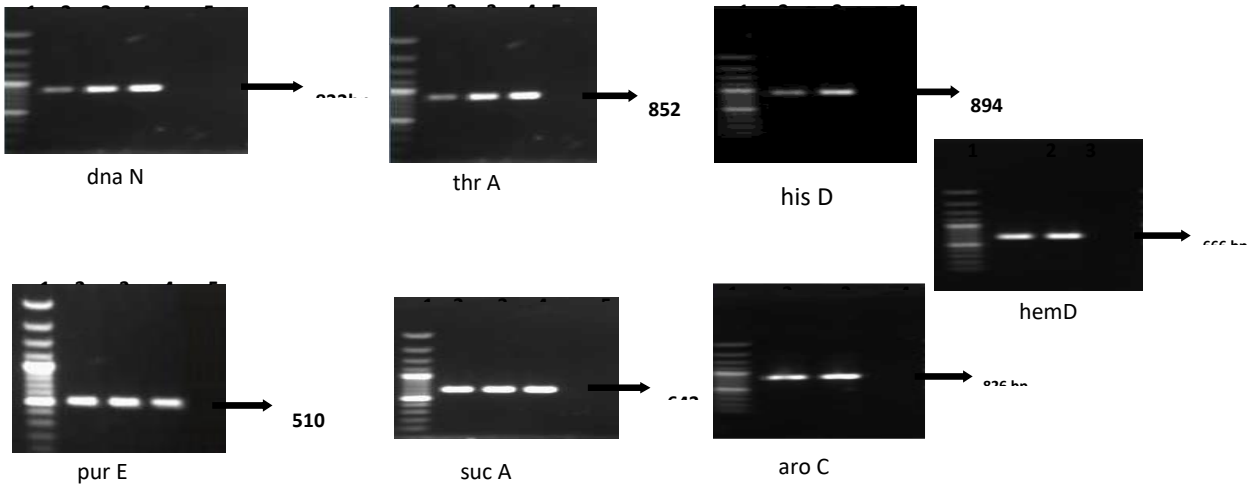


Table 3.20: MLST details

Centre Name	*Salmonella* Typhi		*Salmonella* Paratyphi A
	ST 1	ST 2	ST85
AIIMS	2	25	5
Vellore	1	10	1
Chandigarh	10	15	
Puducherry	1	8	
Hinduja	2	10	2
Sir Gangaram	1	12	1
Apollo		8	
KMC,Kanataka	2	13	2
Total strain studied	19	101	6

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Chapter 4 Non fermenting Gram Negative Bacteria (NFGNB)

Summary of results

The overall isolation rate of Non-fermenting gram negative bacilli was 20.1% during Jan-Dec 2019 across all AMRSN sites. Among which, *Pseudomonas aeruginosa* was the most commonly isolated pathogen (11.7%), followed by *Acinetobacter baumannii* (7.9%) and <1% of *Burkholderia cepacia* (0.2%) and *Stenotrophomonas maltophilia* (0.3%). However, there are differences in the isolation rates based on the clinical settings from where these were isolated. Notably, *P. aeruginosa* was predominantly isolated in wards (12.1%) and ICU (11.9%) compared to OPD (10.8%), while *A. baumannii* was predominant in ICU (21.2%), followed by ward (7.5%) and OPD (2.6%) respectively (Figure 4.1)

However, trend analysis over the years 2016 – 2019 have shown a steady decline in the isolation rates of *P. aeruginosa* from 15% to 12% in 2016 to 2019 respectively. In contrast, isolation trend of *A. baumannii* found to increase from 5% to 8% between 2016 and 2019 respectively. No significant changes in the isolation rates of other pathogens such as *B. cepacia* and *S. maltophilia* have been noted (Figure 4.2).

This was further supported by molecular characterization of drug-resistant *P. aeruginosa* strains, wherein *bla*$_{VIM}$ and *bla*$_{NDM}$are the predominant Metallo beta-lactamases, and *bla*$_{VEB}$ and *bla*$_{PER}$ were the ESBLs found across all centers. Among *A. baumannii* isolates, *bla*$_{PER}$ and *bla*$_{TEM}$ were the predominant genes observed. Whereas, *bla*$_{OXA-23}$and *bla*$_{NDM}$are the predominant Metallo beta-lactamases found across all centers. Considering the phenotypic and molecular profile, it is crucial to choose the appropriate treatment particularly for the management of infection caused by carbapenem resistant organisms.

Acinetobacter baumannii

A. baumannii is resistant to almost all the available earlier drugs and increased antimicrobial resistance has also been implicated in nosocomial infections and hospital outbreaks. Analysis of antimicrobial susceptibility profile of *A. baumannii* showed that the isolates collected from ICU showed reduced susceptibility rates (<12%) to all the tested antibiotics compared to isolates from ward and OPD (Table 4.1), except for minocycline which showed susceptible rate of 50%. Among OPD isolates, amikacin showed comparatively increased susceptibility of 47% than in wards and ICU (10%) (Table 4.1). Of all the agents, minocycline is the only agent showing highest susceptibility of up to 60%, compared to any other agents. Among the various specimens tested against different classes of antibiotics, susceptible rates are less among the isolates from specimens like

LRT, deep infections and superficial infections (Table 4.2). Among BSIs, susceptibility to minocycline, netilmicin and tobramycin were 60%, 45% and 43% respectively. Minocycline has substantial *in-vitro* activity against CRAB as shown by various studies. However, further studies are necessary to consider minocycline as a treatment due to adverse events reported from clinical studies. Among the tested antibiotics, only colistin showed>90% susceptibility. Colistin in combination with other antibiotics such as meropenem are commonly preferred over monotherapy against CRAB. Further, trend analysis of susceptibility profile between the years 2016 and 2019showeddeclingsusceptibility for ceftazidime and cefepime followed by piperacillin-tazobactam, imipenem, meropenem and amikacin. There has been reduced susceptibility to all these antibiotics from 2016 to 2019, (Table 4.3: Figure 4.3).

Molecular characterization of 429 isolates from various regional centers as mentioned in Table 4.4 showed that the co-occurrence of resistance genes was observed predominantly in *A. baumannii* isolates. All the isolates harbored the *bla*$_{\text{OXA-51}}$ like gene, which is intrinsic to *Acinetobacter baumannii*. Among ESBLs, *bla*$_{\text{PER}}$and *bla*$_{\text{TEM}}$ were the predominant genes observed. Whereas, *bla*$_{\text{OXA-23}}$and *bla*$_{\text{NDM}}$ are the predominant Metallo beta-lactamases found across all centers. Co-producers of various AMR genes like ESBLs with carbapenemases and combination of carbapenemases were observed across all the centers. None of the isolates had *bla*$_{\text{OXA-24}}$ like, *bla*$_{\text{OXA-58}}$ like, *bla*$_{\text{IMP}}$ like, *bla*$_{\text{VIM}}$ like, *bla*$_{\text{SIM}}$ like, *bla*$_{\text{KPC}}$ like and *bla*$_{\text{GES}}$ like carbapenemases.

Pseudomonas aeruginosa

Isolation rates among different clinical specimens showed high rates of *P. aeruginosa* from superficial infections and lower respiratory tract infections. Antimicrobial susceptibility testing revealed lower susceptibility rates in isolates from ICU settings (45-55%), followed by Wards (60-70%) and OPD (70-80%) respectively (Table 4.5). More than 90% susceptibility was observed for colistin. Notably, carbapenem susceptibility was seen only in 50% of the isolates from ICU, followed by 35% from ward and 20% from OPD. Similarly, aminoglycoside susceptibility was found to be 45% in ICU, followed 35% and 20% in ward and OPD respectively. However, isolates from CSF and Urine have shown to exhibit lower susceptibility profile to all anti-pseudomonal agents compared to isolates from other specimens. Notably, among the LRT isolates, highest susceptibility was seen for piperacillin/tazobactam (75.4%), followed by cephalosporins (71%), meropenem (73%), amikacin (78%) and tobramycin (80%); however 96% for colistin may not be appropriate for management of lung infections. Similar profile was seen for blood isolates with colistin being the highest susceptible agent, followed by piperacillin/tazobactam, meropenem, aminoglycosides and cephalosporins (Table 4.6).

Trend analysis of antimicrobial susceptibility pattern over a four-year period from 2016-2019 showed that the non-susceptibility to imipenem has increased from 2016 to 2019 with no changes in the meropenem susceptibility. No significant changes were observed for fluoroquinolones and aminoglycosides. Notably, decreasing susceptibility to colistin seems to be rise from 2% in 2016 to 7% in 2019 respectively, which is alarming (Table 4.7, Figure 4.4). Based on the susceptibility profile and the settings where the isolates are likely from, it would be ideal to choose agents as the profile varies with different settings. However, combination agents of any two-antipseudomonal could be preferred to overcome the high resistance rates, which are always recommended for pseudomonal infections.

Molecular characterization of a total of 768 *P. aeruginosa* isolated from various clinical specimens were received at the reference laboratory. The details have been shown in Table 4.8. Of which, 158 were identified (at present) as carbapenem resistant and were screened for the presence of beta lactamase by molecular methods. Of all the beta lactamases screened, *bla*$_{VEB}$ was the most common ESBL and few *bla*$_{SHV}$ genes were identified. Similarly, among the carbapenemases, *bla*$_{NDM}$ was the most common metallo beta lactamse (carbapenemase) identified, followed by *bla*$_{VIM}$ and few *bla*$_{IMP}$. Co-producers of *bla*$_{VIM+}$*bla*$_{NDM}$ were identified in more numbers than individual carbapenemases and as well with ESBL genes such as *bla*$_{GES}$and *bla*$_{VEB}$. Trend analysis shows there has been a shift from *bla*$_{VIM}$ to *bla*$_{NDM}$ producers across different geographical location during the year 2019. However, this needs to be validated with testing of all the pending isolates.

Burkholderia cepacia

Burkholderia cepacia complex (BCC) is an important nosocomial pathogen in hospitalised patients and its antimicrobial resistance being a significant concern. BCC is intrinsically resistant to aminoglycosides, first-and second-generation cephalosporins, anti-pseudomonal penicillins and polymyxins. Also, BCC frequently develops resistance to β-lactams due to presence of inducible chromosomal β-lactamases and altered penicillin-binding proteins. Notably, on primary isolation, the organism may be susceptible to trimethoprim-sulfamethoxazole and antipseudomonal β-lactams *in-vitro*. However, under antimicrobial pressure, resistance rapidly develops and thus makes the treatment challenging. Table 4.9 shows the location-wise susceptibilities of *B. cepacia* across OPD, ward and ICU. Overall, ward and ICU had reduced susceptible rates in comparison to OPD. Some antibiotics such as ceftazidime, carbapenem and ciprofloxacin display some *in vitro* activities against BCC. As per the CLSI 2019 guidelines, the drugs recommended against BCC are ceftazidime, minocycline, meropenem and cotrimoxazole.

For ceftazidime, OPD isolates showed susceptible rates of 92.3% whereas ward and ICU isolates had 90% and 78% respectively. Meropenem showed 86-90% susceptibility rate in OPD, wards and ICU. Table 4.10 shows sample-wise susceptible rates for *B. cepacia*. Among

all agents, trimethoprim-sulfamethoxazole showed highest susceptibility among blood isolates. In contrast, for LRT isolates, minocycline did not show higher susceptibility comparable to blood isolates, which was 68%.; while trimethoprim-sulfamethoxazole, meropenem and minocycline showed >70% susceptibility. Yearly susceptible trends of *B. cepacia* depicted in Table 4.11 and Figure 4.5 showed improved susceptibility between the years 2017 and 2018 for trimethoprim-sulfamethoxazole, meropenem and minocycline, which is in contrast for minocycline, where there was a decline in susceptibility rate from 2017 (85%) to 2019 (75%). Successful treatment using combinations of meropenem with ciprofloxacin and tobramycin has also been reported which can be considered as an alternative for organism resistant to multiple antibiotics. However, the clinical outcome should be studied to understand the true figure.

Stenotrophomonas maltophilia

*Stenotrophomonas maltophilia*is is an emerging multidrug-resistant global opportunistic pathogen. The increasing incidence of nosocomial and community-acquired *S. maltophilia* infections is of particular concern. The preferred treatment for *S. maltophilia* infections has been the use of trimethoprim-sulfamethoxazole and minocycline. Table 4.12 depicts location-wise susceptible trend of *S. maltophilia* across OPD, ward and ICU. There was an increase in susceptibility noted for ceftazidime, with decline in susceptibility rates for levofloxacin, minocycline and trimethoprim-sulfamethoxazole. In case of trimethoprim sulfamethoxazole, susceptibility was less in ICU patients (84%) in comparison to ward and OPD (>90%). In contrast, ceftazidime susceptibility was higher in ICU isolates (71.4%), which was <60% in wards and OPD. Table 4.13 depicts sample-wise susceptible trend of *S. maltophilia* which shows that among LRT samples, ceftazidime had least susceptible rate (70.6%), whereas other agents were >80% susceptible. Overall, minocycline, trimethoprim-sulfamethoxazole and levofloxacin had high susceptible rate of 95%, 90% and 86% respectively. Among blood samples, minocycline showed highest susceptibility of 96.3%. Table 4.14 and Figure 4.6 shows year-wise susceptible trend of *S. maltophilia* from all samples. There were minor changes observed between the years 2016 and 2018.The isolates exhibits 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.

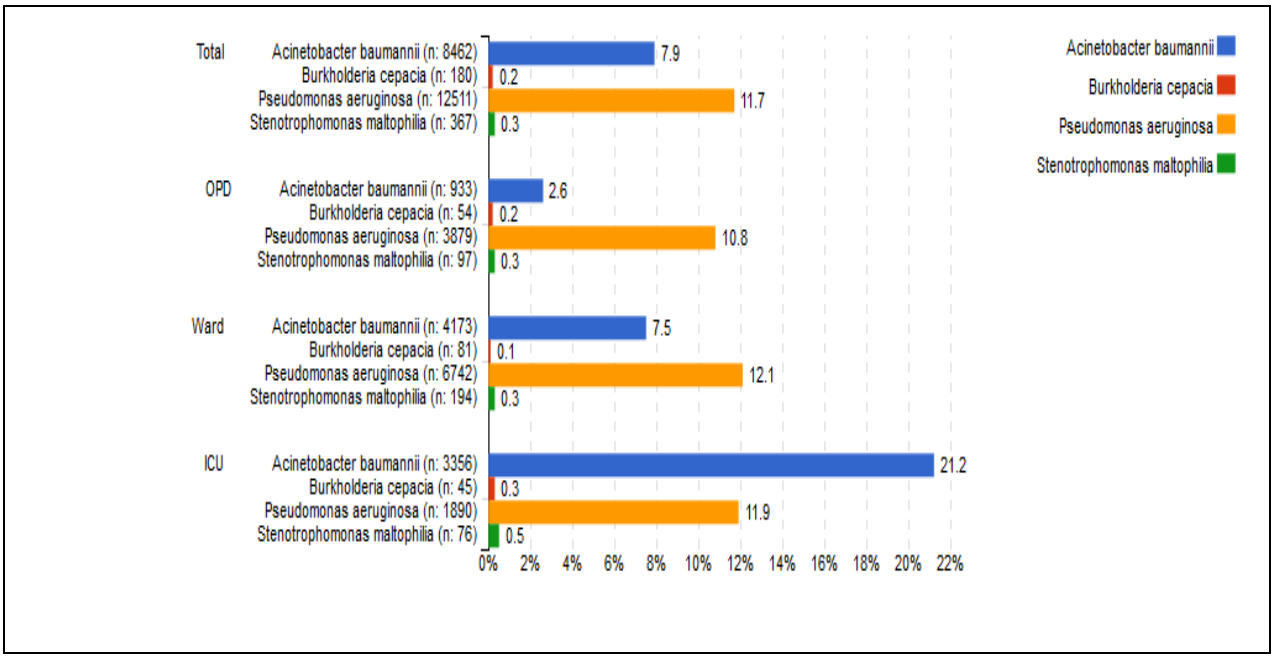

Figure 4.1: Location-wise Isolation pattern of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples across OPD, Ward and ICU

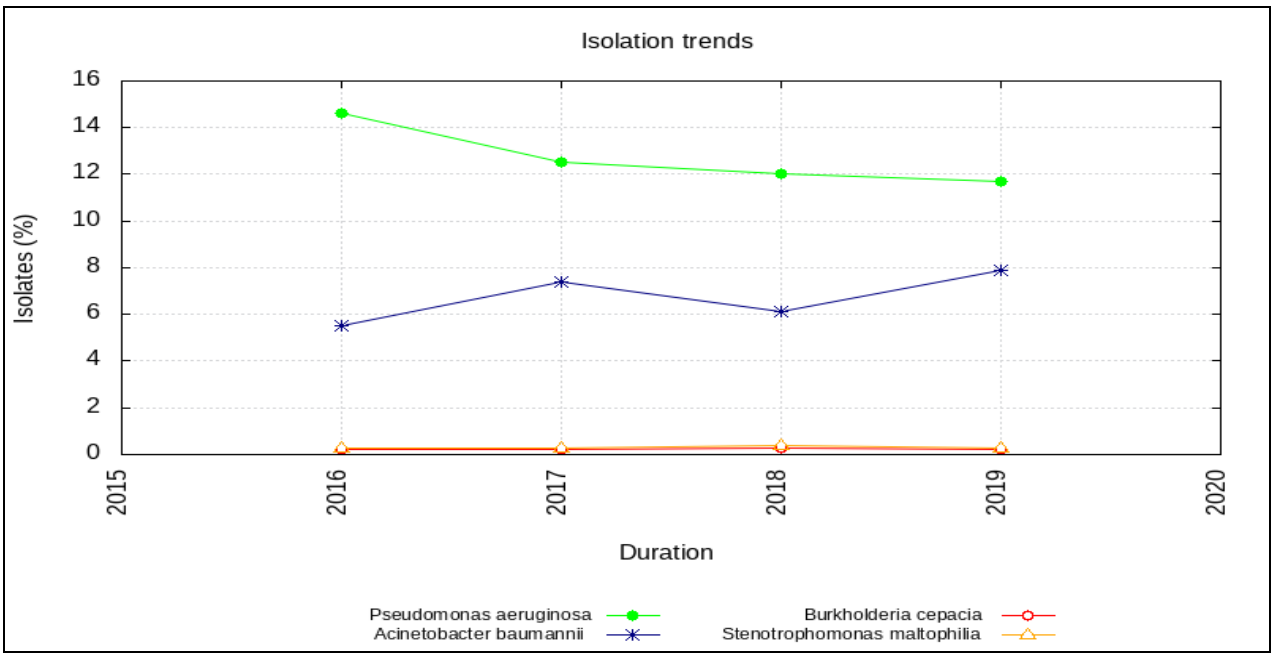

Figure 4.2: Yearly Isolation trend of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples

Table 4.1: Location-wise susceptible percentage of *A. baumannii* isolated from all samples across OPD, Ward and ICU

AMA	Total n=8460	OPD n=931	Ward n=4173	ICU n=3356
	(S %)	(S %)	(S %)	(S %)
Ceftazidime	904/7393 (12.2)	250/728 (34.3)	519/3745 (13.9)	135/2920 (4.6)
Cefepime	1037/8202 (12.6)	274/890 (30.8)	593/4026 (14.7)	170/3286 (5.2)
Piperacillin-tazobactam	1241/7939 (15.6)	324/868 (37.3)	692/3793 (18.2)	225/3278 (6.9)
Imipenem	1095/7200 (15.2)	258/758 (34)	599/3259 (18.4)	238/3183 (7.5)
Meropenem	1740/8327 (20.9)	412/909 (45.3)	934/4116 (22.7)	394/3302 (11.9)
Amikacin	1419/6950 (20.4)	354/774 (45.7)	779/3403 (22.9)	286/2773 (10.3)
Levofloxacin	1489/7777 (19.1)	346/841 (41.1)	792/3726 (21.3)	351/3210 (10.9)
Minocycline	3850/6376 (60.4)	433/578 (74.9)	2093/3138 (66.7)	1324/2660 (49.8)
Colistin	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)

Table 4.2: Sample-wise susceptible percentage of *A. baumannii*

AMA	Blood	LRT	Superficial Infection	Deep Infection	CSF	Urine
	n=1428	n=3386	n=1756	n=617	n=130	n=305
Ceftazidime	277/1333 (20.8)	253/2890 (8.8)	158/1554 (10.2)	57/583 (9.8)	5/88 (5.7)	69/258 (26.7)
Cefepime	314/1392 (22.6)	276/3309 (8.3)	177/1710 (10.4)	70/601 (11.6)	21/130 (16.2)	79/263 (30)
Piperacillin-tazobactam	343/1333 (25.7)	320/3254 (9.8)	220/1626 (13.5)	75/523 (14.3)	30/122 (24.6)	116/298 (38.9)
Imipenem	319/1234 (25.9)	274/3018 (9.1)	204/1421 (14.4)	54/409 (13.2)	19/123 (15.4)	112/268 (41.8)
Meropenem	429/1419 (30.2)	495/3328 (14.9)	362/1725 (21)	96/615 (15.6)	26/130 (20)	142/289 (49.1)
Amikacin	350/1187 (29.5)	430/2865 (15)	265/1378 (19.2)	121/567 (21.3)	13/58 (22.4)	116/271 (42.8)
Levofloxacin	389/1297 (30)	446/3216 (13.9)	293/1611 (18.2)	82/551 (14.9)	17/85 (20)	115/254 (45.3)
Minocycline	754/1141 (66.1)	1336/2632 (50.8)	867/1272 (68.2)	379/535 (70.8)	41/75 (54.7)	136/173 (78.6)
Colistin	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)

Table 4.3: Yearly susceptible trend of *A. baumannii* isolated from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=396	Total n=3361	Total n=4550	Total n=8462
	(S%)	(S%)	(S%)	(S%)
Ceftazidime	56/328 (17.1)	356/3204 (11.1)	575/4165 (13.8)	905/7397 (12.2)
Cefepime	67/318 (21.1)	369/3302 (11.2)	587/4458 (13.2)	1038/8205 (12.7)
Piperacillin-tazobactam	94/335 (28.1)	485/3189 (15.2)	760/4495 (16.9)	1242/7943 (15.6)
Imipenem	104/334 (31.1)	502/3348 (15)	818/4518 (18.1)	1095/7204 (15.2)
Meropenem	100/331 (30.2)	616/3289 (18.7)	953/4179 (22.8)	1741/8331 (20.9)
Amikacin	102/347 (29.4)	638/3314 (19.3)	877/3796 (23.1)	1420/6954 (20.4)
Levofloxacin	104/312 (33.3)	887/3042 (29.2)	959/4048 (23.7)	1489/7781 (19.1)
Minocycline	0/0	926/1380 (67.1)	2393/3726 (64.2)	3852/6379 (60.4)
Colistin	0/0	0/0	0/0	0/0

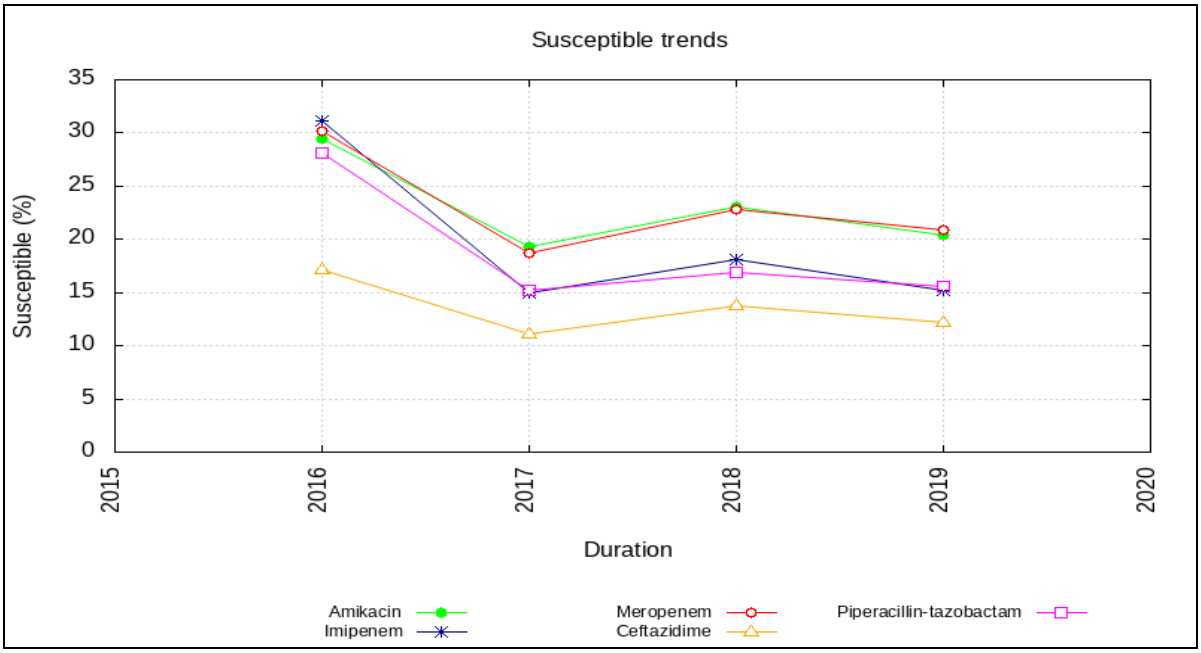

Figure 4.3: Yearly susceptible trend of *A. baumannii* isolated from all samples

Table 4.4: Molecular characterization of carbapenem resistant *A. baumannii* collected across India during the year 2019

Centers (n)	Total tested	OXA-51	OXA-23 only	NDM only	OXA-23 + NDM	OXA-23 + PER	OXA-23 + TEM	OXA-23 + NDM + TEM	OXA-23 + NDM + PER	OXA-23 + PER + TEM	OXA-23 + NDM + TEM + PER	Negative
CMC (n=60)	60	60	21	0	17	11	5	0	3	3	0	0
MGIMS (n=80)	62	62	27	0	18	16	0	1	0	0	0	0
TMC (n=40)	34	34	14	1	14	3	0	0	2	0	0	0
AIIMS, Jodhpur (n=77)	63	63	41	0	5	8	7	0	2	0	0	0
Sir Ganga Ram Hospital (n=60)	58	58	22	0	10	4	8	6	4	3	1	0
Kasturba medical college (n=21)	21	21	11	0	7	2	0	1	0	0	0	0
Nizam's Institute of Medical science (n=64)	27	27	9	0	7	7	1	0	2	1	0	0
King George medical university (n=4)	2	2	0	0	2	0	0	0	0	0	0	0
JIPMER (n=55)	47	47	16	0	16	5	7	0	1	0	0	2
Apollo hospital (n=36)	24	24	8	0	4	6	3	0	3	0	0	0
Armed Forces Medical college (n=38)	28	28	10	0	5	7	0	0	5	0	0	1
Assam medical college (n=26)	22	22	7	1	2	9	2	1	0	0	0	0
Lokmanya Tilak hospital (n=73)	61	61	26	1	20	13	0	0	0	0	1	0
Regioanl Institute of Medical science (n=10)	5	5	1	0	1	1	1	1	0	0	0	0
IPGMER (n=7)	7	7	3	0	1	0	2	1	0	0	0	0
PD Hindhuja (n=33)	12	12	7	0	3	0	2	0	0	0	0	0
Total (n=684)	533	533 (100%)	223 (42%)	3 (1%)	115 (22%)	98 (18%)	44 (8%)	16 (3%)	19 (4%)	7 (1%)	5 (1%)	3 (1%)

Table 4.5: Location-wise susceptible percentage of *Pseudomonas aeruginosa* isolated from all samples (except faeces) across OPD, Ward and ICU.

AMA	Total n=12507	OPD n=3879	Ward n=6738	ICU n=1890
	(S %)	(S %)	(S %)	(S %)
Ceftazidime	7476/11852 (63.1)	2682/3665 (73.2)	3906/6389 (61.1)	888/1798 (49.4)
Cefepime	7588/11913 (63.7)	2692/3646 (73.8)	3977/6426 (61.9)	919/1841 (49.9)
Piperacillin-tazobactam	8334/11303 (73.7)	2911/3525 (82.6)	4349/6029 (72.1)	1074/1749 (61.4)
Imipenem	6356/10108 (62.9)	2281/3079 (74.1)	3295/5403 (61)	780/1626 (48)
Meropenem	8185/12115 (67.6)	2932/3715 (78.9)	4290/6547 (65.5)	963/1853 (52)
Amikacin	8283/12205 (67.9)	2941/3813 (77.1)	4282/6548 (65.4)	1060/1844 (57.5)
Gentamicin	5762/9266 (62.2)	2083/2914 (71.5)	2926/4837 (60.5)	753/1515 (49.7)
Tobramycin	4569/6664 (68.6)	1592/1972 (80.7)	2487/3813 (65.2)	490/879 (55.7)
Ciprofloxacin	6239/10823 (57.6)	2209/3371 (65.5)	3276/5905 (55.5)	754/1547 (48.7)
Levofloxacin	6104/10800 (56.5)	2198/3368 (65.3)	3084/5697 (54.1)	822/1735 (47.4)
Colistin	1722/1854 (92.9)	422/465 (90.8)	919/984 (93.4)	381/405 (94.1)

Table 4.6: Sample-wise susceptible percentage of *Pseudomonas aeruginosa*

AMA	Blood	LRT	Superficial Infection	Deep Infection	CSF	Urine
	n=868	n=3549	n=3867	n=955	n=102	n=1754
Ceftazidime	571/846 (67.5)	2443/3418 (71.5)	2285/3646 (62.7)	583/929 (62.8)	53/100 (53)	679/1579 (43)
Cefepime	567/835 (67.9)	2413/3368 (71.6)	2344/3695 (63.4)	563/894 (63)	56/101 (55.4)	769/1655 (46.5)
Piperacillin-tazobactam	598/805 (74.3)	2569/3262 (78.8)	2580/3420 (75.4)	598/854 (70)	63/94 (67)	966/1622 (59.6)
Imipenem	431/635 (67.9)	1623/2463 (65.9)	2226/3410 (65.3)	481/706 (68.1)	50/93 (53.8)	815/1623 (50.2)
Meropenem	613/855 (71.7)	2476/3395 (72.9)	2644/3785 (69.9)	642/940 (68.3)	55/101 (54.5)	838/1682 (49.8)
Amikacin	616/850 (72.5)	2729/3493 (78.1)	2489/3787 (65.7)	637/950 (67.1)	41/84 (48.8)	873/1708 (51.1)
Gentamicin	480/674 (71.2)	1600/2242 (71.4)	1853/3060 (60.6)	423/646 (65.5)	29/78 (37.2)	757/1566 (48.3)
Tobramycin	306/428 (71.5)	1733/2149 (80.6)	1451/2244 (64.7)	262/420 (62.4)	35/62 (56.5)	322/691 (46.6)
Ciprofloxacin	425/644 (66)	1814/2695 (67.3)	2076/3587 (57.9)	543/921 (59)	34/82 (41.5)	692/1676 (41.3)
Levofloxacin	505/758 (66.6)	2168/3212 (67.5)	1818/3325 (54.7)	416/753 (55.2)	40/87 (46)	544/1451 (37.5)
Colistin	180/194 (92.8)	418/437 (95.7)	528/583 (90.6)	145/149 (97.3)	20/20 (100)	224/237 (94.5)

Table 4.7: Yearly susceptible trend of *Pseudomonas aeruginosa* isolated from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=1057	Total n=5689	Total n=8882	Total n=12511
	(S%)	(S%)	(S%)	(S%)
Ceftazidime	624/1035 (60.3)	3604/5506 (65.5)	5665/8601 (65.9)	7479/11855 (63.1)
Cefepime	585/981 (59.6)	3076/5005 (61.5)	5260/8286 (63.5)	7592/11917 (63.7)
Piperacillin-tazobactam	705/1036 (68.1)	3759/5452 (68.9)	6034/8499 (71)	8338/11307 (73.7)
Imipenem	810/1017 (79.6)	4061/5516 (73.6)	5629/8379 (67.2)	6360/10112 (62.9)
Meropenem	651/970 (67.1)	3492/5085 (68.7)	5735/8294 (69.1)	8189/12119 (67.6)
Amikacin	693/1030 (67.3)	3866/5611 (68.9)	6020/8749 (68.8)	8287/12209 (67.9)
Gentamicin	402/776 (51.8)	2528/4251 (59.5)	4078/6465 (63.1)	5766/9270 (62.2)
Tobramycin	579/957 (60.5)	2955/4366 (67.7)	3809/5602 (68)	4569/6664 (68.6)
Ciprofloxacin	436/842 (51.8)	2932/5071 (57.8)	4815/8028 (60)	6243/10827 (57.7)
Levofloxacin	536/958 (55.9)	3238/5353 (60.5)	4795/8219 (58.3)	6108/10804 (56.5)
Colistin	711/723 (98.3)	1729/1740 (99.4)	985/1077 (91.5)	1722/1854 (92.9)

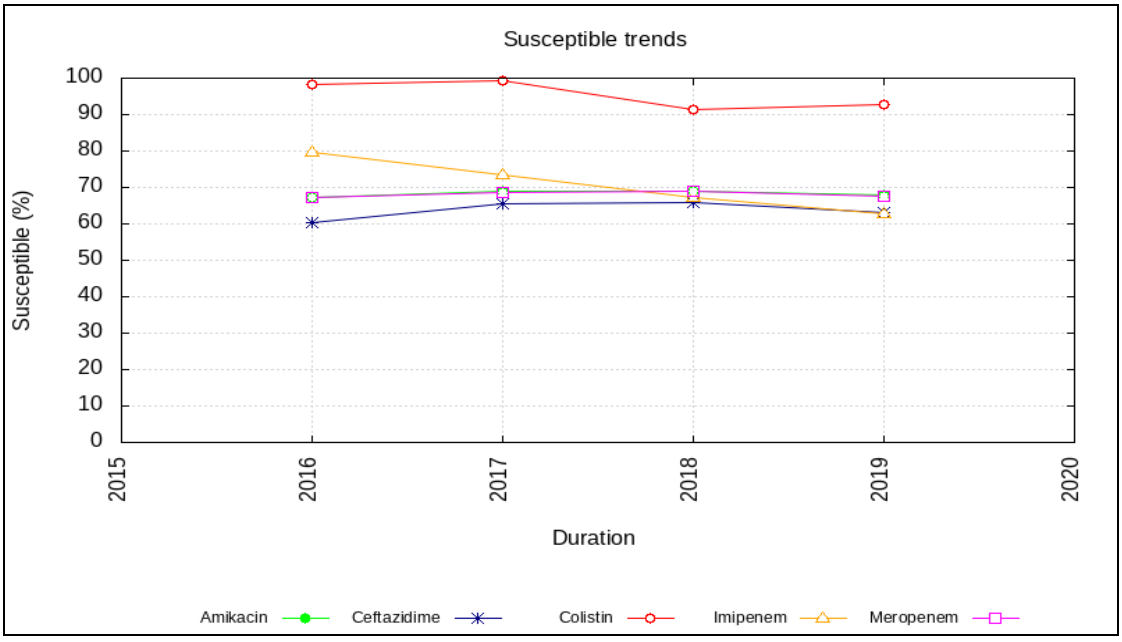

Figure 4.4: Yearly susceptible trend of *Pseudomonas aeruginosa* isolated from all samples.

Table 4.8: Molecular characterization of carbapenem resistant *P. aeruginosa* collected across India during the year 2019

Centers	*P. aeruginosa*	ESBL				Class A Carbapenemase		Class B carbapenemase (MβLs)				Co-producers
	Total (R tested)	SHV	TEM	VEB	PER	KPC	GES	SPM	IMP	VIM	NDM	Others
CMC	24 (24)	1	-	2	-	-	2	-	5	1	1	PER&NDM-1 TEM&VIM-3 VEB&IMP-1 VEB&NDM-1 VEB&VIM-2 IMP&VEB,GES-1
AIIMS	56 (11)	-	-	1	-	-	-	-	-	6	2	-
JIPMER	70 (6)	1	-	-	-	-	-	-			1	-
PGIMER	38(7)	-	-		-	-	-	-	-	2	-	-
TATA MEDICAL CENTRE	40 (35)	-	-	3	-	-	-	-	-	4	4	VIM&NDM-7 VIM,NDM&GES-1 VEB&VIM,NDM-8 VEB&NDM-1
SIR GANGARAM	60 (14)	-		1	-	-	-	-	-	4	4	NDM&VIM-3 VEB&NDM,VIM-1
MGIMS	80 (14)	-	-		-	-	-	-	-			-
APOLLO	56 (11)	-	-	-	-	-	-	-	-	-	1	NDM&VEB-3 VEB&VIM,NDM-1
P.D.HINDUJA	77 (11)	-	-	-	-	-	2	-	-	-	1	GES&NDM-4 GES,VEB&NDM-1 GES,VIM&NDM-3
NIMS	70 (5)	-	-	-	-	-		-	-		2	VEB&NDM-1
SKIMS	1 (1)	-	-	-	-	-					-	-
NODEL(KMC	16 (2)	-	-	-	-	-	1	-	-	-	-	GES&NDM-1
AMC	45 (4)	-	-		-	-	-	-	-		3	VIM&NDM-1
AFMC	54 (4)	-	-	-	-	-	-	-	-	-	4	-
RIIMS	5 (1)	-	-	-	-	-	-	-	-	-	-	GES&VEB-1
KGMU	7 (2)	-	-	-	-	-	-	-	-	-	1	VEB&NDM-1
LTMMC	69(6)	-	-	1	-	-	-	-	-	-	1	-
Total	**768 (158)**	**2 (1%)**	**-**	**8 (5%)**	**-**	**-**	**5 (3%)**	**-**	**5 (3%)**	**17 (11%)**	**25 (16%)**	**47 (30%)**

Table 4.9: Location-wise susceptible percentage of *Burkholderia cepacia* isolated from all samples across OPD, Ward and ICU.

AMA	*Burkholderia cepacia*			
	Total n=180	**OPD n=54**	**Ward n=81**	**ICU n=45**
	(S %)	**(S %)**	**(S %)**	**(S %)**
Ceftazidime	155/177 (87.6)	48/52 (92.3)	72/80 (90)	35/45 (77.8)
Chloramphenicol	3/3	2/2	1/1	*0/0
Levofloxacin	70/124 (56.5)	21/32 (65.6)	36/59 (61)	13/33 (39.4)
Meropenem	160/180 (88.9)	48/54 (88.9)	73/81 (90.1)	39/45 (86.7)
Minocycline	132/173 (76.3)	43/51 (84.3)	60/79 (75.9)	29/43 (67.4)
Ticarcillin-clavulanic acid	36/102 (35.3)	8/26 (30.8)	25/46 (54.3)	3/30 (10)
Trimethoprim-sulfamethoxazole	163/176 (92.6)	51/54 (94.4)	74/77 (96.1)	38/45 (84.4)

Table 4.10: Sample-wise susceptible percentage of *Burkholderia cepacia*

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection	Urine
	n=180	**n=84**	**n=28**	**n=14**	**n=12**	**n=19**
Ceftazidime	155/177 (87.6)	74/83 (89.2)	21/28 (75)	12/13 (-)	11/11 (-)	18/19 (-)
Chloramphenicol	3/3 (-)	0/0 (-)	*0/0 (-)	0/0 (-)	2/2 (-)	0/0 (-)
Levofloxacin	70/124 (56.5)	37/56 (66.1)	*5/16 (-)	6/11 (-)	7/9 (-)	11/17 (-)
Meropenem	160/180 (88.9)	72/84 (85.7)	25/28 (89.3)	12/14 (-)	11/12 (-)	19/19 (-)
Minocycline	132/173 (76.3)	62/81 (76.5)	19/28 (67.9)	10/14 (-)	8/11 (-)	15/16 (-)
Ticarcillin-clavulanic acid	36/102 (35.3)	19/49 (38.8)	3/14 (-)	3/6 (-)	3/7 (-)	6/13 (-)
Trimethoprim-sulfamethoxazole	163/176 (92.6)	77/82 (93.9)	23/27 (85.2)	12/14 (-)	12/12 (-)	18/18 (-)

Table 4.11: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples.

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=18	Total n=112	Total n=197	Total n=180
	(S%)	(S%)	(S%)	(S%)
Ceftazidime	5/13	73/101 (72.3)	137/192 (71.4)	155/177 (87.6)
Chloramphenicol	0/0	0/0	1/1	3/3
Levofloxacin	0/0	4/13	34/66 (51.5)	70/124 (56.5)
Meropenem	7/14	83/111 (74.8)	140/171 (81.9)	160/180 (88.9)
Minocycline	14/16	89/104 (85.6)	146/185 (78.9)	132/173 (76.3)
Ticarcillin-clavulanic acid	0/0	0/9	4/51 (7.8)	36/102 (35.3)
Trimethoprim-sulfamethoxazole	8/9	84/109 (77.1)	179/192 (93.2)	163/176 (92.6)

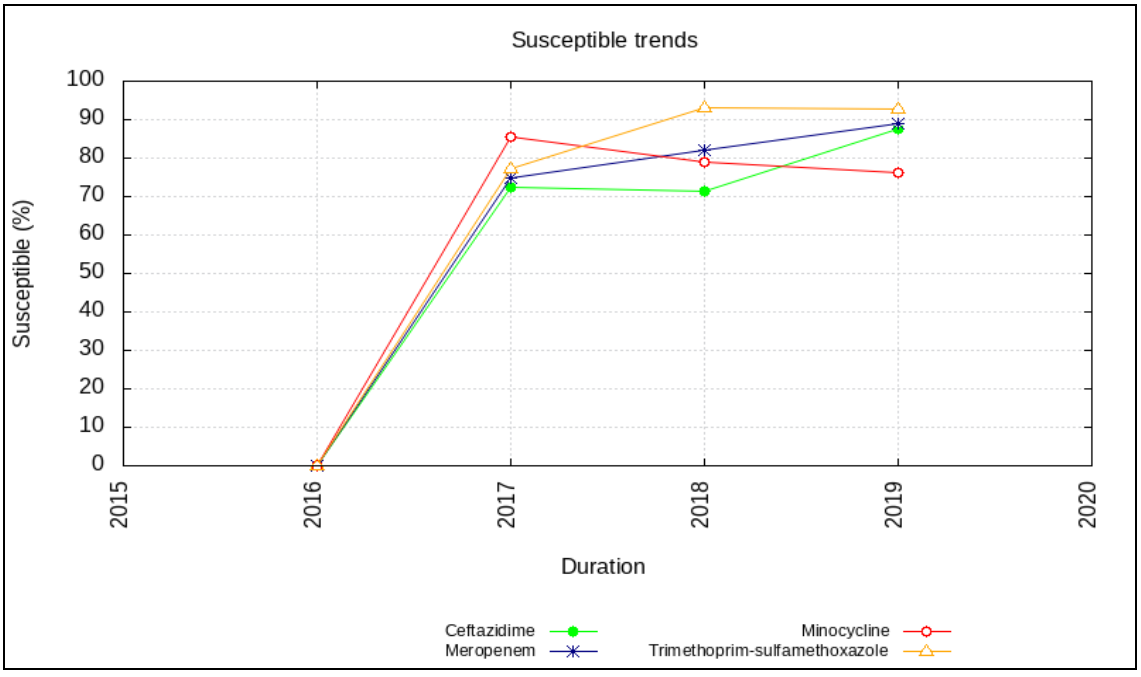

Figure 4.5: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples.

Table 4.12: Location-wise susceptible percentage of *Stenotrophomonas maltophilia* isolated from all samples across OPD, Ward and ICU.

AMA	*Stenotrophomonas maltophilia*			
	Total n=367	OPD n=97	Ward n=194	ICU n=76
	(S %)	(S %)	(S %)	(S %)
Ceftazidime	46/73 (63)	19/32 (59.4)	12/20 (60)	15/21 (71.4)
Chloramphenicol	3/3	0/0	3/3	0/0
Levofloxacin	218/254 (85.8)	74/81 (91.4)	98/115 (85.2)	46/58 (79.3)
Minocycline	331/350 (94.6)	86/90 (95.6)	178/187 (95.2)	67/73 (91.8)
Ticarcillin-clavulanic acid	59/68 (86.8)	27/29 (93.1)	15/21 (71.4)	17/18
Trimethoprim-sulfamethoxazole	327/365 (89.6)	89/96 (92.7)	175/194 (90.2)	63/75 (84)

Table 4.13: Sample-wise susceptible percentage of *Stenotrophomonas maltophilia*.

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection	Urine
	n=366	n=114	n=141	n=33	n=30	n=14
Ceftazidime	45/72 (62.5)	6/15 (-)	24/34 (70.6)	2/7 (-)	1/1 (-)	3/5 (-)
Chloramphenicol	3/3 (-)	1/1 (-)	0/0 (-)	1/1 (-)	1/1 (-)	0/0 (-)
Levofloxacin	217/253 (85.8)	65/72 (90.3)	82/99 (82.8)	20/25 (80)	17/17 (-)	11/14 (-)
Minocycline	330/349 (94.6)	105/109 (96.3)	132/140 (94.3)	26/30 (86.7)	23/26 (88.5)	11/11 (-)
Ticarcillin-clavulanic acid	58/67 (86.6)	9/11 (-)	31/34 (91.2)	6/8 (-)	1/1 (-)	3/5 (-)
Trimethoprim-sulfamethoxazole	326/364 (89.6)	109/114 (95.6)	121/139 (87.1)	28/33 (84.8)	27/30 (90)	12/14 (-)

Table 4.14: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples.

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=23	Total n=157	Total n=309	Total n=367
	(S%)	(S%)	(S%)	(S%)
Ceftazidime	0/0	15/27 (55.6)	42/63 (66.7)	46/73 (63)
Chloramphenicol	0/0	0/0	1/2	3/3
Levofloxacin	23/23 (100)	126/152 (82.9)	224/256 (87.5)	218/254 (85.8)
Minocycline	21/23 (91.3)	143/151 (94.7)	271/298 (90.9)	331/350 (94.6)
Ticarcillin-clavulanic acid	0/0	19/26 (73.1)	45/60 (75)	59/68 (86.8)
Trimethoprim-sulfamethoxazole	7/8	132/150 (88)	255/307 (83.1)	327/365 (89.6)

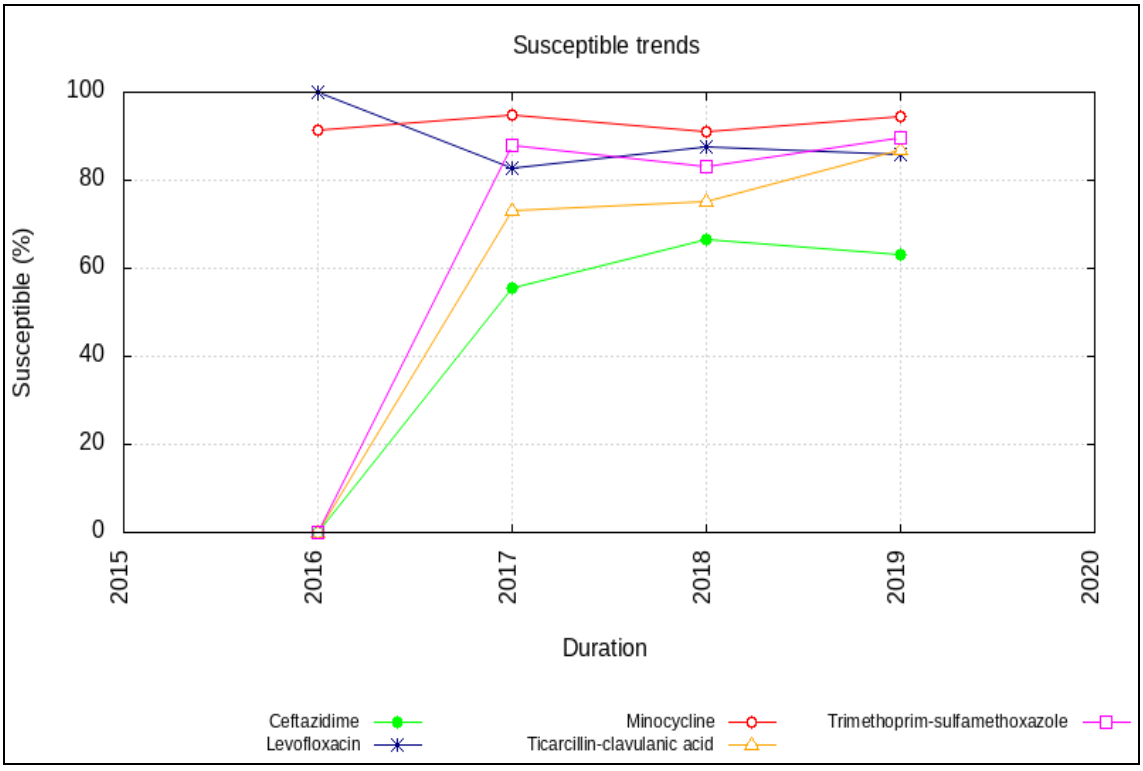

Figure 4.6: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples.

Chapter 5 Diarrheal pathogens

Summary of results

A total of 468 faecal pathogens were isolated during the year 2019. The predominant species identified was *Shigella spp* and *Aeromonas spp* (16%) as observed in previous year. *Salmonella spp* and *Vibrio spp* was isolated in 6% and 5% respectively. The location wise isolation pattern showed that the pathogens are predominantly seen in OPD and wards. However, few isolates of *Aeromonas spp*, *Shigella spp* and *Vibrio spp* were obtained from ICU settings except *Salmonella spp* (Figure 5.1). Species wise distributions of faecal pathogens are given in Table 5.1. The isolation trend over the period of four years (2014 – 2019) shows decreasing trend in the isolation of *Aeromonas spp*. whereas, there is no significant change in the isolation trend of *Shigella spp*, *Vibrio spp* and *Salmonella spp* over the years was observed (Figure 5.2).

Trend analysis over the years 2016 – 2019 showed that *Aeromonas spp* had higher susceptibility to tetracycline and decreased susceptibility to carbapenems. However significant change was not observed. The trend analysis of *S. flexneri* showed that susceptibility to ampicillin and norfloxacin seems to be decreasing and susceptibility to trimethoprim-sulfamethoxazole is increasing. Similarly, year-wise susceptibility trend of *S. sonnei* exhibited higher ampicillin susceptibility compared to *S. flexneri*. Notably, both the species has higher susceptibility to cefixime. Among *Vibrio spp*, no change in the susceptibility of trimethoprim-sulfamethoxazole and tetracycline was observed. However, ampicillin susceptibility decreased over the years 2017 – 2019.

Molecular characterization of drug resistant *Shigella spp* identified *dhfr*A and *sul*II as the predominant genes which confer resistant to trimethoprim and sulfamethoxazole. Among beta-lactamases, *bla*$_{OXA}$ gene was predominantly seen. AmpC genes and PMQR genes were also identified in few isolates. Various resistance genes were identified in *Aeromonas spp*, isolated from environmental sample. Among Non-Typhoidal *Salmonella*, no acquired AMR genes were detected. Finally, the phenotypic and molecular data suggests that third generation cephalosporins and azithromycin are the drug of choice for *Shigella* and *Salmonella spp*. For *Vibrio*, tetracycline or third generation cephalosporins are effective. Whereas, third generation cephalosporins or fluoroquinolones can be used for *Aeromonas spp*. Further, clinical breakpoints for azithromycin need to be defined as this has been currently considered as an alternative therapy for all enteric pathogens. Also clinical evidence for the efficacy of azithromycin is limited. Therefore, more isolates should be tested for azithromycin MIC which would help to define breakpoints in the future.

Aeromonas spp

In 2019, *Aeromonas spp* collected from all specimens showed only 11% susceptibility to ciprofloxacin, but showed >80% susceptibility to cefixime, meropenem, norfloxacin and tetracycline. Whereas, 75% susceptibility was observed for imipenem (Table 5.2). The four years susceptibility trend showed that tetracycline had greater susceptibility (>85%) over the years. Susceptibility of ciprofloxacin was comparatively less throughout the years. Carbapenem such as imipenem and meropenem showed decreasing susceptibility trend, this is due to the less number of isolates tested (Figure 5.3). However, significant change in the susceptibility trend over the years was not observed. The year wise antibiotic susceptibility percentage was given in Table 5.3.

Mostly, *Aeromonas* associated diarrhea are self-limited and generally managed with supportive therapy. However, definite therapy should be adjusted based on the local susceptibility profile. Third generation cephalosporins, fluoroquinolones and aminoglycosides remain as options to treat severe diarrhea. Also, treatment failure may occur in other severe infections while on treatment with third-generation cephalosporins or carbapenem due to the chromosome encoded inducible AmpC and MBL gene-carrying aeromonads. The antimicrobial therapy may differ depending on the site of infection since Aeromonas are popularly called as Jack of all trades due to its ubiquitous nature. Notably, usage of ampicillin should be avoided, as all species of clinical aeromonads are resistant to ampicillin except for *A. trota*.

In addition, molecular characterization of *Aeromonas* isolated from environmental water samples showed the presence of antimicrobial resistance genes such as *bla*$_{\text{MOX3}}$, *mph*A, *mph*E, *cat*B3, *arr*2, *sul*1 and *dfr*A1. This highlights the role of environment in the transmission of antimicrobial resistance genes (ARGs) and thus needs to be monitored to understand the transmission dynamics of these ARGs.

Shigella spp

S. flexneri and *S. sonnei* was the predominant serogroup isolated in 2019. Few *S. boydii* were isolated and *S. dysenteriae* was not isolated in the year 2019. The antibiotic susceptibility varies between the *Shigella spp*. Ampicillin susceptibility varies between *S. flexneri* (25%) and *S. sonnei* (73%) as observed earlier. Both *S. flexneri* and *S. sonnei* showed decreased susceptibility to trimethoprim-sulfamethoxazole (23% and 8%), nalidixic acid (5.7% and 0%) and norfloxacin (22% and 0%) respectively. These suggest that ampicillin, co-trimoxazole and fluroquinolones should not be recommended unless susceptibility is known or expected based on local surveillance. Whereas, the isolates showed higher susceptibility to cefixime (79% and 91%) as shown in Table 5.4. Therefore, third generation cephalosporins can be used as the first line therapy. For resistant isolates,

azithromycin can be used as a second-line oral therapy for both children and adults. Whereas, emerging resistance to azithromycin was being observed.

Year-wise susceptibility trend of *S. flexneri* was shown in Figure 5.4 and Table 5.5. The trend analysis of *S. flexneri* showed that ampicillin and norfloxacin susceptibility seems to be decreasing from 45% in 2017 to 25% in 2019 and 50% in 2017 to 22% in 2019 respectively. Whereas susceptibility to trimethoprim-sulfamethoxazole is increasing from 10% in 2017 to 23% in 2019, this could be due to the limited use of this antibiotic in the recent years. Cefixime showed higher susceptibility (>80%) compared to other antibiotics over the last three years. Similarly, year-wise susceptibility trend of *S. sonnei* was shown in Figure 5.5 and Table 5.6. Notably ampicillin susceptibility was higher in *S. sonnei* in contrast to *S. flexneri*. Susceptibility to cefixime was observed to be >90%. No significant change in the susceptibility trend between 2017 and 2019 was observed.

A total of 58 *Shigella* isolates were characterized for the presence of AMR genes such as *dhfr*A, *sul*II, *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{CTX\text{-}M\text{-}1}$, AmpCs and *qnr*A/B/S by PCR in the year 2019. Majority of the isolates carried *dhfr*A and *sul*II genes which confer resistant to trimethoprim/sulfamethoxazole. Among beta-lactamases, *bla*$_{OXA}$ gene was predominantly seen followed by *bla*$_{TEM}$ and *bla*$_{CTX\text{-}M}$ as expected. While, AmpC genes were identified only in few isolates. Further, plasmid mediated quinolone resistance (PMQR) genes such as *qnr*B and/or *qnr*S were identified in 21% of the tested isolates. The results are shown in the Table 5.7. This molecular observation correlates with the phenotypic results. There was no change in the resistance gene profile of *S. flexneri* and *S. sonnei* was seen when compared to last years. Monitoring of resistance to third generation cephalosporins and macrolides are particularly important because these antibiotics are among the few therapeutic options commonly used for moderate to severe *Shigella* infections.

Vibrio spp

The isolation rate of *Vibrio spp* was usually lesser than other enteric pathogens identified. In 2019, *V. cholerae* showed 47%, 56% and 74% susceptibility to trimethoprim-sulfamethoxazole, ampicillin and norfloxacin respectively (Table 5.8). Therefore, this should be used only when the susceptibility is known. Only less number of isolates were tested for nalidixic acid and norfloxacin. For treatment of *V. cholerae*, rehydration (oral/IV) is essential and antibiotics are just an adjunctive therapy. Generally, tetracycline/ doxycycline is being used for cholera infections. However, doxycycline should not have recommended in children and pregnant women. Notably, tetracycline susceptibility was higher (95%) compared to other antibiotics. The year-wise susceptibility of *V. cholerae* was shown in Table 5.9 and Figure 5.6. No change in the susceptibility of trimethoprim-sulfamethoxazole and tetracycline was observed. However, ampicillin susceptibility decreased from 71% in 2017 to 56% in 2019 which needs to be monitored routinely.

Recently, azithromycin has shown to be clinically superior to tetracycline in treating cholera infections in children and can be considered as a first-line therapy. Similarly, erythromycin is clinically superior to ciprofloxacin and considered as a second-line therapy. Thus, tetracycline or azithromycin appear more effective than some of the other antibiotics tested, but the choice of which antibiotic to use will depend on local drug resistance.

Non-Typhoidal *Salmonella*

Among Non-Typhoidal *Salmonella* (NTS), *S*. Typhimurium was the predominant species. *Salmonella spp* was found to be highly resistant to ciprofloxacin and pefloxacin but showed 80% to 100% susceptibility to other tested antibiotics such as ampicillin, chloramphenicol, co-trimoxazole, ceftriaxone and azithromycin. Otherwise, similar to *Shigella*, bacterial dysentery caused by NTS can be treated with ceftriaxone and azithromycin as this has greater susceptibility profile. Totally, 52 *Salmonella* isolates were received from other centers and none of the isolates showed positive for AMR genes by PCR and the resistance to fluroquinolones could be due to mutation in QRDR which needs to be study to correlate with the phenotypic resistance profile.

Further, a total of 40 Diarrheagenic *E. coli* isolates has been received from RIMS, Imphal in 2019 and the molecular PCR is pending.

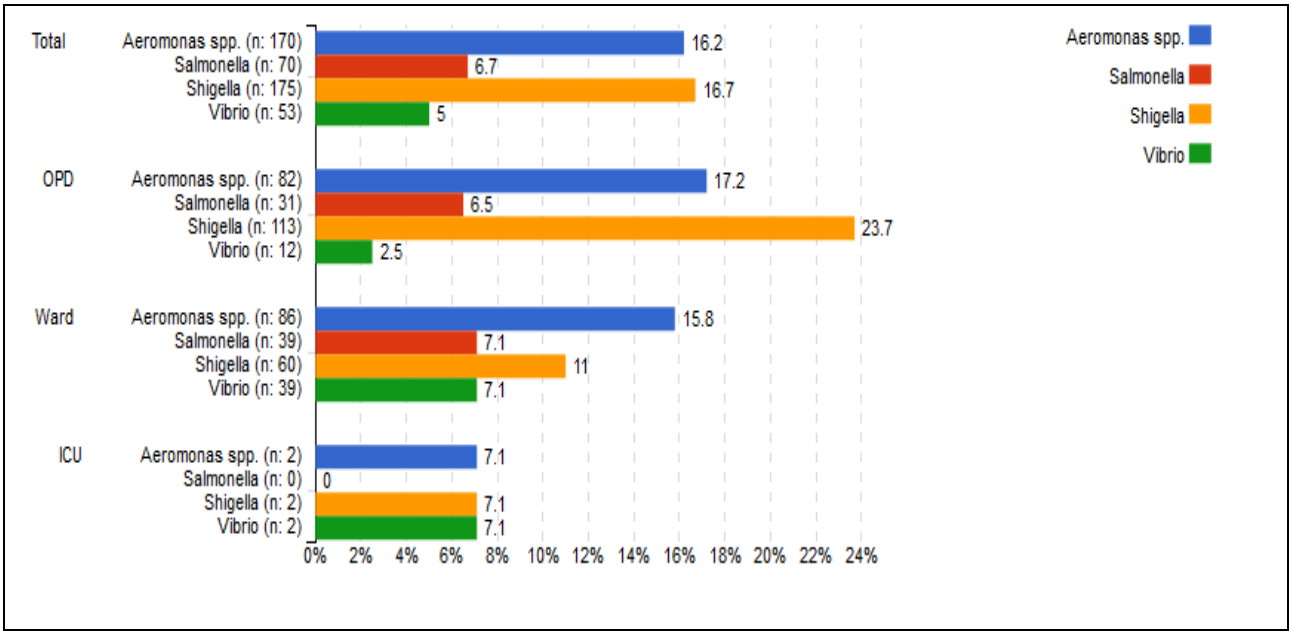

Figure 5.1: Location-wise Isolation pattern of *Aeromonas* species, *Salmonella* faecal, *Shigella* and *Vibrio* isolated from Faeces across OPD, Ward and ICU.

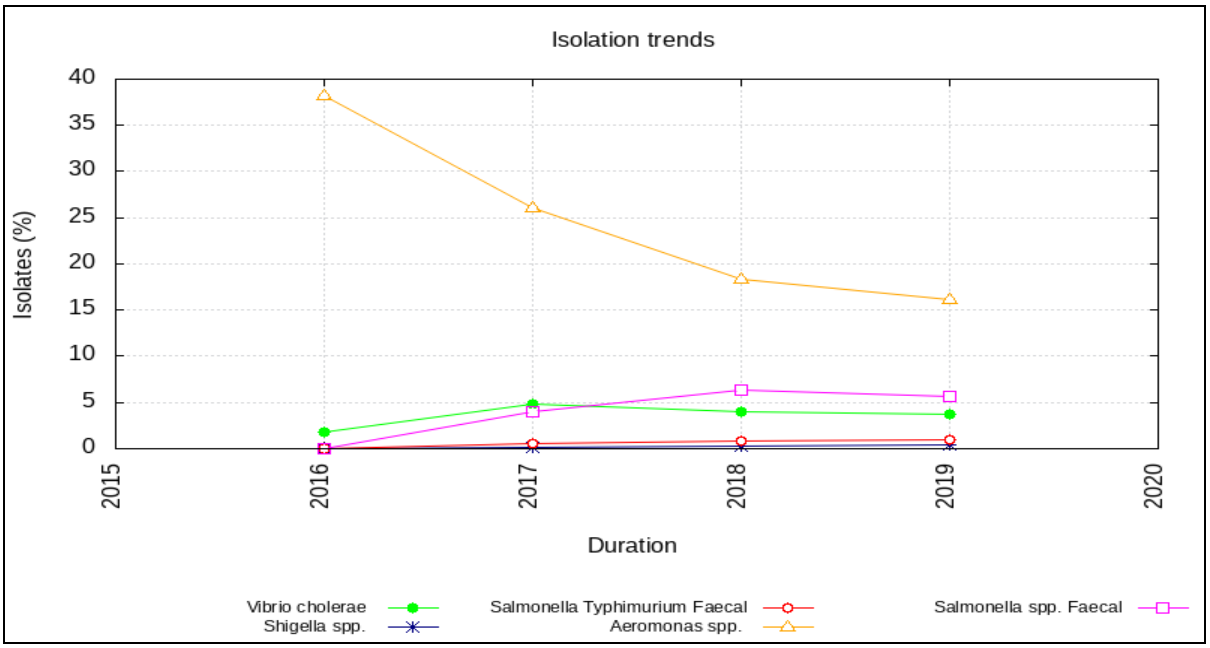

Figure 5.2: Yearly Isolation trends of *Aeromonas* species, *Salmonella spp* faecal, *Salmonella* Typhimurium faecal, *Shigella spp* and *Vibrio cholerae* isolated from Faeces.

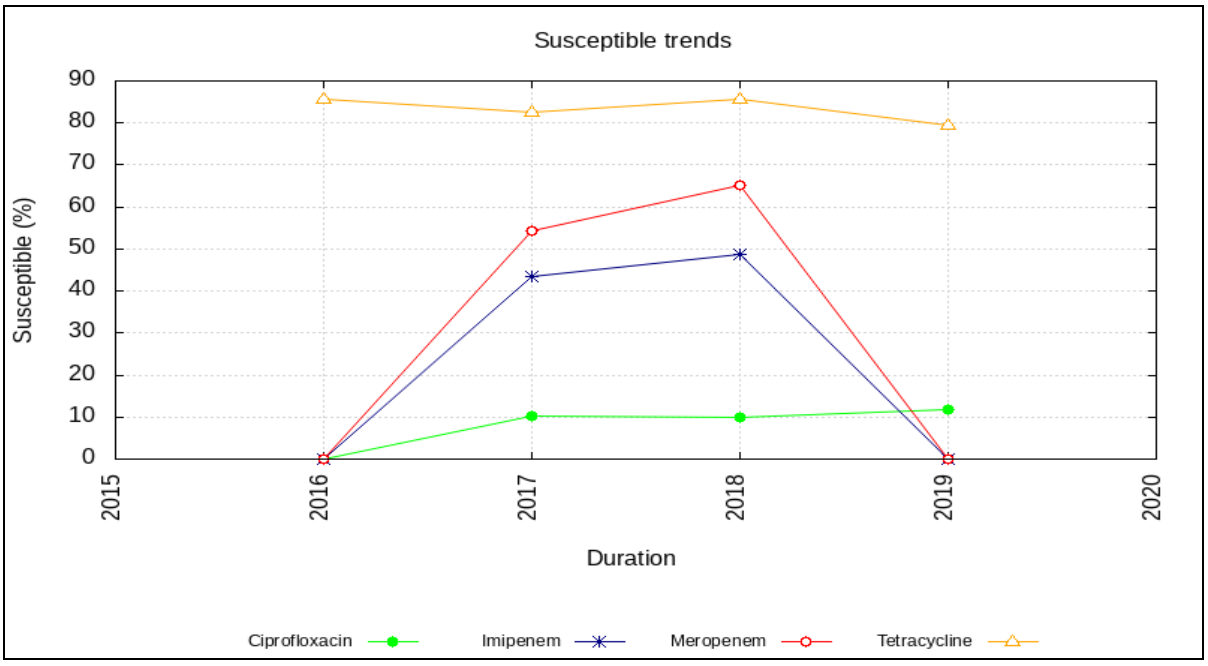

Figure 5.3: Yearly susceptible trends of *Aeromonas spp*

Table 5.1: Isolation rates of Faecal isolates isolated in 2019

Isolate	Total positive cultures 'n' = 107387	
	n	%
Salmonella	74	0.1
Salmonella enteritidis	4	0
Salmonella heidelberg	0	-
Salmonella newport	0	-
Salmonella typhimurium faecal	11	0
Salmonella spp. faecal	59	0.1
Shigella	176	0.2
Shigella boydii	11	0
Shigella dysenteriae	8	0
Shigella flexneri	96	0.1
Shigella sonnei	57	0.1
Shigella spp.	4	0
Vibrio	60	0.1
Vibrio cholerae	46	0
Vibrio parahaemolyticus	0	-
Vibrio spp.	14	0
Aeromonas spp.	221	0.2
Arizona spp.	0	-
Campylobacter jejuni	0	-
Clostridium difficile	0	-
Escherichia coli Diarrhoeagenic	132	0.1
Plesiomonas shigelloides	1	0
Yersinia enterocolitica	0	-

Table 5.2: Susceptible pattern of *Aeromonas spp*

AMA	All Specimens
	Aeromonas spp. **n=221**
Cefixime	28/33 (84.8)
Ciprofloxacin	23/202 (11.4)
Imipenem	30/40 (75)
Meropenem	32/38 (84.2)
Norfloxacin	188/211 (89.1)
Tetracycline	163/205 (79.5)

Table 5.3: Yearly susceptible trends of *Aeromonas spp*

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

Table 5.4: Susceptible pattern of *Shigella* species

AMA	Faeces		
	Shigella boydii **n=11**	***Shigella flexneri*** **n=95**	***Shigella sonnei*** **n=57**
Ampicillin	5/11 (-)	24/94 (25.5)	42/57 (73.7)
Cefixime	8/10 (-)	73/92 (79.3)	52/57 (91.2)
Nalidixic acid	1/2 (-)	2/35 (5.7)	0/8 (-)
Norfloxacin	2/2 (-)	8/36 (22.2)	3/9 (-)
Trimethoprim-sulfamethoxazole	1/11 (-)	22/95 (23.2)	5/57 (8.8)

Table 5.5: Yearly susceptible trends of *Shigella flexneri*

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

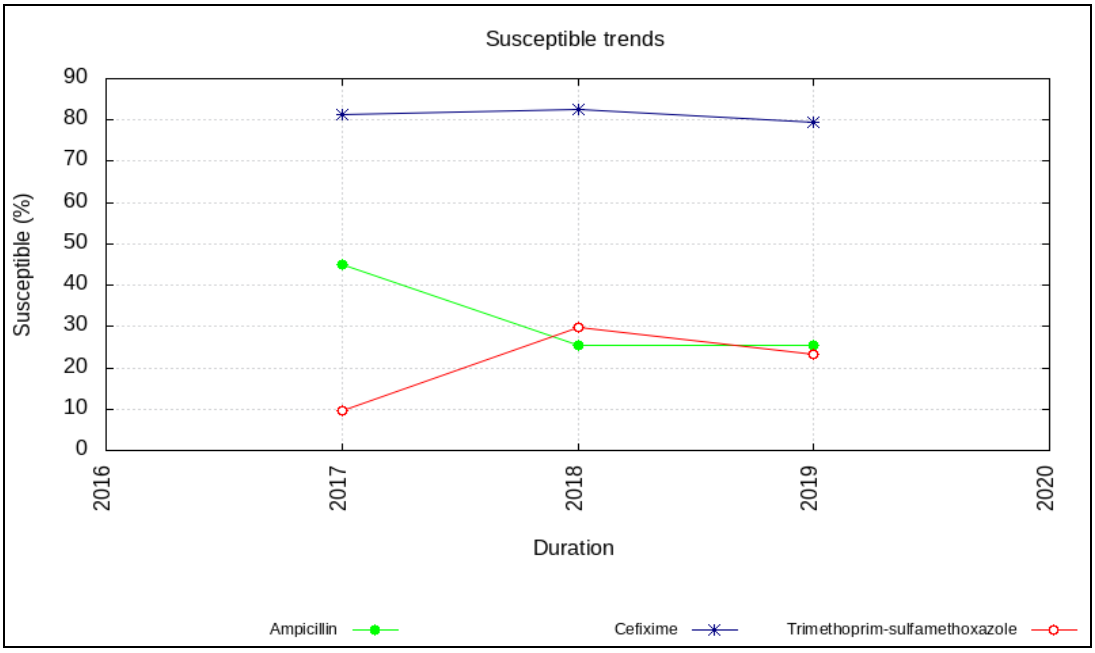

Figure 5.4: Yearly susceptible trends of *Shigella flexneri*

Table 5.6: Yearly susceptible trends of *Shigella sonnei*

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

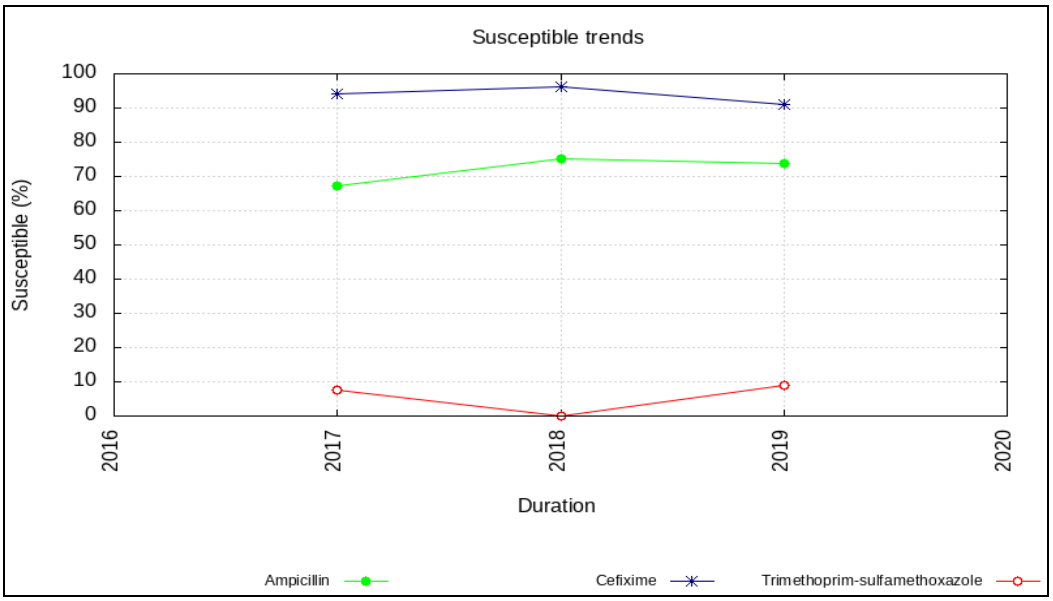

Figure 5.5: Yearly susceptible trends of *Shigella sonnei*

Table 5.7: Diarrheal pathogens received at Nodal center (CMC) for the year 2019

Centre (n)	Total tested	*dhfr*A	*sul*II	*bla*$_{OXA}$	*bla*$_{TEM}$	*bla*$_{CTX-M-1}$	*qnr*S/B
AMCH, Dibrugarh, Assam (n = 10)	10	9	2	1	2	0	2
RIMS, Imphal (n = 15)	7	7	4	0	1	1	2
Ganga Ram Hospital (n = 6)	6	6	3	0	0	2	0
CMC, Vellore (n = 35)	35	22	21	16	1	0	8
Total (n = 66)	58	44 (76%)	30 (52%)	17 (29%)	4 (7%)	3 (5%)	12 (21%)

Table 5.8: Susceptible pattern of *Vibrio cholerae* and *Vibrio spp*

AMA	Faeces	
	Vibrio cholera **n=39**	***Vibrio spp.*** **n=14**
Ampicillin	22/39 (56.4)	10/14 (-)
Nalidixic acid	0/5 (-)	0/0 (-)
Norfloxacin	29/39 (74.4)	13/14 (-)
Tetracycline	36/38 (94.7)	14/14 (-)
Trimethoprim-sulfamethoxazole	18/38 (47.4)	14/14 (-)

Table 5.9: Yearly susceptible trends of *Vibrio cholerae*

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

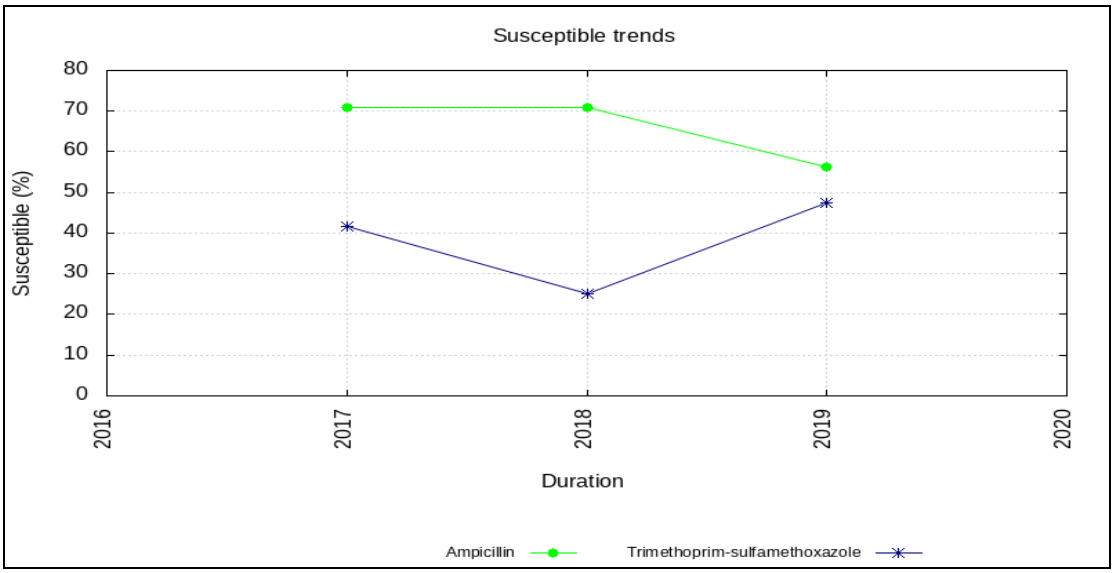

Figure 5.6: Yearly susceptible trends of *Vibrio cholerae*

Chapter 6 Staphylococci and Enterococci

Summary of results

Staphylococcus aureus

The overall proportion of MRSA in 2019 across the country was 42.1%, which is higher than the rate reported in 2018 (38.6%) (Figure 6.1 and Table 6.1). There were significant differences observed between the various zones of India, the highest in the North (52.2%), followed by east (47.4%), west (46.6%) and central region (44.6%). Southern zone (33.9%) demonstrated much lower MRSA rates, with JIPMER recording the lowest rate at 29.4% (Table 6.3). This variation may be indicative of the differences in the antibiotic prescription practices and usage in the different regions. Although there has been a gradually declining trend in the MRSA rates from 2016-2018 in JIPMER (28% in 2015, 23.5% in 2017 and 21% in 2018), the rate increased in 2019 to 29.4% (Table 6.6 and Figure 6.3). The reason for this increase is not known but may reflect the type of patients and procedures carried out in the hospital. 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 to erythromycin. The unusual occurrence of tigecycline and teicoplanin resistance was observed in MSSA isolates (0.3 % and 0.8 %) although this could reflect methodological errors (Table 6.8 and Figure 6.4).

The resistance rates of MRSA to non-beta lactam antibiotics were significantly higher when compared to MSSA (Table 6.1). This was particularly observed for ciprofloxacin, clindamycin and mupirocin. Moreover, mupirocin resistance among MRSA isolates showed a sudden increase in 2019 (11%) when compared to the previous years where it had remained at around 3-5%. The remarkable finding was the increased susceptibility rates of MRSA to most other antibiotics as compared to 2018 (Table 6.6 and Figure 6.3).

Most laboratories depend on cefoxitin disc (30μg) diffusion to identify MRSA. It has been observed that this test tends to miss out some of the MRSA isolates. This feature was noticed with both JIPMER isolates as well as those received as part of EQAS from regional centres. Some of the centres identified MRSA based on VITEK results. A discordance was found between cefoxitin and oxacillin results. As per the data shared by ICMR, MRSA rate based on cefoxitin DD results was 42.1% whereas, the rate was 39.4% based on oxacillin MIC results (Table 6.1). This 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.

The phenotypically identified MRSA were further subjected to genotypic confirmation by *mecA* gene identification by PCR of randomly selected isolates from all centres. However, in less than 1% of MRSA, besides *mec*A gene, *mec*C gene was also found negative in these isolates by PCR. Recently, plasmid mediated *mec*B and *mec*D genes have been reported in *S. aureus* which may complicate detection methods even further. 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*A or *erm*C gene 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. This may explain the absence of *erm* genes in many erythromycin resistant isolates tested this year. Resistance to the high-level mupirocin (200µg) was mostly conferred by *mupA* gene (Table 6.19).

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 JIPMER and other centres combined, the hVISA prevalence was found to be 5.7% (78/1359). Even among JIPMER isolates, the rate was 5.7% which is significantly less than the 12.4% rate reported in the earlier years. As susceptibility to daptomycin and tigecycline continue to be close to 100% among MRSA isolates, these antimicrobials may be considered as alternative agents besides vancomycin and linezolid. This may also remove some of the selection pressure on antimicrobial resistant genes as exerted by these agents.

MIC creep

MIC creep for the anti MRSA antibiotics among JIPMER isolates will be presented first taking 2018 as the index year. The number of MRSA isolates from some of the centres sent for EQAS in 2019 was too low to determine MIC creep center wise. Hence this calculation was restricted to only 8 centres with sufficient number of isolates.

For vancomycin, the MIC_{50} for isolates from JIPMER and AIIMS, New Delhi was 0.5 which was slightly higher than the previous year (0.25). This increased trend was also seen among isolates from CMC Vellore and PGI, Chandigarh (0.19 to 0.25). The values remained the same in TMC, Kolkata, MGIMS, Sevagram and PD Hinduja, Mumbai (0.19, 0.25 and 0.25 respectively). The isolates from SGRH, New Delhi showed a declining trend from 0.38 to 0.25. MIC_{50} of the other anti MRSA antibiotics also showed minor variations when compared to 2018 values.

Coagulase negative staphylococci (CoNS)

Most of the CoNS isolates were obtained from pus and blood samples. 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* and *S. epidermidis*, followed by *S. hominis* and *S. lugdunensis*. The species which showed maximum resistance to most antibiotics was *S. haemolyticus* followed by *S. hominis* (Table 6.9). No significant resistance was observed to vancomycin. However, 19.2% of *S. haemolyticus* isolates (23/120) revealed heteroresistance to vancomycin when tested by PAP-AUC analysis. Linezolid resistance was observed in 2.5% of CoNS isolates overall, with the highest rates seen in *S. haemolyticus* and *S. hominis*. No linezolid resistance was observed in *S. lugdunensis* and *S. saprophyticus*. All the linezolid resistant CoNS isolates tested were positive for the *cfr* gene (Table 6.19). Although establishing clinical relevance of CoNS may be difficult, in situations like device related infections or where they are isolated from sterile sites, beta lactam antibiotics may have very little role based on the high rates of cefoxitin resistance. Between vancomycin and linezolid, the latter may be a better option as heteroresistance to vancomycin has been detected in a significant number of isolates.

Enterococci

As per published data, *E. faecalis* is usually the commonest species followed by *E. faecium*. However, in 2019 *E. faecium* was the predominant species accounting for 50.4% of the total followed by *E. faecalis* (Table 6.12). This reversal of species predominance was noted in the North and east zones while in the west and south, *E. faecalis* was the more common species (Table 6.17). While *E. faecalis* was the major species from superficial and deep infections, *E. faecium* dominated in blood and CSF samples (Table 6.12). A few other species of *Enterocccus* spp were also isolated including *E. gallinarum, E. casseliflavus, E. avium* and *E. raffinosus.*

The possible reason for emergence of *E. faecium* as the predominant species may reflect the increased usage of vancomycin in hospitalized patients. As a larger percentage of *E. faecium* are vancomycin resistant. They may have a survival advantage when compared to *E. faecalis*. Much higher rates of resistance were observed in *E. faecium* for all the other antibiotics as well. No substantial differences in these rates for most antibiotics except for ampicillin and high-level gentamicin in *E. faecalis*, where a disproportionately higher percentage of resistance was observed in north and eastern zones compared to the other regions (Table 6.17 and 6.18).

Isolates from blood (both the species) appear to be more resistant when compared to isolates from superficial and deep infections. As empirical therapy, ampicillin may still be

an option in superficial and deep infections as *E. faecalis* is the more common species from these sites and also shows a good rate of susceptibility to this antibiotic. However, when it comes to blood stream infections and meningitis, particularly following neurosurgical procedures, vancomycin or linezolid may be better options as it has been noticed that *E. faecium* is the more common species from these infections. For urinary tract infections, ampicillin or nitrofurantoin may still be useful. Fosfomycin resistance was encountered in 5.2% of *E. faecalis* urine isolates compared to 12.5% last year and may be an option in serious infections (Table 6.13).

Vancomycin resistance was 9.7% overall. However, as seen from Table 6.12, it was almost 6 times higher in *E. faecium* compared to *E. faecalis* (17.4% vs 2.8% respectively). Overall, VRE rates have shown a declining trend, starting at 7% in 2015 and lowering to 4% in 2018. The sudden increase to 9.7% in 2019 may be due to the higher number of *E. faecium* isolates this year (Tables 6.15 and 6.16). In all VRE from our center as well as other regional centers, the resistance was mediated solely by *van*A gene. Few isolates exhibited van B genotype. No other *van* genes were detected (Table 6.19). Linezolid resistance was observed in 0.8% of the *E. faecalis* isolates and 2.9% in *E. faecium* isolates. It was seen in both vancomycin sensitive as well as vancomycin resistant isolates.

Results of biocide resistance of (*qac*A/B and *smr)* genes from MRSA, MRCoNS and VRE isolates

222 isolates of MRSA, 328 *S. haemolyticus* and 184 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 9% (20/222) and 1.8% (4/222) respectively. In *Enterococcus*, *qac*A/B was detected in 2.1 % (4/184) isolates while none had *smr* genes. Among CoNS, *S. haemolyticus* was chosen as it was the commonest and the most resistant species. Of the 328 isolates tested, *qac*A/B genes were detected in 114 isolates (34.8%), *smr* genes in 118 (3.4%) while a combination of both was detected in 93 isolates (28.4%).

Detailed analysis of results (For January to December 2019)

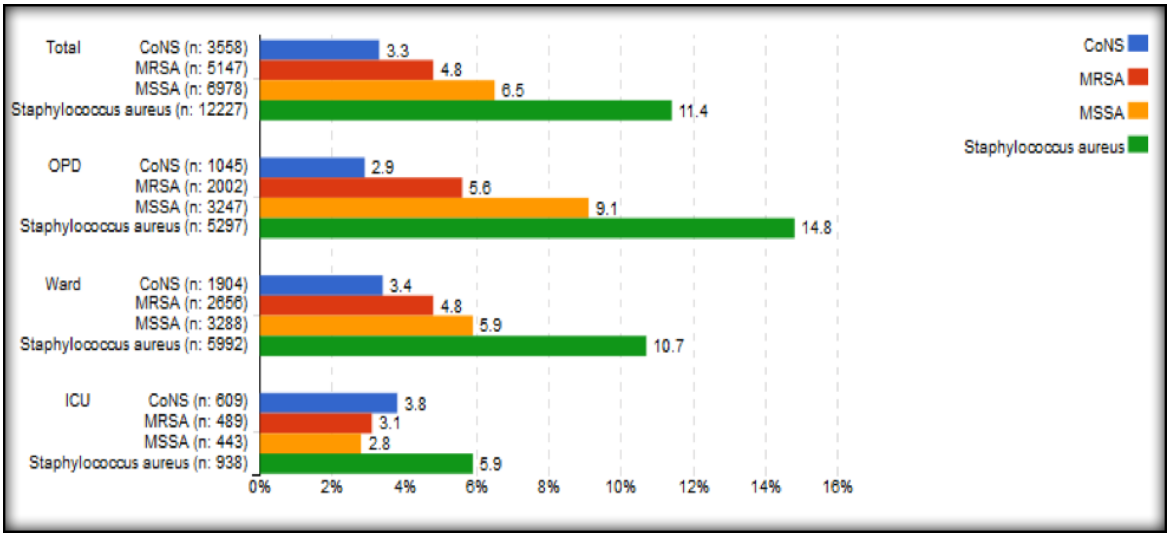

Figure 6.1: Location-wise Isolation pattern of *Staphylococcus aureus*, CoNS, MRSA, MSSA isolated from All Samples across OPD, Ward and ICU

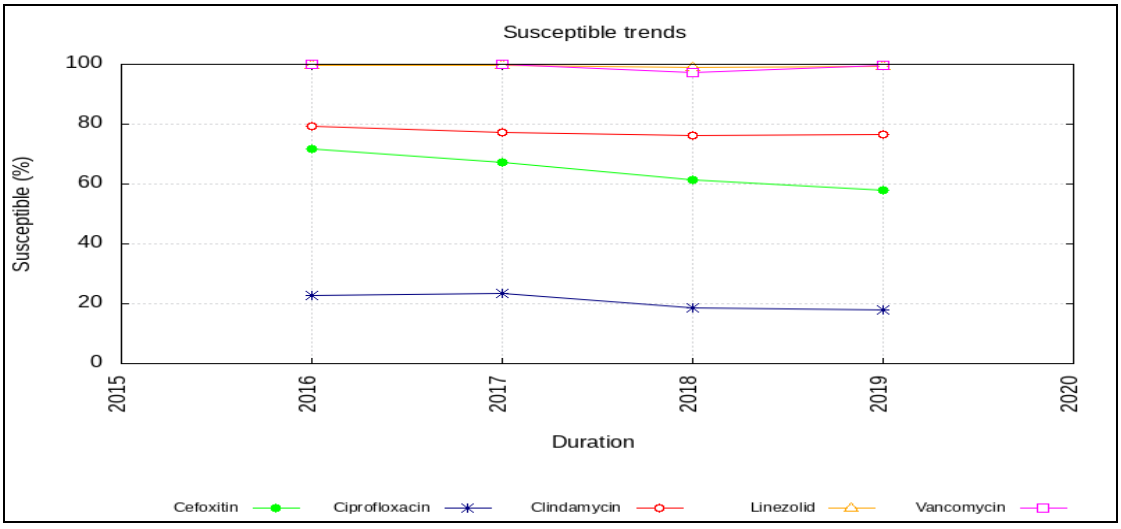

Figure 6.2: Year wise susceptibility trends of *Staphylococcus aureus* from all samples

Table 6.1: Percentage susceptibility of *S. aureus*, MRSA, MSSA and CoNS isolated from all samples

AMA	All Specimens			
	S. aureus **n=12226**	**MSSA n=6979**	**MRSA n=5148**	**CoNS n=3558**
Cefoxitin	6209/10771 (57.7)	6208/6208 (100)	0/4546 (0)	918/3285 (27.9)
Ciprofloxacin	1983/11111 (17.8)	1582/6404 (24.7)	396/4619 (8.6)	1172/2784 (42.1)
Clindamycin	9112/11891 (76.6)	5808/6789 (85.6)	3238/5008 (64.7)	2055/3496 (58.8)
Erythromycin	4779/11888 (40.2)	3515/6847 (51.3)	1241/4952 (25.1)	815/3502 (23.3)
Linezolid	11370/11457 (99.2)	6386/6401 (99.8)	4899/4965 (98.7)	3329/3416 (97.5)
Mupirocin High Level	4624/4892 (94.5)	2776/2821 (98.4)	1828/2050 (89.2)	0/0 (-)
Oxacillin	2272/3760 (60.4)	2188/2188 (100)	84/1572 (5.3)	10/11 (-)
Penicillin	456/6936 (6.6)	409/3689 (11.1)	0/3210 (0)	266/2595 (10.3)
Teicoplanin	6172/6247 (98.8)	3382/3410 (99.2)	2722/2768 (98.3)	1324/1378 (96.1)
Tetracycline	9209/10262 (89.7)	5354/5757 (93)	3804/4446 (85.6)	2656/3265 (81.3)
Tigecycline	2895/2907 (99.6)	1605/1610 (99.7)	1276/1282 (99.5)	287/292 (98.3)
Trimethoprim-sulfamethoxazole	7874/11320 (69.6)	4727/6434 (73.5)	3103/4815 (64.4)	1683/3421 (49.2)
Vancomycin	6925/6925 (100)	3950/3950 (100)	2927/2927 (100)	1688/1688 (100)

Table 6.2: Location-wise susceptibility of MRSA, MSSA, CoNS from all samples

AMA	*Staphylococcus aureus*				*MSSA*				*MRSA*				*CoNS*			
	Total n=12227	**OPD n=5297**	**Ward n=5992**	**ICU n=938**	**Total n=6978**	**OPD n=3247**	**Ward n=3288**	**ICU n=443**	**Total n=5147**	**OPD n=2002**	**Ward n=2656**	**ICU n=489**	**Total n=3558**	**OPD n=1045**	**Ward n=1904**	**ICU n=609**
	(S %)	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**	**(S %)**
Cefoxitin	6225/10754 (57.9)	2938/4715 (62.3)	2913/5267 (55.3)	374/772 (48.4)	6207/6207 (100)	2933/2933 (100)	2903/2903 (100)	371/371 (100)	16/4544 (0.4)	5/1782 (0.3)	9/2362 (0.4)	2/400 (0.5)	918/3286 (27.9)	388/983 (39.5)	428/1745 (24.5)	102/558 (18.3)
Ciprofloxacin	1983/11110 (17.8)	956/4910 (19.5)	897/5448 (16.5)	130/752 (17.3)	1581/6403 (24.7)	782/3033 (25.8)	704/3014 (23.4)	95/356 (26.7)	396/4617 (8.6)	171/1832 (9.3)	190/2394 (7.9)	35/391 (9)	1172/2785 (42.1)	456/888 (51.4)	542/1411 (38.4)	174/486 (35.8)
Clindamycin	9112/11891 (76.6)	4130/5170 (79.9)	4413/5882 (75)	569/839 (67.8)	5807/6788 (85.5)	2724/3166 (86)	2745/3233 (84.9)	338/389 (86.9)	3237/5006 (64.7)	1370/1957 (70)	1639/2604 (62.9)	228/445 (51.2)	2055/3496 (58.8)	691/1022 (67.6)	1061/1875 (56.6)	303/599 (50.6)
Erythromycin	4780/11888 (40.2)	2170/5155 (42.1)	2275/5825 (39.1)	335/908 (36.9)	3515/6846 (51.3)	1642/3190 (51.5)	1635/3221 (50.8)	238/435 (54.7)	1241/4950 (25.1)	515/1924 (26.8)	630/2559 (24.6)	96/467 (20.6)	814/3502 (23.2)	296/1025 (28.9)	423/1879 (22.5)	95/598 (15.9)
Linezolid	11371/11457 (99.2)	4960/4993 (99.3)	5502/5543 (99.3)	909/921 (98.7)	6385/6400 (99.8)	3002/3009 (99.8)	2948/2956 (99.7)	435/435 (100)	4898/4963 (98.7)	1916/1939 (98.8)	2513/2544 (98.8)	469/480 (97.7)	3328/3416 (97.4)	1000/1020 (98)	1762/1807 (97.5)	566/589 (96.1)
Mupirocin HL	4623/4891 (94.5)	2316/2411 (96.1)	2082/2234 (93.2)	225/246 (91.5)	2775/2820 (98.4)	1472/1496 (98.4)	1188/1206 (98.5)	115/118 (97.5)	1828/2050 (89.2)	830/900 (92.2)	888/1022 (86.9)	110/128 (85.9)	1/1	NT	NT	NT
Oxacillin	2272/3760 (60.4)	1031/1615 (63.8)	1043/1775 (58.8)	198/370 (53.5)	2188/2188 (100)	988/988 (100)	1010/1010 (100)	190/190 (100)	84/1572 (5.3)	43/627 (6.9)	33/765 (4.3)	8/180 (4.4)	10/11	7/8	2/2	1/1
Penicillin	456/6936 (6.6)	212/3095 (6.8)	207/3280 (6.3)	37/561 (6.6)	409/3688 (11.1)	185/1779 (10.4)	194/1664 (11.7)	30/245 (12.2)	0/3208 (0)	0/1295 (0)	0/1599 (0)	0/314 (0)	266/2595 (10.3)	113/838 (13.5)	121/1306 (9.3)	32/451 (7.1)
Teicoplanin	6172/6247 (98.8)	2801/2832 (98.9)	2928/2965 (98.8)	443/450 (98.4)	3381/3409 (99.2)	1621/1636 (99.1)	1550/1562 (99.2)	210/211 (99.5)	2720/2766 (98.3)	1144/1160 (98.6)	1347/1371 (98.2)	229/235 (97.4)	1324/1378 (96.1)	454/463 (98.1)	697/733 (95.1)	173/182 (95.1)
Tetracycline	9211/10262 (89.8)	4151/4544 (91.4)	4400/4942 (89)	660/776 (85.1)	5353/5756 (93)	2562/2764 (92.7)	2449/2630 (93.1)	342/362 (94.5)	3804/4444 (85.6)	1561/1749 (89.3)	1928/2286 (84.3)	315/409 (77)	2654/3265 (81.3)	817/985 (82.9)	1397/1730 (80.8)	440/550 (80)
Tigecycline	2895/2907 (99.6)	1317/1322 (99.6)	1298/1302 (99.7)	280/283 (98.9)	1605/1610 (99.7)	774/777 (99.6)	686/687 (99.9)	145/146 (99.3)	1276/1282 (99.5)	533/535 (99.6)	609/611 (99.7)	134/136 (98.5)	287/292 (98.3)	112/114 (98.2)	133/134 (99.3)	42/44 (95.5)
Trimethoprim-sulfamethoxazole	7875/11320 (69.6)	3479/4956 (70.2)	3809/5475 (69.6)	587/889 (66)	4726/6433 (73.5)	2224/3039 (73.2)	2195/2975 (73.8)	307/419 (73.3)	3103/4813 (64.5)	1232/1882 (65.5)	1595/2467 (64.7)	276/464 (59.5)	1682/3421 (49.2)	521/1016 (51.3)	881/1816 (48.5)	280/589 (47.5)
Vancomycin	6926/6926 (100)	2928/2928 (100)	3508/3508 (100)	490/490 (100)	3950/3950 (100)	1808/1808 (100)	1912/1912 (100)	226/226 (100)	2927/2927 (100)	1092/1092 (100)	1573/1573 (100)	262/262 (100)	1687/1687 (100)	480/480 (100)	931/931 (100)	276/276 (100)

Table 6.3: Susceptibility pattern of *Staphylococcus aureus* isolated from all samples except faeces and urine across different regions of India

Antibiotic	National (n=11645)		North (n=2897)		Central (n=556)		East (n=1415)		West (n=2096)		South (n=4681)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Tigecycline	2853/2865 (99.6)	97.7-100	411/414 (99.3)	99.5	220/222 (99.1)	98.9-100	259/259 (100)	100	667/669 (99.7)	99.6-100	1296/1301 (99.6)	97.7-100
Vancomycin	6839/6839 (100)	95.6-100	1683/1683 (100)	100-100	226/226 (100)	100-100	278/278 (100)	100-100	1217/1217 (100)	100-100	3435/3435 (100)	100-100
Linezolid	10847/10929 (99.2)	90.2-100	2763/2780 (99.4)	98.8-100	551/552 (99.8)	99.5-100	1377/1403 (98.1)	90.2-99.7	2058/2068 (99.5)	98.9-100	4098/4126 (99.3)	96.7-99.9
Teicoplanin	5867/5936 (98.8)	74.2-100	1798/1821 (98.7)	80.8-100	285/289 (98.6)	96.1-100	729/749 (97.3)	74.2-100	1197/1214 (98.6)	88.6-100	1858/1863 (99.7)	98.3-100
Mupirocin High Level	4292/4515 (95.1)	78.5-100	1233/1246 (99)	93.3-100	94/97 (96.9)	96.9	775/939 (82.5)	78.5-100	345/353 (97.7)	97.6	1845/1880 (98.1)	98.1
Tetracycline	8801/9754 (90.2)	71.6-98.7	1948/2182 (89.3)	74.9-98.7	464/534 (86.9)	86.6-87.4	1240/1407 (88.1)	71.6-91.7	1707/1914 (89.2)	85.1-95.9	3442/3717 (92.6)	89.3-97.1
Clindamycin	8732/11314 (77.2)	35.7-97.3	2159/2880 (75)	43.5-91.7	435/554 (78.5)	73.4-88.4	939/1411 (66.5)	35.7-81.6	1558/2086 (74.7)	57-92.1	3641/4383 (83.1)	65-97.3
Trimethoprim-sulfamethoxazole	7475/10754 (69.5)	34.1-88.5	1364/2198 (62.1)	34.1-74.9	284/542 (52.4)	49.6-57.9	888/1399 (63.5)	38.3-70	1396/2022 (69)	63.4-85.5	3543/4593 (77.1)	57.8-88.5
Oxacillin	2235/3705 (60.3)	46.4-70.3	650/1167 (55.7)	46.4-60.8	113/223 (50.7)	47.3-65.9	166/248 (66.9)	67.6	442/729 (60.6)	47.7-64.1	864/1338 (64.6)	63.9-70.3
Cefoxitin	5908/10186 (58)	32.4-71.4	919/1923 (47.8)	33.7-56.6	201/363 (55.4)	55.4	741/1408 (52.6)	32.4-66.9	1028/1924 (53.4)	41.4-63.9	3019/4568 (66.1)	56.7-71.4
Erythromycin	4601/11323 (40.6)	14.8-58.8	1015/2817 (36)	21.5-44	189/535 (35.3)	26.8-52.2	329/1401 (23.5)	14.8-35.2	715/2031 (35.2)	30-44.5	2353/4539 (51.8)	37.7-58.8
Ciprofloxacin	1807/10547 (17.1)	3.2-37.3	434/2716 (16)	3.2-30.1	48/554 (8.7)	6.3-9.9	443/1404 (31.6)	14.5-37.3	153/1880 (8.1)	6.3-12.1	729/3993 (18.3)	11.5-21.9
Penicillin	384/6398 (6)	2-19.4	78/2042 (3.8)	2-6.1	20/355 (5.6)	5.8	95/1267 (7.5)	5.2-19.4	95/1768 (5.4)	3.1-8	96/966 (9.9)	2.6-10.6

Table 6.4: Year wise susceptibility trends of *Staphylococcus aureus* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=960	Total n=5708	Total n=8567	Total n=12227
	(S%)	(S%)	(S%)	(S%)
Cefoxitin	686/958 (71.6)	3805/5668 (67.1)	4814/7842 (61.4)	6225/10754 (57.9)
Ciprofloxacin	191/838 (22.8)	1224/5260 (23.3)	1483/8017 (18.5)	1983/11110 (17.8)
Clindamycin	729/921 (79.2)	4235/5475 (77.4)	6386/8380 (76.2)	9112/11891 (76.6)
Erythromycin	492/955 (51.5)	2755/5570 (49.5)	3553/8028 (44.3)	4780/11888 (40.2)
Linezolid	860/863 (99.7)	5424/5445 (99.6)	7977/8071 (98.8)	11371/11457 (99.2)
Mupirocin High Level	573/584 (98.1)	2971/3012 (98.6)	3656/3742 (97.7)	4623/4891 (94.5)
Oxacillin	*0/0	314/438 (71.7)	1168/2121 (55.1)	2272/3760 (60.4)
Penicillin	60/737 (8.1)	267/3519 (7.6)	246/4047 (6.1)	456/6936 (6.6)
Teicoplanin	877/880 (99.7)	5233/5257 (99.5)	6469/6622 (97.7)	6172/6247 (98.8)
Tetracycline	669/738 (90.7)	3492/3860 (90.5)	6184/6975 (88.7)	9211/10262 (89.8)
Tigecycline	0/0	433/435 (99.5)	1456/1463 (99.5)	2895/2907 (99.6)
Trimethoprim-sulfamethoxazole	513/852 (60.2)	3064/4306 (71.2)	4695/7490 (62.7)	7875/11320 (69.6)
Vancomycin	565/565 (100)	2602/2602 (100)	4565/4565 (100)	6926/6926 (100)

Table 6.5: Susceptibility pattern of MRSA isolated from all samples except faeces and urine across different regions of India

Antibiotic	National (n=4890)		North (n=1396)		Central (n=262)		East (n=672)		West (n=977)		South (n=1583)	
	n (%)	Range (%)	n (%)	Range (%)	n (%)	Range (%)	n (%)	Range (%)	n (%)	Range (%)	n (%)	Range (%)
Tigecycline	1259/1265 (99.5)	97.1-100	219/221 (99.1)	99.1	120/121 (99.2)	98.9-100	89/89 (100)	100	272/272 (100)	100-100	559/562 (99.5)	97.1-100
Vancomycin	2888/2888 (100)	100-100	868/868 (100)	100-100	125/125 (100)	100-100	100/100 (100)	100	584/584 (100)	100-100	1211/1211 (100)	100-100
Linezolid	4655/4717 (98.7)	89.7-100	1321/1332 (99.2)	98.5-100	259/259 (100)	100-100	647/666 (97.1)	89.7-99.7	958/968 (99)	97.9-100	1470/1492 (98.5)	96.2-99.8
Teicoplanin	2597/2638 (98.4)	93-100	856/871 (98.3)	96.8-100	143/146 (97.9)	94.2-100	285/296 (96.3)	93-100	531/540 (98.3)	95.9-100	782/785 (99.6)	98-100
Mupirocin High Level	1676/1864 (89.9)	61.8-100	595/605 (98.3)	94.9-100	50/52 (96.2)	96.2	359/503 (71.4)	61.8-100	149/154 (96.8)	96.6	523/550 (95.1)	95.1
Tetracycline	3629/4205 (86.3)	66.7-97.8	983/1145 (85.9)	71.4-97.8	205/249 (82.3)	81.1-83	571/670 (85.2)	66.7-95.4	775/877 (88.4)	83.4-92.6	1095/1264 (86.6)	77.4-96.5
Clindamycin	3103/4752 (65.3)	29.9-96	874/1387 (63)	30-84.8	183/261 (70.1)	61.2-85.4	378/670 (56.4)	29.9-78.2	595/974 (61.1)	37.8-89.1	1073/1460 (73.5)	56.3-96
Trimethoprim-sulfamethoxazole	2957/4565 (64.8)	27.1-88.9	661/1157 (57.1)	27.1-67.4	127/256 (49.6)	43.6-60.2	366/665 (55)	36.8-58.6	666/942 (70.7)	54.3-87.6	1137/1545 (73.6)	47.4-88.9
Erythromycin	1201/4698 (25.6)	6.2-41.6	369/1349 (27.4)	12.3-37.6	71/246 (28.9)	21.7-41.6	83/666 (12.5)	6.2-28.6	220/935 (23.5)	19.3-30.9	458/1502 (30.5)	24.4-36.9
Ciprofloxacin	360/4369 (8.2)	0.9-27.5	105/1282 (8.2)	0.9-15.7	9/261 (3.4)	3.1-3.6	144/666 (21.6)	8-27.5	26/870 (3)	1.9-3.8	76/1290 (5.9)	1.5-8.2
Oxacillin	81/1551 (5.2)	0-57.6	0/517 (0)	0-0	19/129 (14.7)	0-57.6	5/87 (5.7)	6	3/290 (1)	0-1.4	54/528 (10.2)	8.4-18.7
Penicillin	37/2968 (1.2)	0-15.6	8/1062 (0.8)	0.5-1.7	2/158 (1.3)	1.3	17/609 (2.8)	0.7-15.6	6/821 (0.7)	0-1.4	4/318 (1.3)	0-2.8

Table 6.6: Year wise susceptibility trends of MRSA from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=272	Total n=1870	Total n=3417	Total n=5147
	(S%)	(S%)	(S%)	(S%)
Cefoxitin	0/272 (0)	4/1867 (0.2)	6/3034 (0.2)	0/4544 (0)
Ciprofloxacin	23/228 (10.1)	165/1718 (9.6)	323/3194 (10.1)	396/4617 (8.6)
Clindamycin	167/259 (64.5)	1067/1802 (59.2)	2057/3345 (61.5)	3237/5006 (64.7)
Erythromycin	72/270 (26.7)	494/1813 (27.2)	817/3201 (25.5)	1241/4950 (25.1)
Linezolid	225/228 (98.7)	1779/1794 (99.2)	3200/3268 (97.9)	4898/4963 (98.7)
Mupirocin High Level	139/144 (96.5)	852/873 (97.6)	1238/1297 (95.5)	1828/2050 (89.2)
Oxacillin	0/0	8/132 (6.1)	29/982 (3)	84/1572 (5.3)
Penicillin	0/180 (0)	0/1111 (0)	0/1959 (0)	0/3208 (0)
Teicoplanin	240/242 (99.2)	1719/1735 (99.1)	2821/2929 (96.3)	2720/2766 (98.3)
Tetracycline	141/181 (77.9)	983/1193 (82.4)	2372/2832 (83.8)	3804/4444 (85.6)
Tigecycline	0/0	133/133 (100)	601/608 (98.8)	1276/1282 (99.5)
Trimethoprim-sulfamethoxazole	99/223 (44.4)	851/1332 (63.9)	1677/2979 (56.3)	3103/4813 (64.5)
Vancomycin	137/137 (100)	667/667 (100)	1554/1554 (100)	2927/2927 (100)

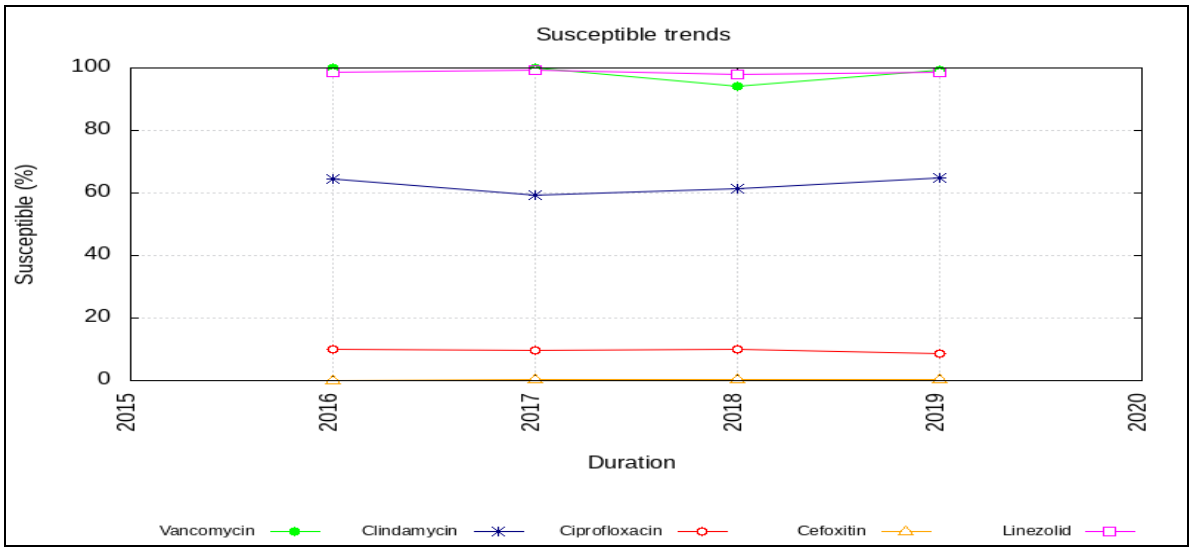

Figure 6.3: Year wise susceptibility trends of MRSA from All Samples

Table 6.7: Susceptibility pattern of MSSA isolated from All samples across different regions of India

Antibiotic	National (n=6658)		North (n=1458)		Central (n=285)		East (n=742)		West (n=1097)		South (n=3076)	
	n (%)	Range(%)	n(%)	Range(%)	n(%)	Range(%)	n(%)	Range(%)	n(%)	Range(%)	n(%)	Range(%)
Oxacillin	2154/2154 (100)	100-100	650/650 (100)	100-100	94/94 (100)	100	161/161 (100)	100	439/439 (100)	100-100	810/810 (100)	100-100
Cefoxitin	5891/5891 (100)	100-100	915/915 (100)	100-100	198/198 (100)	100	738/738 (100)	100-100	1025/1025 (100)	100-100	3015/3015 (100)	100-100
Linezolid	6111/6125 (99.8)	91.3-100	1404/1407 (99.8)	99.3-100	283/284 (99.6)	98.8-100	730/737 (99.1)	91.3-100	1081/1081 (100)	100-100	2613/2616 (99.9)	99.5-100
Tigecycline	1582/1587 (99.7)	98.1-100	192/192 (100)	100	93/94 (98.9)	98.8	170/170 (100)	100	393/395 (99.5)	99.4-100	734/736 (99.7)	98.1-100
Vancomycin	3905/3905 (100)	100-100	792/792 (100)	100-100	94/94 (100)	100	178/178 (100)	100	626/626 (100)	100-100	2215/2215 (100)	100-100
Teicoplanin	3205/3232 (99.2)	84-100	907/914 (99.2)	97.9-100	135/136 (99.3)	98-100	444/453 (98)	97.2-100	652/660 (98.8)	84-100	1067/1069 (99.8)	98.6-100
Mupirocin High Level	2597/2631 (98.7)	94.4-100	635/637 (99.7)	98.9-100	44/45 (97.8)	97.8	416/436 (95.4)	94.4-100	187/190 (98.4)	98.4	1315/1323 (99.4)	99.4
Tetracycline	5124/5493 (93.3)	80.5-100	948/1019 (93)	80.5-99.4	252/276 (91.3)	89.5-95.3	669/737 (90.8)	82.2-93.9	915/1017 (90)	85.8-100	2340/2444 (95.7)	93.6-98.4
Clindamycin	5568/6473 (86)	47.8-98.1	1257/1451 (86.6)	63.8-97	245/284 (86.3)	83.3-93	561/741 (75.7)	47.8-89.8	947/1092 (86.7)	77.5-94.1	2558/2905 (88.1)	71.8-98.1
Trimethoprim-sulfamethoxazole	4476/6123 (73.1)	41.3-92.6	691/1021 (67.7)	41.9-80.6	153/277 (55.2)	55.2-55.4	522/734 (71.1)	41.3-81.1	717/1061 (67.6)	59.2-83.7	2393/3030 (79)	62-92.6
Erythromycin	3378/6541 (51.6)	28.7-70.2	637/1428 (44.6)	31.7-53.9	115/282 (40.8)	31.1-62.8	246/735 (33.5)	28.7-38.7	490/1076 (45.5)	37.2-51.8	1890/3020 (62.6)	44-70.2
Ciprofloxacin	1442/6094 (23.7)	4.8-50	328/1394 (23.5)	4.8-50	39/284 (13.7)	10.5-15.2	299/738 (40.5)	17.8-50	125/991 (12.6)	9.6-19.1	651/2687 (24.2)	15.5-27.7
Penicillin	344/3396 (10.1)	4.1-30.8	69/973 (7.1)	4.1-13.1	18/195 (9.2)	9.5	78/658 (11.9)	9-30.8	88/934 (9.4)	7.5-11.8	91/636 (14.3)	14-15.1

Table 6.8: Year wise susceptibility trends of MSSA from All Samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=686	Total n=3819	Total n=5086	Total n=6978
	(S%)	(S%)	(S%)	(S%)
Cefoxitin	686/686 (100)	3801/3801 (100)	4808/4808 (100)	6207/6207 (100)
Ciprofloxacin	168/609 (27.6)	1051/3524 (29.8)	1153/4767 (24.2)	1581/6403 (24.7)
Clindamycin	561/661 (84.9)	3162/3666 (86.3)	4293/4973 (86.3)	5807/6788 (85.5)
Erythromycin	419/684 (61.3)	2251/3739 (60.2)	2722/4794 (56.8)	3515/6846 (51.3)
Linezolid	634/634 (100)	3630/3636 (99.8)	4726/4751 (99.5)	6385/6400 (99.8)
Mupirocin High Level	434/440 (98.6)	2119/2139 (99.1)	2414/2441 (98.9)	2775/2820 (98.4)
Oxacillin	0/0	306/306 (100)	1139/1139 (100)	2188/2188 (100)
Penicillin	59/557 (10.6)	248/2393 (10.4)	218/2068 (10.5)	409/3688 (11.1)
Teicoplanin	636/636 (100)	3509/3517 (99.8)	3594/3634 (98.9)	3381/3409 (99.2)
Tetracycline	528/557 (94.8)	2508/2665 (94.1)	3763/4089 (92)	5353/5756 (93)
Tigecycline	0/0	300/302 (99.3)	855/855 (100)	1605/1610 (99.7)
Trimethoprim-sulfamethoxazole	414/629 (65.8)	2202/2959 (74.4)	2985/4451 (67.1)	4726/6433 (73.5)
Vancomycin	428/428 (100)	1935/1935 (100)	2993/2993 (100)	3950/3950 (100)

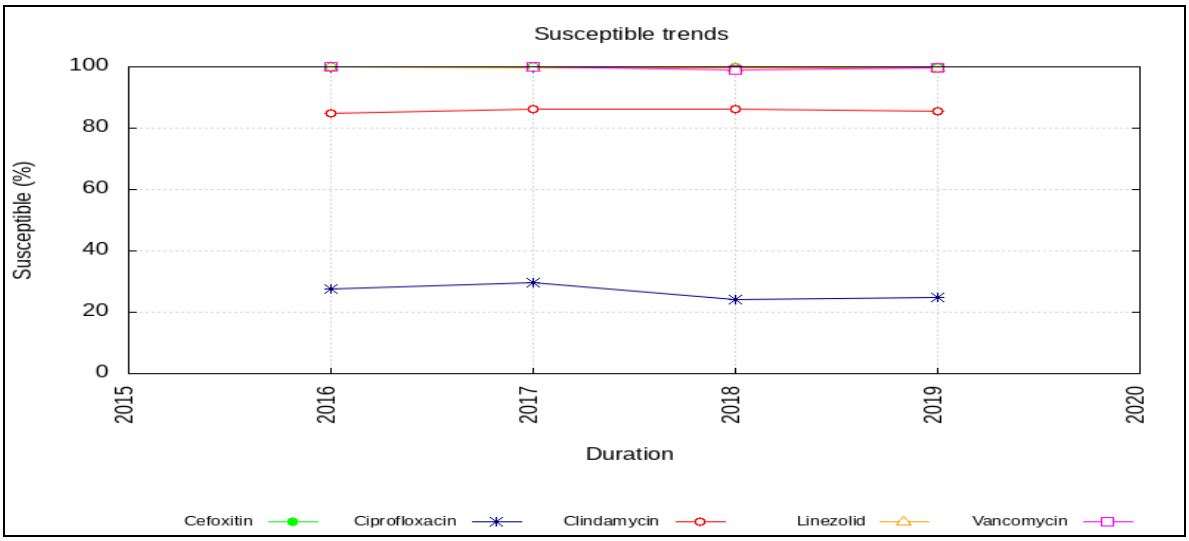

Figure 6.4: Year wise susceptibility trends of MSSA from All Samples

CoNS

Table 6.9: Susceptibility percentages of CoNS isolated from all specimens

AMA	All Specimens					
	Staphylococcus epidermidis **n=701**	***Staphylococcus haemolyticus*** **n=802**	***Staphylococcus hominis*** **n=428**	***Staphylococcus lugdunensis*** **n=76**	***Staphylococcus saprophyticus*** **n=26**	***Staphylococcus spp.*** **n=1523**
Cefoxitin	225/615 (36.6)	129/715 (18)	112/368 (30.4)	40/74 (54.1)	12/21 (57.1)	399/1490 (26.8)
Ciprofloxacin	354/688 (51.5)	178/771 (23.1)	200/413 (48.4)	51/75 (68)	22/25 (88)	366/810 (45.2)
Clindamycin	486/692 (70.2)	376/793 (47.4)	276/424 (65.1)	44/74 (59.5)	11/19 (-)	860/1491 (57.7)
Erythromycin	190/689 (27.6)	106/790 (13.4)	96/422 (22.7)	31/74 (41.9)	7/18 (-)	384/1506 (25.5)
Linezolid	675/689 (98)	732/755 (97)	395/407 (97.1)	75/75 (100)	22/22 (100)	1427/1465 (97.4)
Penicillin	51/587 (8.7)	21/563 (3.7)	65/341 (19.1)	18/73 (24.7)	4/22 (18.2)	107/1006 (10.6)
Teicoplanin	354/369 (95.9)	234/239 (97.9)	133/152 (87.5)	16/16 (-)	15/15 (-)	569/584 (97.4)
Tetracycline	578/676 (85.5)	618/760 (81.3)	320/406 (78.8)	64/74 (86.5)	14/17 (-)	1059/1329 (79.7)
Tigecycline	130/131 (99.2)	67/69 (97.1)	55/56 (98.2)	0/0 (-)	2/2 (-)	33/34 (97.1)
Trimethoprim-sulfamethoxazole	342/684 (50)	313/763 (41)	196/404 (48.5)	45/74 (60.8)	15/26 (57.7)	770/1467 (52.5)
Vancomycin	325/325 (100)	580/580 (100)	273/273 (100)	12/12 (-)	13/13 (-)	484/484 (100)

Table 6.10: Susceptibility pattern of CoNS isolated from all samples except faeces and urine across different regions of India

Antibiotic	National (n=3363)		North (n=1229)		Central (n=118)		East (n=282)		West (n=1013)		South (n=721)	
	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range
Vancomycin	1659/1659 (100)	100-100	730/730 (100)	100-100	13/13 (-)	-	129/129 (100)	100-100	526/526 (100)	100-100	261/261 (100)	100
Tigecycline	278/283 (98.2)	97.3-100	0/0 (-)	-	13/14 (-)	-	120/121 (99.2)	99.1	140/143 (97.9)	97.3-100	5/5 (-)	-
Linezolid	3150/3231 (97.5)	84.6-99.8	1135/1151 (98.6)	85.4-99.8	107/115 (93)	92.8	259/280 (92.5)	84.6-96.6	956/981 (97.5)	94.2-99.2	693/704 (98.4)	98.2-98.8
Teicoplanin	1191/1243 (95.8)	75.3-100	293/313 (93.6)	75.3-100	30/37 (81.1)	78.8	214/230 (93)	88-100	633/642 (98.6)	95.6-100	21/21 (100)	0
Tetracycline	2516/3082 (81.6)	61.5-97.9	983/1159 (84.8)	62.2-97.9	92/118 (78)	78.1	221/279 (79.2)	61.5-84.3	754/945 (79.8)	64.4-89.8	466/581 (80.2)	78.2-83.3
Clindamycin	1949/3315 (58.8)	23.1-73.5	716/1228 (58.3)	33.7-72.1	63/117 (53.8)	54.9	179/279 (64.2)	23.1-72.9	605/998 (60.6)	49-73.5	386/693 (55.7)	54.8-59.3
Trimethoprim-sulfamethoxazole	1579/3235 (48.8)	30.8-72.5	512/1152 (44.4)	42.4-68.3	58/117 (49.6)	50	151/273 (55.3)	30.8-72.5	523/984 (53.2)	48.4-59.9	335/709 (47.2)	44.7-48.1
Ciprofloxacin	1084/2614 (41.5)	8.6-56.5	487/1153 (42.2)	9.9-49.9	45/116 (38.8)	39.3	140/281 (49.8)	42.3-56.5	313/788 (39.7)	8.6-46.1	99/276 (35.9)	37.8
Cefoxitin	857/3109 (27.6)	14.2-71.4	342/1148 (29.8)	17.4-42.2	16/113 (14.2)	14.2	63/189 (33.3)	21.6-71.4	257/949 (27.1)	20.6-29.7	179/710 (25.2)	24.1-28.9
Erythromycin	768/3324 (23.1)	3.8-31.9	289/1225 (23.6)	15-31.9	17/117 (14.5)	15	55/278 (19.8)	3.8-25.5	216/988 (21.9)	16.8-26.4	191/716 (26.7)	24-28.6
Penicillin	239/2412 (9.9)	6-15.7	108/1105 (9.8)	9.7-9.9	14/112 (12.5)	12.5	27/220 (12.3)	8.5-15.7	88/944 (9.3)	6-12	2/31 (6.5)	0

Table 6.11: Year wise susceptibility trends of CoNS from All Samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=490	Total n=2830	Total n=4016	Total n=3558
	(S%)	(S%)	(S%)	(S%)
Cefoxitin	173/490 (35.3)	930/2810 (33.1)	982/3574 (27.5)	918/3286 (27.9)
Ciprofloxacin	159/335 (47.5)	986/2236 (44.1)	1145/3015 (38)	1172/2785 (42.1)
Clindamycin	297/488 (60.9)	1613/2782 (58)	2151/3952 (54.4)	2055/3496 (58.8)
Erythromycin	148/488 (30.3)	742/2679 (27.7)	755/3459 (21.8)	814/3502 (23.2)
Linezolid	375/381 (98.4)	2638/2680 (98.4)	3796/3900 (97.3)	3328/3416 (97.4)
Oxacillin	0/0	3/3	13/14	10/11
Penicillin	58/224 (25.9)	223/1227 (18.2)	185/2021 (9.2)	266/2595 (10.3)
Teicoplanin	335/336 (99.7)	2212/2236 (98.9)	2912/3083 (94.5)	1324/1378 (96.1)
Tetracycline	176/226 (77.9)	1177/1358 (86.7)	2236/2811 (79.5)	2654/3265 (81.3)
Tigecycline	0/1	165/167 (98.8)	434/441 (98.4)	287/292 (98.3)
Trimethoprim-sulfamethoxazole	199/379 (52.5)	923/1940 (47.6)	1579/3452 (45.7)	1682/3421 (49.2)
Vancomycin	86/86 (100)	718/718 (100)	1679/1679 (100)	1687/1687 (100)

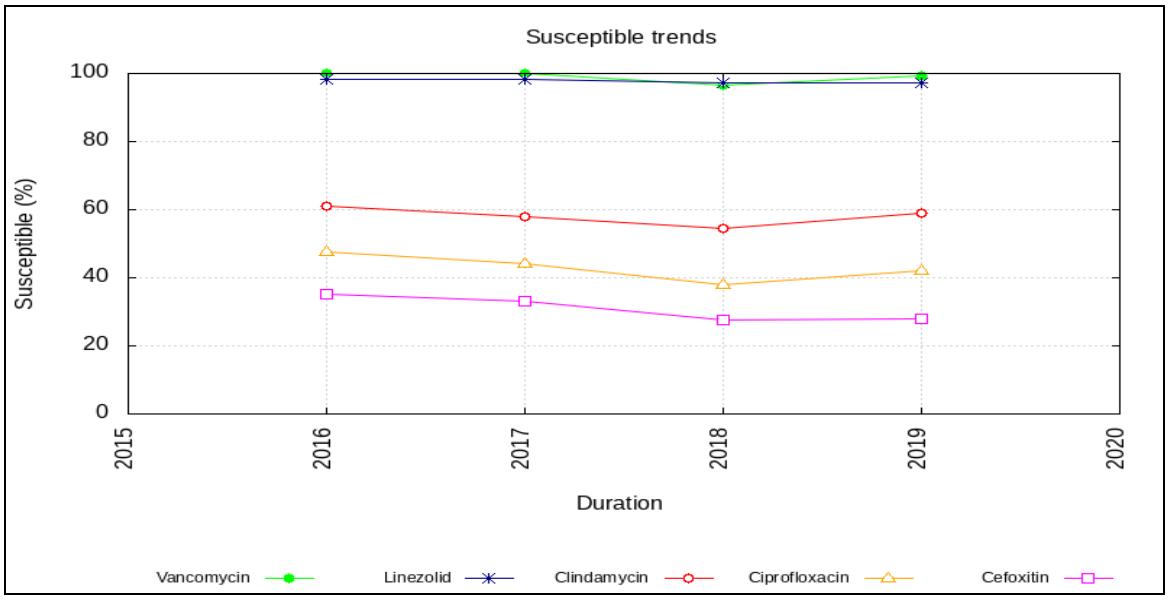

Figure 6.5: Year wise susceptibility trends of CoNS from All Samples

Enterococci

Table 6.12: Susceptibility pattern of Enterococci from all samples except urine

AMA	All Specimens (except urine)		Blood		Superficial Infection		Deep Infection		CSF	
	***Enterococcus faecalis* n=1416**	***Enterococcus faecium* n=1440**	***Enterococcus faecalis* n=301**	***Enterococcus faecium* n=539**	***Enterococcus faecalis* n=562**	***Enterococcus faecium* n=450**	***Enterococcus faecalis* n=261**	***Enterococcus faecium* n=149**	***Enterococcus faecalis* n=*16**	***Enterococcus faecium* n=30**
Ampicillin	1001/1248 (80.2)	183/1195 (15.3)	166/246 (67.5)	63/436 (14.4)	402/487 (82.5)	59/379 (15.6)	220/252 (87.3)	30/128 (23.4)	4/13 (-)	3/28 (10.7)
Gentamicin HL	749/1320 (56.7)	441/1243 (35.5)	99/252 (39.3)	102/406 (25.1)	334/542 (61.6)	185/433 (42.7)	154/256 (60.2)	61/133 (45.9)	0/15 (-)	3/29 (10.3)
Linezolid	1358/1374 (98.8)	1381/1425 (96.9)	284/291 (97.6)	513/535 (95.9)	546/550 (99.3)	437/447 (97.8)	253/255 (99.2)	142/146 (97.3)	15/16 (-)	29/30 (96.7)
Teicoplanin	1293/1322 (97.8)	1156/1410 (82)	255/262 (97.3)	404/522 (77.4)	521/531 (98.1)	386/446 (86.5)	247/252 (98)	131/147 (89.1)	11/11 (-)	11/28 (39.3)
Vancomycin	1367/1407 (97.2)	1156/1430 (80.8)	290/299 (97)	402/530 (75.8)	542/557 (97.3)	384/449 (85.5)	255/260 (98.1)	131/149 (87.9)	14/15 (-)	13/30 (43.3)

Table 6.13: Susceptibility pattern of Enterococci from Urine

AMA	Urine	
	Enterococcus faecalis **n=1467**	***Enterococcus faecium*** **n=1244**
Ampicillin	982/1207 (81.4)	229/1081 (21.2)
Ciprofloxacin	161/977 (16.5)	79/983 (8)
Fosfomycin	668/705 (94.8)	NT
Gentamicin HL	656/1127 (58.2)	390/1135 (34.4)
Linezolid	1357/1367 (99.3)	1170/1203 (97.3)
Nitrofurantoin	1288/1416 (91)	557/1218 (45.7)
Teicoplanin	1277/1299 (98.3)	1039/1214 (85.6)
Vancomycin	1413/1441 (98.1)	1048/1237 (84.7)

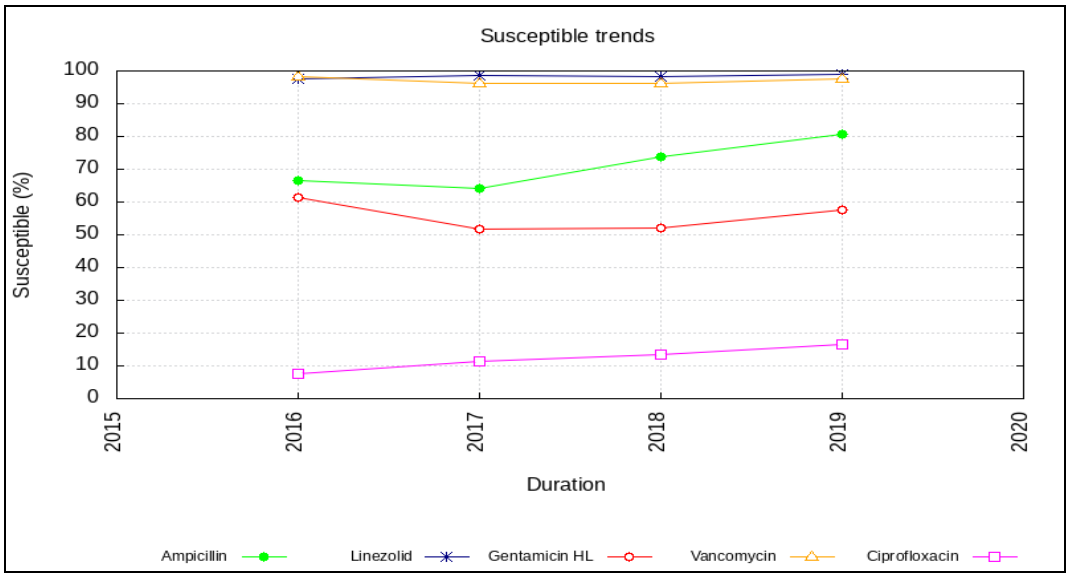

Figure 6.6: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

Table 6.14: Susceptibility pattern of Enterococci from all samples across OPD, Ward and ICU

AMA	*Enterococcus faecalis*				*Enterococcus faecium*			
	Total n=2889	OPD n=1155	Ward n=1546	ICU n=188	Total n=2686	OPD n=576	Ward n=1623	ICU n=487
	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)
Ampicillin	1989/2461 (80.8)	880/995 (88.4)	1031/1321 (78)	78/145 (53.8)	412/2278 (18.1)	165/522 (31.6)	219/1418 (15.4)	28/338 (8.3)
Ciprofloxacin	161/981 (16.4)	99/462 (21.4)	59/464 (12.7)	3/55 (5.5)	79/984 (8)	49/276 (17.8)	27/588 (4.6)	3/120 (2.5)
Fosfomycin	668/705 (94.8)	294/303 (97)	344/372 (92.5)	30/30 (100)	0/0	0/0	0/0	0/0
Gentamicin HL	1409/2452 (57.5)	612/932 (65.7)	739/1352 (54.7)	58/168 (34.5)	832/2380 (35)	225/529 (42.5)	520/1447 (35.9)	87/404 (21.5)
Linezolid	2721/2747 (99.1)	1069/1078 (99.2)	1472/1484 (99.2)	180/185 (97.3)	2553/2630 (97.1)	555/563 (98.6)	1538/1583 (97.2)	460/484 (95)
Nitrofurantoin	1292/1420 (91)	671/712 (94.2)	573/649 (88.3)	48/59 (81.4)	558/1219 (45.8)	216/348 (62.1)	296/734 (40.3)	46/137 (33.6)
Teicoplanin	2576/2627 (98.1)	1035/1041 (99.4)	1388/1427 (97.3)	153/159 (96.2)	2197/2626 (83.7)	500/552 (90.6)	1333/1596 (83.5)	364/478 (76.2)
Vancomycin	2785/2854 (97.6)	1119/1134 (98.7)	1487/1532 (97.1)	179/188 (95.2)	2205/2669 (82.6)	516/573 (90.1)	1326/1615 (82.1)	363/481 (75.5)

Table 6.15: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019
	Total n=126	Total n=1034	Total n=2014	Total n=2889
	(S%)	(S%)	(S%)	(S%)
Ampicillin	82/123 (66.7)	633/987 (64.1)	1338/1813 (73.8)	1989/2461 (80.8)
Ciprofloxacin	3/40 (7.5)	41/358 (11.5)	87/641 (13.6)	161/981 (16.4)
Fosfomycin	*0/0	209/222 (94.1)	469/536 (87.5)	668/705 (94.8)
Gentamicin HL	73/119 (61.3)	512/993 (51.6)	982/1890 (52)	1409/2452 (57.5)
Linezolid	123/126 (97.6)	998/1011 (98.7)	1832/1863 (98.3)	2721/2747 (99.1)
Nitrofurantoin	38/40 (95)	352/375 (93.9)	710/763 (93.1)	1292/1420 (91)
Teicoplanin	124/126 (98.4)	992/1030 (96.3)	1889/1970 (95.9)	2576/2627 (98.1)
Vancomycin	123/125 (98.4)	978/1016 (96.3)	1921/2000 (96.1)	2785/2854 (97.6)

Table 6.16: Year wise susceptibility trends of *Enterococcus faecium* from all samples

AMA	Year-2016 Total n=180 (S%)	Year-2017 Total n=937 (S%)	Year-2018 Total n=1476 (S%)	Year-2019 Total n=2686 (S%)
Ampicillin	56/178 (31.5)	172/860 (20)	214/1213 (17.6)	412/2278 (18.1)
Ciprofloxacin	2/34 (5.9)	10/230 (4.3)	26/446 (5.8)	79/984 (8)
Gentamicin HL	27/102 (26.5)	208/812 (25.6)	360/1247 (28.9)	832/2380 (35)
Linezolid	170/179 (95)	860/910 (94.5)	1352/1411 (95.8)	2553/2630 (97.1)
Nitrofurantoin	16/33 (48.5)	181/251 (72.1)	259/509 (50.9)	558/1219 (45.8)
Teicoplanin	158/179 (88.3)	740/926 (79.9)	1148/1461 (78.6)	2197/2626 (83.7)
Vancomycin	156/178 (87.6)	697/914 (76.3)	1139/1465 (77.7)	2205/2669 (82.6)

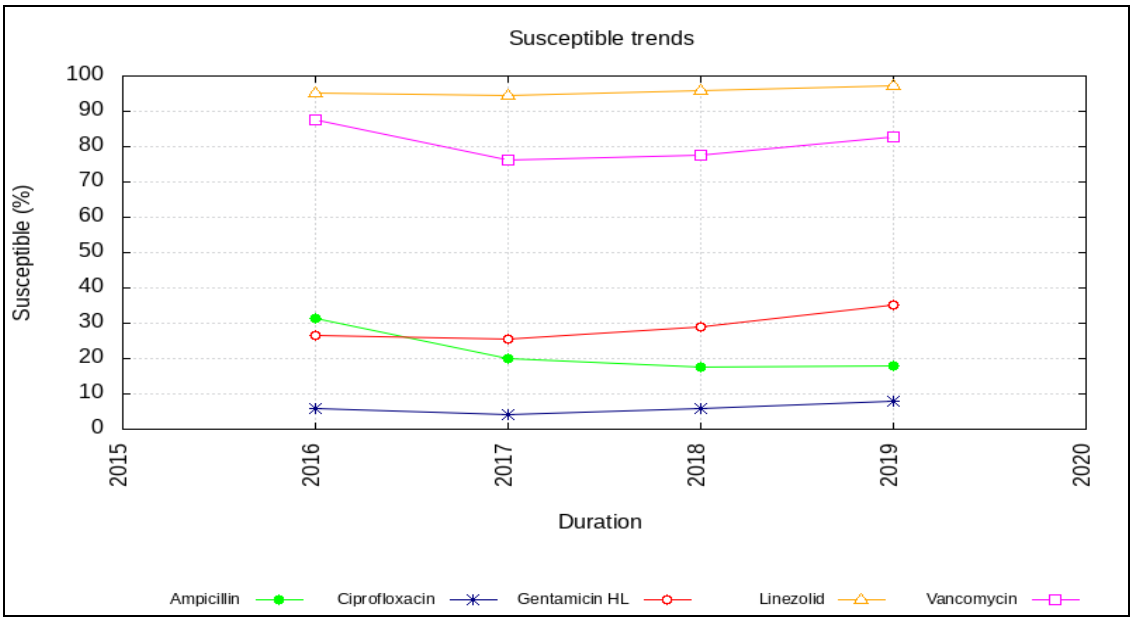

Figure 6.7: Year wise susceptibility trends of *Enterococci faecium* from all samples

Table 6.17: Susceptibility Percentage of *Enterococcus faecalis* from Total (Except Faeces & Urine)

Antibiotic	National (n=1418)		North (n=253)		Central (n=19)		East (n=73)		West (n=165)		South (n=908)	
	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range	n (%)	%Range
Linezolid	1358/1374 (98.8)	90.5-100	229/234 (97.9)	97.3-100	19/19 (-)	-	69/72 (95.8)	90.5-100	158/162 (97.5)	95.9-98.8	883/887 (99.5)	98-100
Teicoplanin	1293/1322 (97.8)	82.4-100	158/168 (94)	82.4-100	19/19 (-)	-	70/73 (95.9)	90.9-100	156/158 (98.7)	97.9-98.8	890/904 (98.5)	91.8-100
Vancomycin	1367/1407 (97.2)	73-100	228/245 (93.1)	73-100	19/19 (-)	-	70/73 (95.9)	90.9-100	157/162 (96.9)	93.8-98.8	893/908 (98.3)	97-100
Ampicillin	1001/1248 (80.2)	20-95.3	122/250 (48.8)	20-93.2	5/6 (-)	-	33/56 (58.9)	90.5	121/142 (85.2)	83.9-95.3	720/794 (90.7)	82-92
Gentamicin HL	749/1320 (56.7)	13.6-78.9	97/249 (39)	34.7-50	7/9 (-)	-	32/73 (43.8)	13.6-57.7	89/152 (58.6)	54.7-78.9	524/837 (62.6)	42.9-63.8

Table 6.18: Susceptibility Percentage of *Enterococcus faecium* from Total (Except Faeces & Urine)

Antibiotic	National (n=1428)		North (n=387)		Central (n=50)		East (n=129)		West (n=148)		South (n=714)	
	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range	n(%)	%Range
Linezolid	1368/1412 (96.9)	86.1-100	374/387 (96.6)	93.1-98.6	43/48 (89.6)	86.1	121/128 (94.5)	94.4-98.4	137/144 (95.1)	89.7-96.7	693/705 (98.3)	96.8-100
Teicoplanin	1147/1398 (82)	63.4-100	273/365 (74.8)	63.4-78.5	35/49 (71.4)	72.2	113/128 (88.3)	75.9-100	121/144 (84)	82.6-89.7	605/712 (85)	68.9-95.7
Vancomycin	1146/1417 (80.9)	65.9-100	282/381 (74)	65.9-76.3	35/50 (70)	69.4	114/129 (88.4)	75.9-100	121/146 (82.9)	79.3-82.8	594/711 (83.5)	69.7-90.2
Gentamicin HL	436/1230 (35.4)	15-50	83/379 (21.9)	15-50	9/21 (42.9)	0	42/119 (35.3)	31.8-37.5	41/133 (30.8)	25.8	261/578 (45.2)	25-48.9
Ampicillin	183/1182 (15.5)	5.4-38.7	36/383 (9.4)	6.9-25	2/14 (-)	-	30/86 (34.9)	38.7	17/131 (13)	5.4	98/568 (17.3)	7.3-23.4

Table 6.19: Antibiotic resistance genes phenotypically resistant isolates of *S. aureus*, CoNS and enterococci from nodal and regional centers

S.No	Phenotypic resistance	Genes detected	Nodal center (No. positive /no tested)	Regional centers (No. positive /no tested)	National (No. positive /no tested)
1	Methicillin resistant *S. aureus* (MRSA)	*mec*A	*mec*A: 102/105 (97.1%)	*mec*A: 286/287 (99.7%)	*mec*A: 388/392 (99.0%)
2	Erythromycin resistance (*S. aureus*)	*erm* A, *erm* B and *erm* C	*erm* A :35/145 (24.1%) *erm* B :0/145 (0%) *erm* C :72/145 (49.6%) Negative for ermA, B, C:38/145 (26.2%)	*erm* A: 10 /482 (2.1%) *erm* B: 0/482 (0%) *erm* C: 121/482 (25.1%) ermA and C: 9/482 (1.9%) Negative for ermA, B, C: 342 /482 (70.9%)	*erm* A: 45/627 (7.2%) *erm* B: 0/627 (0%) *erm* C: 193/627 (30.8%) ermA and C: 9/482 (1.9%) Negative for ermA, B, C: 380/627 (60.6%)
3	Mupirocin resistance (*S. aureus*)	*mup*A and mupB	*mup* A: 30/35 (85.7%) *mup* B : 0/35 (0%)	*mup* A :5/5 *mup* B :0/5	*mup* A :35/40 (87.5%) *mup* B :0/40 (0%)
4	Linezolid resistant MRSA and MR CoNS	*cfr*	*cfr*: 1/1 (MRSA) 3/3 (CoNS)	*cfr* :1/1(MRSA)	*cfr*: 2/2 (MRSA) 3/3 (CoNS)
5	Vancomycin resistant Enterococci (VRE)	*van*A, *van*B, *van*C_1/C_2	*van*A :97/97 (100%) *van*B :0/97 (0%) *van*C_1/C_2 :0/97 (0%)	*van*A :85/85 (100%) *van*B: 0/85 (0%) *van*C_1/C_2: 0/85 (0%)	*van*A :182/182 (100%) *van*B: 0/182 (0%) *van*C_1/C_2: 0/182 (0%)

Chapter 7 Fungal pathogens

Summary of the results

A total of 2191 yeast isolates were included during the study period, of those 33% (737) were isolated from blood. *Candida albicans* (n=623, 27.6%) and *C. tropicalis* (595, 26.3%) were the two major yeast species isolated. The isolation rate of *C. albicans* and *C. tropicalis* were lesser in the reporting year compared to last year, whereas isolation rate of *C. parapsilosis*, *C. glabrata* and *C. utilis* remained the same (Figure 7.1). In ICUs, *C. tropicalis* was most common (34.4%), followed by *C. albicans* (28.6%) and *C. parapsilosis* (14.3%). In wards, *C. albicans* (30.3%), *C. tropicalis* (29.1%) and *C. utilis* (12.2%) were common isolates. The incidence of *C. auris* significantly increased this year (111, 4.9%) compared to previous year (n=25, 2.1%) (p=0.019) (Table 7.1). *C. auris* was isolated from nine centers (North-48, East-7, West-14, South-42) compared to five centers in previous year. Distribution of *C. albicans* and *C. tropicalis* among different wards are almost similar but in ICUs, *C. tropicalis* precedes *C. albicans* (Figure 7.2). Isolation of *Wicker hamomyces anamolous* among the top ten isolated yeasts is a matter of concern (Table 7.1). Antifungal susceptibility profile of *Candida* species of all specimens revealed; fluconazole susceptibility of 97.7% -*C. utilis*, 92.1% - *C. tropicalis* and 90.9% - *C. albicans;* voriconazole susceptibility 95.1% -*C. albicans,* 94.1% - *C. tropicalis,* 89.1% - *C. glabrata*. More than 94% of *C. albicans* and *C. tropicalis* were susceptible to echinocandins. *C. auris* is the most resistant species exhibiting resistance to fluconazole (94.6%) and voriconazole (78.3%). Susceptibility to echinocandins, the treatment of choice for *C. auris* infection were not better (caspofungin - 69.4%, anidulafungin - 88.2% and micafungin - 91.4%) (Figure 7.7.6). Whereas *C. parapsilosis* which was earlier considered to be less susceptible to echinocandins were mostly susceptible to echinocandins compared to other *Candida* species (Table 7.2). Though *C. utilis*is emerging species, majority of them were susceptible to all major class of antifungals compared to any other *Candida* species (Table 7.2 and Figure 7.7.5). Although the 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 (Figure 7.7.1 and 7.7.2). *C. tropicalis* is the predominant isolate from blood and urine followed by *C. albicans* and other yeast species (Table 7.3 and 7.4). However, *C. tropicalis* isolated from blood were more susceptible to different antifungals compared to isolates obtained from urine (Table 7.3 and 7.4). *C. albicans* was predominantly isolated from genital samples (Table 7.5). Decrease in susceptibility to majority of the antifungals among *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata* needs to be cautiously monitored (Table 7.7.1, 7.7.2, 7.7.3 and 7.7.4).

Aspergillus flavus is the most commonly isolated mold followed by *A. fumigatus. A. flavus* is less susceptible to amphotericin B and caspofungin compared to *A. fumigatus* (Table 7.6). Azole resistant *Aspergillus* causing concerns in western world is not noted in our strains. Molecular mechanisms of resistance among *C. tropicalis* (azole resistance) and *C. auris* (azole and echinocandin resistance) were evaluated. In *C. tropicalis*, over-expression of efflux pumps, mutation in ergosterol pathway genes and transcription factor were responsible for development of resistance. Azole resistance among *C. auris* was due to mutation at 395th position (A to T) in the DNA leading to substitution of tyrosine to phenylalanine at 132nd position in amino acid in the *ERG11* gene. We identified two novel mutations, T1904A (n=2) and T1903C (n=4), leading to substitution of phenylalanine to tyrosine and phenylalanine to lysine at 635th position in *FKS* gene responsible for echinocandin resistance among Indian *C. auris* isolates.We also found previously reported transition mutation C1916T (n=3) (serine to phenylalanine at 639th position) among our echinocandin resistant *C. auris* isolates. Further, a high baseline chitin level in conjunction with an adaptive *FKS1* mutation was found to impart a high-level cross-echinocandin resistance. The study reveals the adjunctive roles played by transcriptional upregulation of *Chs1* and *FKS1* genes in promoting echinocandin resistance in *C. auris*. We also evaluated the phenotypic and molecular responses of *C. auris* to various oxidative and osmotic stresses. Fluconazole susceptible *C. auris* isolates were more tolerant to both hyper-osmotic and oxidative stress compared to the resistant group. *Hog 1* gene expression was upregulated upon oxidative stress exposure to fluconazole susceptible *C. auris* clinical isolates unlike resistant *C. auris* group. FAFLP analysis of the isolates of largest outbreak of *C. krusei* candidemia in pediatric unit revealed relations of the clinical strains with isolates from environment and hands of healthcare workers. Amongst the azole group of antifungals tested against outbreak isolates of *C. krusei* exhibited MIC <= 0.5 mg/L against itraconazole, voriconazole and posaconazole except for two isolates which showed MICs of>= 2 mg/L against voriconazole.

Clinical relevance

Fungal infection among hospitalized patients is significantly increasing. Though the majority of these infections are caused by few common species, the number of rare species is increasing implying requirement of newer treatment strategies. Isolation of *C. auris*, a multidrug resistant yeast, from many regional centers indicates its spread across the country. *C. auris*is known to cause outbreaks and persists in the hospital environment for prolonged period increasing the probability of contracting infection in patients. Therefore, appropriate disinfection and decontamination strategies must be practiced reducing the menace of *C. auris*. Reduced susceptibility to commonly used antifungals among most frequently isolated fungal species such as *C. tropicalis, C. albicans* and *C. parapsilosis* restricts treatment options. Molecular studies revealed significant changes at molecular

level leading to better adaptation of these fungal species to currently used antifungals. Fluconazole acts by inhibiting 14-α lanosterol demethylase. However, mutation in the gene responsible for the production of this enzyme imparts resistance to azoles. There is a need to develop of rapid methods to detect this resistance that may help in proper management. Similarly, we also evaluated the underlying molecular mechanism responsible for azole and echinocandin resistance among *C. auris* clinical isolates. We found mutations in the genes responsible for the production of enzymes where these drugs act. Echinocandin resistant *C. auris* isolates had mutation in different part of*FKS* gene, emphasizing its ability to adapt various antifungal stresses. These findings help in understanding the underlying resistance mechanism which in turn helps in choosing appropriate antifungal. We also explored the outbreak situation in pediatric ward due to *C. krusei* and found that hospital surfaces and hands of health care workers were contaminated by this yeast. Thereby, transmitting among various patients in the ward via fomites and hands of health care workers causing infection among susceptible patients. Periodic disinfection of the fomites and adherence to hand hygiene by the health care workers can significntly reduce the fungal infection.

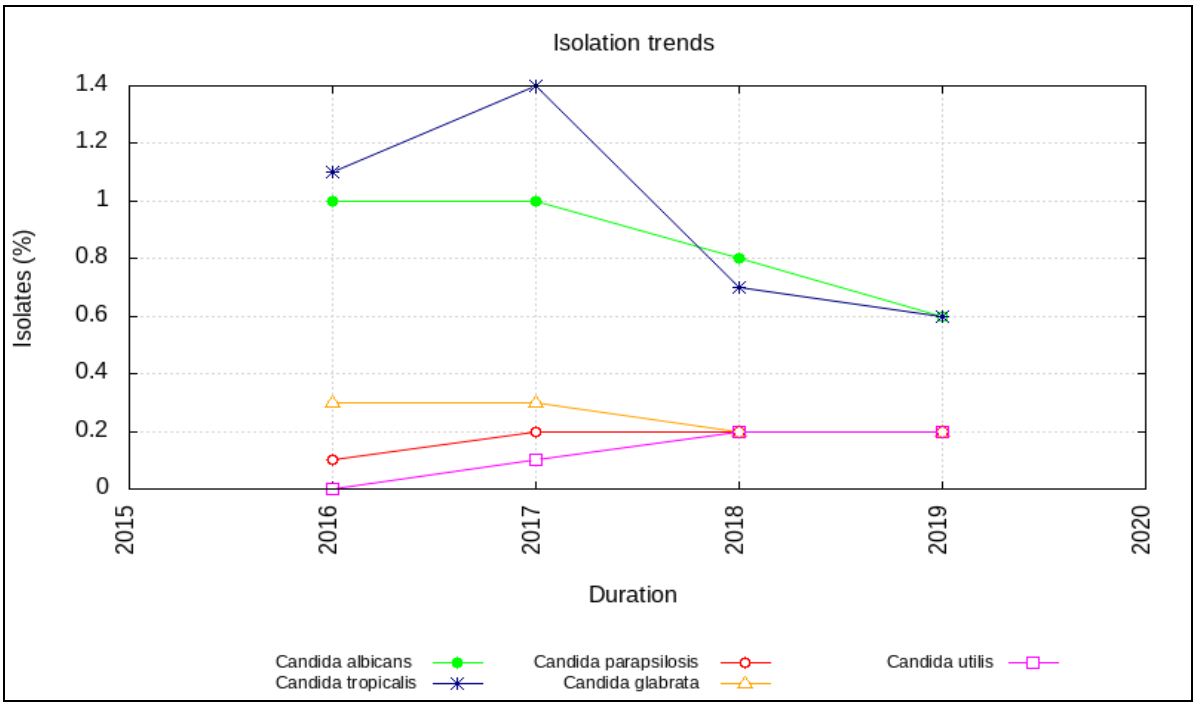

Figure 7.1: Yearly trends for isolation of *Candida* species isolated from all samples.

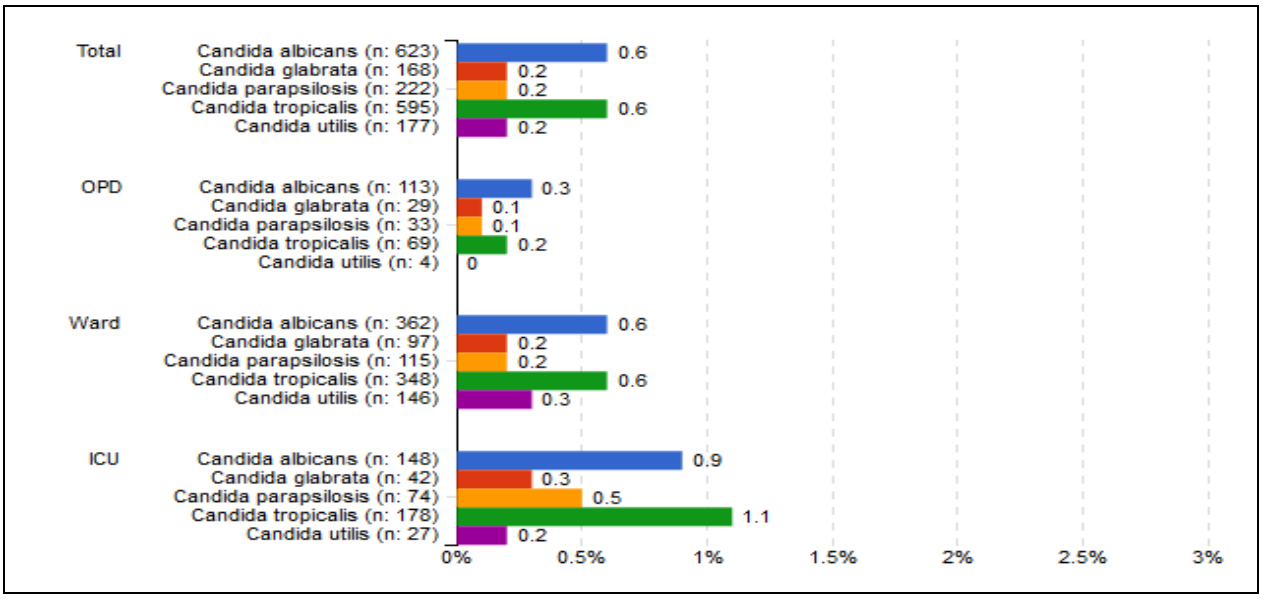

Figure 7.2: Location-wise pattern of *Candida* species isolated from all samples across OPD, Wards and ICUs.

Table 7.1: *Candida* species isolated from all samples across OPD, Ward and ICUs.

	All Specimen			
	Total	**OPD**	**Ward**	**ICU**
Candida albicans	623/107387 (0.58)	113/35753 (0.31)	362/55782 (0.64)	148/15852 (0.93)
Candida tropicalis	595/107387 (0.55)	69/35753 (0.19)	348/55782 (0.62)	178/15852 (1.12)
Candida parapsilosis	222/107387 (0.20)	33/35753 (0.09)	115/55782 (0.20)	74/15852 (0.46)
Candida utilis	177/107387 (0.16)	4/35753 (0.01)	146/55782 (0.26)	27/15852 (0.17)
Candida glabrata	168/107387 (0.15)	29/35753 (0.08)	97/55782 (0.17)	42/15852 (0.26)
Candida auris	111/107387 (0.10)	11/35753 (0.03)	65/55782 (0.11)	35/15852 (0.22)
Wickerhamomyces anomalus	99/107387 (0.1)	0/0 (-)	90/55782 (0.16)	9/15852 (0.05)
Candida krusei	91/107387 (0.08)	18/35753 (0.05)	60/55782 (0.16)	13/15852 (0.08)
Candida pelliculosa	22/107387 (0.02)	3/35753 (0.008)	11/55782 (0.01)	8/15852 (0.05)
Candida kefyr	13/107387 (0.01)	5/35753 (0.01)	7/55782 (0.01)	1/15852 (0.006)
Candida haemulonii	5/107387 (0.004)	1/35753 (0.002)	4/55782 (0.007)	0/0 (-)

Table 7.2: Susceptible pattern of *Candida* species isolated from all samples

AMA	All Specimens									
	Candida albicans **n=622**	***Candida auris*** **n=111**	***Candida glabrata*** **n=168**	***Candida guilliermondii*** **n=*15**	***Candida kefyr*** **n=*13**	***Candida krusei*** **n=91**	***Candida parapsilosis*** **n=222**	***Candida pelliculosa*** **n=22**	***Candida tropicalis*** **n=595**	***Candida utilis*** **n=177**
Anidulafungin	116/124 (93.5)	30/34 (88.2)	45/48 (93.8)	*0/2 (-)	*3/3 (-)	45/47 (95.7)	68/69 (98.6)	*2/2 (-)	197/215 (91.6)	151/161 (93.8)
Caspofungin	470/497 (94.6)	68/98 (69.4)	74/129 (57.4)	*0/0 (-)	*8/9 (-)	44/83 (53)	186/193 (96.4)	*8/9 (-)	485/510 (95.1)	165/176 (93.8)
Fluconazole	562/618 (90.9)	6/111 (5.4)	97/143 (67.8)	*7/15 (-)	*12/12 (-)	5/73 (6.8)	162/221 (73.3)	16/21 (76.2)	548/595 (92.1)	173/177 (97.7)
Micafungin	416/418 (99.5)	64/70 (91.4)	95/96 (99)	*2/6 (-)	*7/7 (-)	35/42 (83.3)	137/142 (96.5)	*7/7 (-)	350/353 (99.2)	52/52 (100)
Voriconazole	561/590 (95.1)	17/75 (22.7)	123/138 (89.1)	*11/13 (-)	*13/13 (-)	90/91 (98.9)	180/193 (93.3)	*18/18 (-)	529/562 (94.1)	172/172 (100)

Table 7.3: Susceptible pattern of *Candida* species isolated from blood

AMA	Blood								
	Candida albicans **n=190**	***Candida auris*** **n=75**	***Candida glabrata*** **n=90**	***Candida guilliermondii*** **n=*12**	***Candida krusei*** **n=58**	***Candida parapsilosis*** **n=189**	***Candida pelliculosa*** **n=22**	***Candida tropicalis*** **n=387**	***Candida utilis*** **n=173**
Anidulafungin	75/79 (94.9)	23/26 (88.5)	31/32 (96.9)	*0/1 (-)	42/44 (95.5)	62/63 (98.4)	*2/2 (-)	176/186 (94.6)	148/158 (93.7)
Caspofungin	154/170 (90.6)	42/62 (67.7)	44/73 (60.3)	*0/0 (-)	26/55 (47.3)	156/163 (95.7)	*8/9 (-)	318/338 (94.1)	161/172 (93.6)
Fluconazole	177/189 (93.7)	5/75 (6.7)	53/76 (69.7)	*4/12 (-)	5/58 (8.6)	133/188 (70.7)	16/21 (76.2)	368/387 (95.1)	169/173 (97.7)
Micafungin	114/115 (99.1)	39/40 (97.5)	45/46 (97.8)	*1/5 (-)	*9/15 (-)	112/117 (95.7)	*7/7 (-)	200/203 (98.5)	52/52 (100)
Voriconazole	170/178 (95.5)	15/52 (28.8)	60/70 (85.7)	*8/10 (-)	57/58 (98.3)	149/161 (92.5)	*18/18 (-)	347/360 (96.4)	168/168 (100)

Table 7.4: Susceptible pattern of *Candida* species isolated from Urine

AMA	Urine				
	Candida albicans **n=93**	***Candida auris*** **n=23**	***Candida glabrata*** **n=*18**	***Candida parapsilosis*** **n=*10**	***Candida tropicalis*** **n=100**
Anidulafungin	*5/5 (-)	*2/2 (-)	*5/5 (-)	*2/2 (-)	*12/13 (-)
Caspofungin	78/81 (96.3)	15/23 (65.2)	*9/15 (-)	*9/9 (-)	81/83 (97.6)
Fluconazole	78/91 (85.7)	0/23 (0)	*13/14 (-)	*8/10 (-)	88/100 (88)
Micafungin	78/79 (98.7)	*14/19 (-)	*15/15 (-)	*9/9 (-)	78/78 (100)
Voriconazole	80/87 (92)	*1/15 (-)	*13/16 (-)	*9/9 (-)	87/99 (87.9)

Table 7.5: Susceptible pattern of *Candida* species isolated from genital samples

AMA	Genital		
	Candida albicans **n=85**	***Candida glabrata*** **n=17**	***Candida tropicalis*** **n=13**
Anidulafungin	0/0 (-)	0/0 (-)	0/0 (-)
Caspofungin	0/0 (-)	0/0 (-)	0/0 (-)
Fluconazole	79/84 (94)	0/17 (-)	13/13 (-)
Micafungin	0/0 (-)	0/0 (-)	0/0 (-)
Voriconazole	81/85 (95.3)	15/17 (-)	13/13 (-)

Table 7.6: Susceptible pattern of *Aspergillus* species isolated from all samples

AMA	All Specimens	
	Aspergillus flavus **n=34 (%)**	***Aspergillus fumigatus*** **n=27 (%)**
Amphotericin B	19/27 (70.4)	21/21(100)
Caspofungin	28/33 (84.8)	25/27(92.6)
Itraconazole	34/34 (100)	27/27(100)
Posaconazole	34/34 (100)	27/27(100)
Voriconazole	34/34(100)	27/27(100)

Figures 7.7 (7.7.1 to 7.7.6): Susceptibility trend of various Candida species from all samples

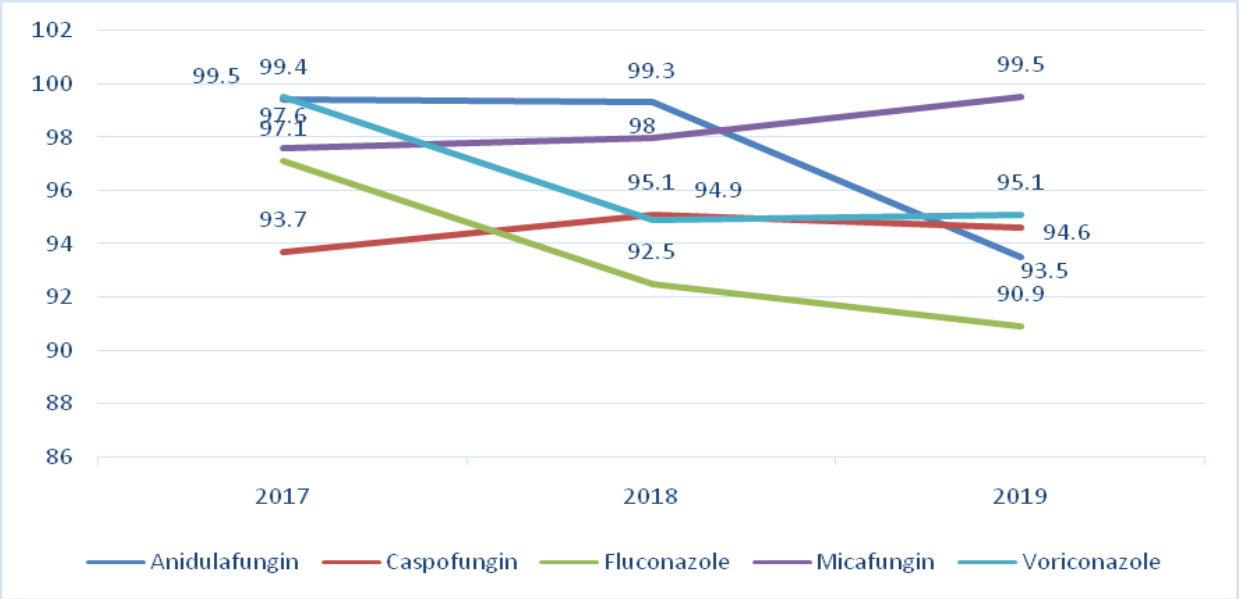

Figure 7.7.1: Susceptibility trend of *Candida albicans* from all samples

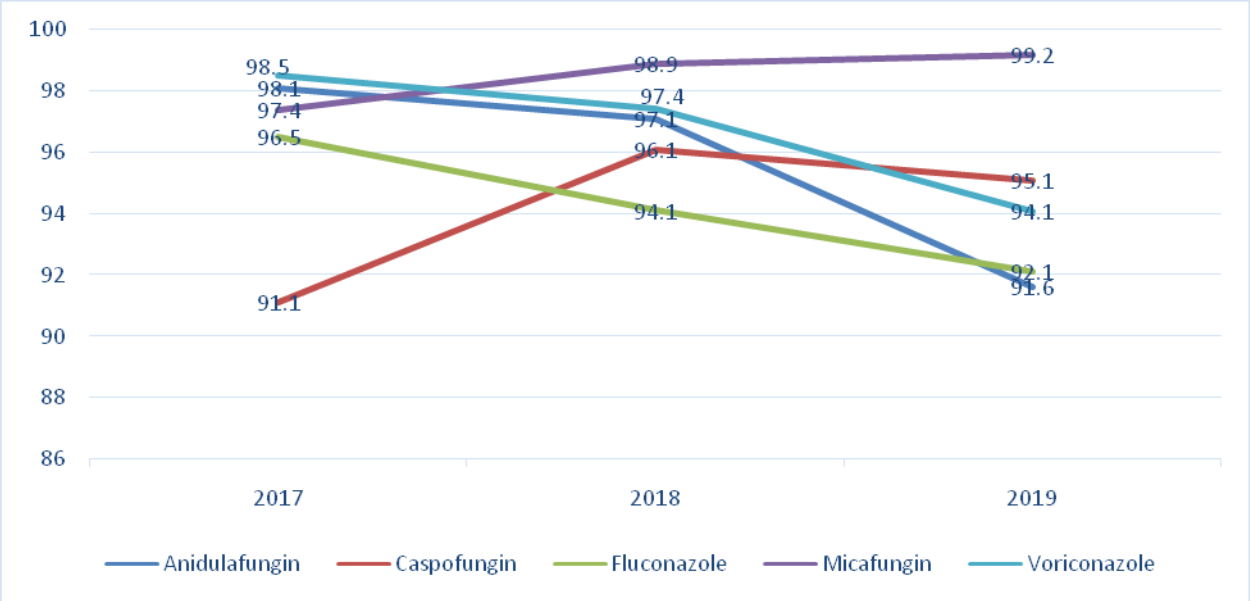

Figure 7.7.2: Susceptibility trend of *Candida tropicalis* from all samples

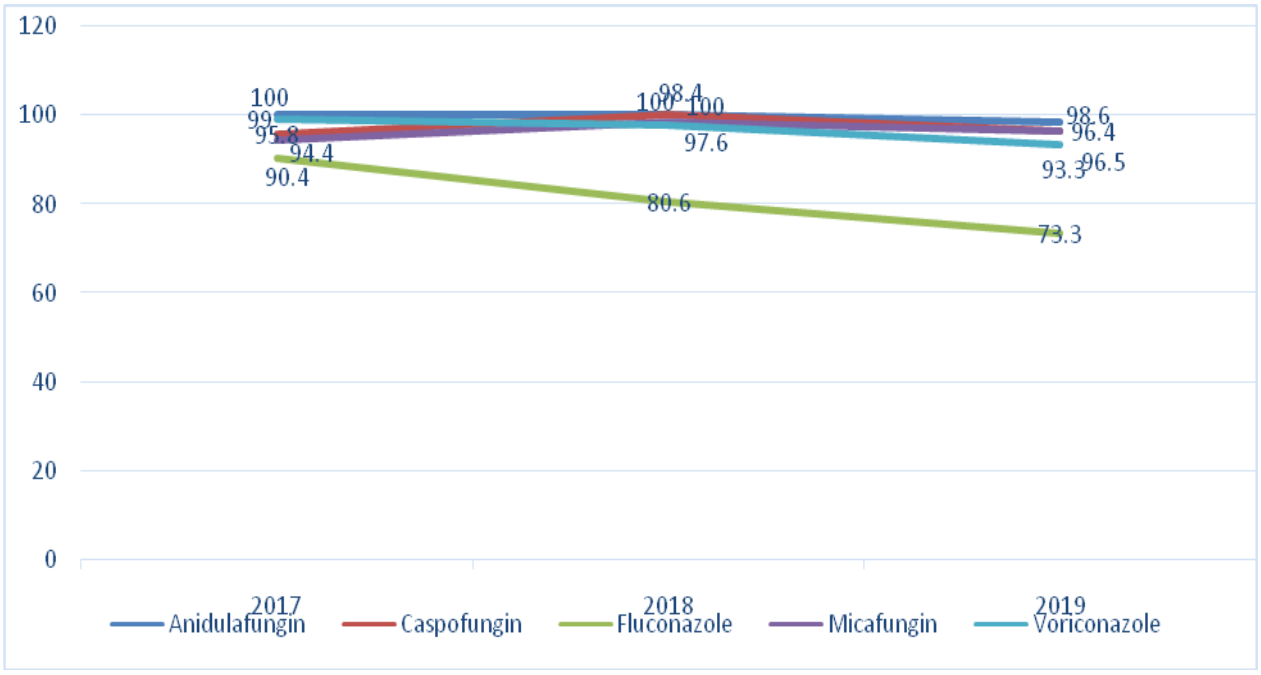

Figure7.7.3: Susceptibility trend of *Candida parapsilosis* from all samples

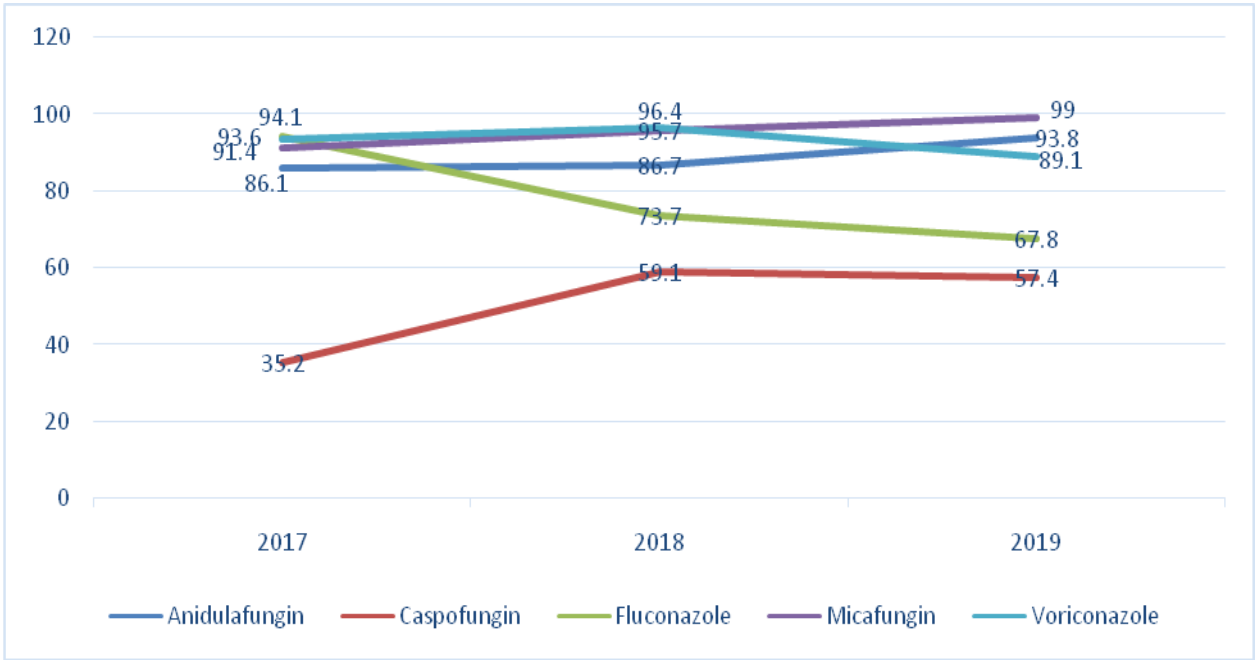

Figure7.7.4: Susceptibility trend of *Candida glabrata* from all samples

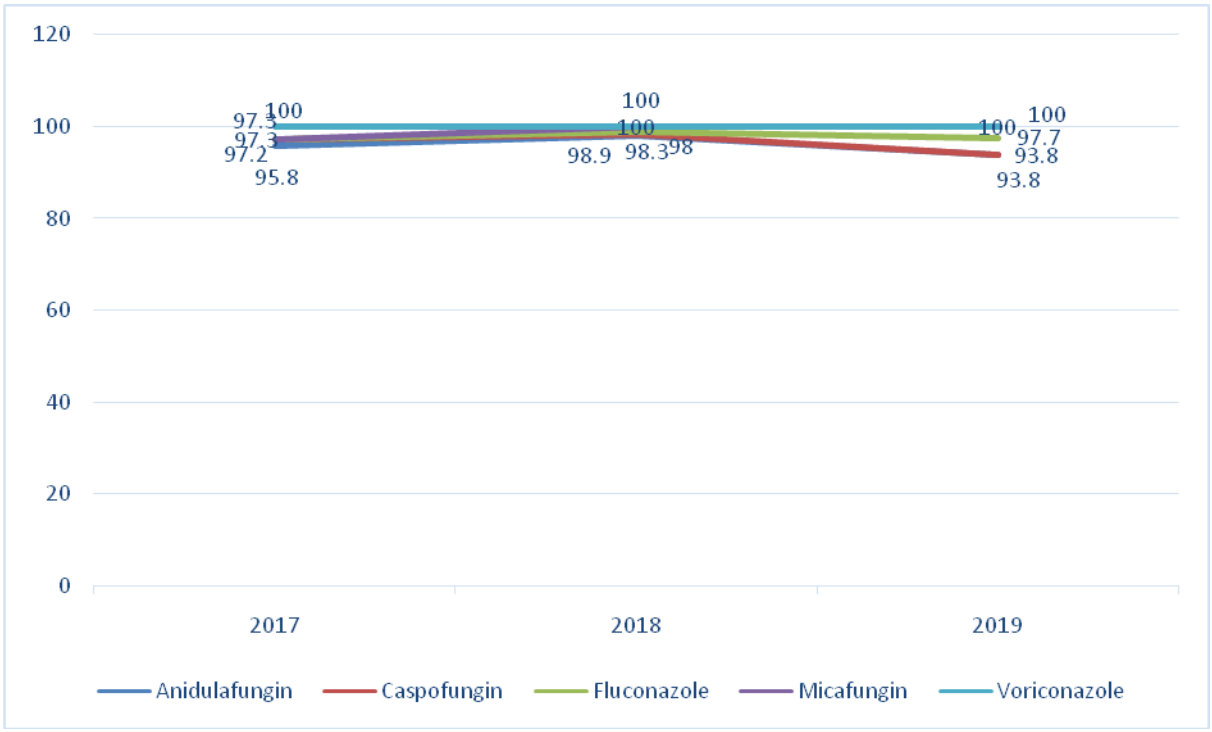

Figure7.7.5: Susceptibility trend of *Candida utilis* from all samples

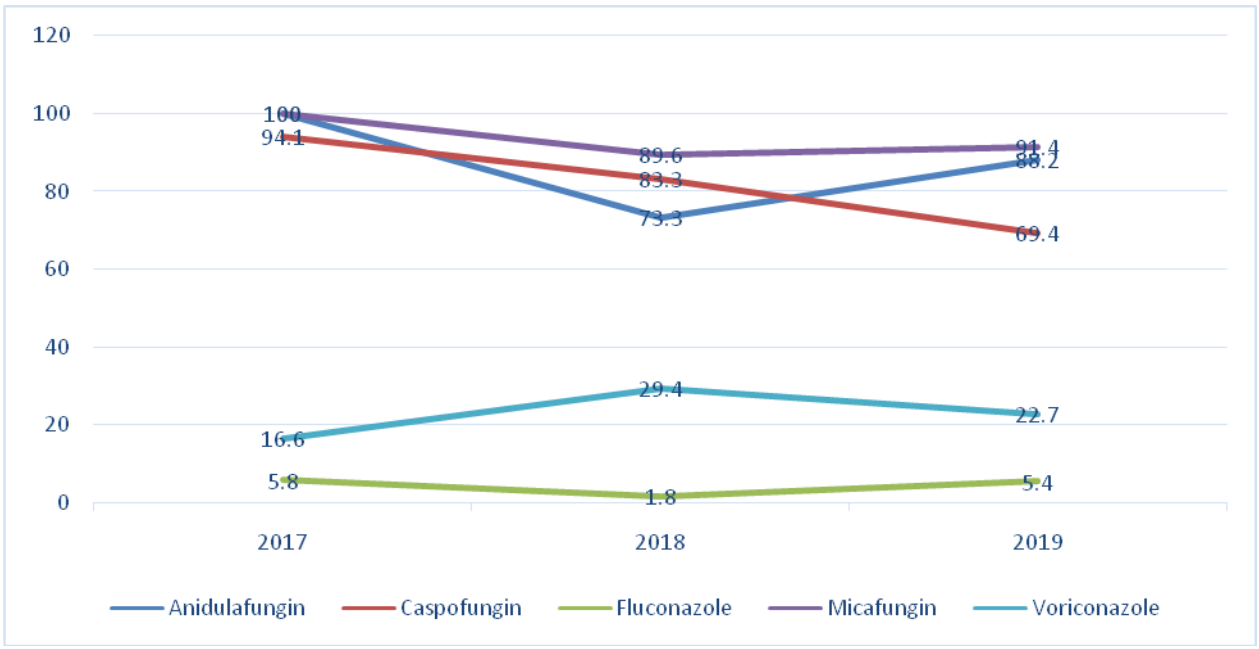

Figure 7.7.6: Susceptibility trend of *Candida auris* from all samples

7.8 Molecular method results

7.8.1: Molecular mechanism of fluconazole resistance in *C. tropicalis:*

*7.8.1.1:*Evaluation of reference genes for RT-qPCR based expression analysis in *Candida tropicalis* following azole treatment [1]

The present study investigated the expression stability levels of ten genes including *ACT1*, *EF1*, *GAPDH*, *PGK1*, *RDN5.8*, *RDN18*, *RDN28*, *SDHA*, *TUB1*, and *UBC13*for their suitability in fluconazole treated/untreated *C. tropicalis* cells(**Error! Reference source not found.**7.8.1.1a).The stability levels of these genes were examined by the ΔΔCT method and five independent software including hkgFinder, geNorm, NormFinder, BestKeeper, and RefFinder software. To determine the expression stability of the reference genes in fluconazole treated *C. tropicalis*, the CT values were compared between the untreated control (u) and fluconazole treated (t) cells utilizing the formula: average CT Change =CT(u) - CT(t). For the validation of reference genes 6 target genes [ABC transporter genes (*CDR1* and *CDR2*), Multi drug resistance gene (*MDR1*), Squalene epoxidase (*ERG1*), $\Delta^{5,6}$-desaturase (*ERG3*), Lanosterol C14α demethylase (*ERG11*)] related to azole resistance were also studied. Figure 7.8.1.1a represents the CT distributions of 10 candidate reference genes in 60 samples [30 isolates (20 resistant and 10 susceptible)] of *C. tropicalis* in the presence and absence of fluconazole. Two ribosomal RNA subunits *RDN18* and *RDN28*, *EF1*, *SDHA*, *UBC13*, and *GAPDH* were the highly stable genes with CT changes <0.5. Whereas*ACT1, PGK1*, *RDN5.8*, and *TUB1* were comparatively less stable reference genes with CT changes >0.5. **Error! Reference source not found.**7.8.1.1b shows the $2^{-\Delta\Delta CT}$ values of the 10 candidate reference genes and indicates that *EF1*, *SDHA*, *RDN18*, *RDN28*, *UBC13*, and *GAPDH* were the most stable, while *ACT1, PGK1*, *RDN5.8,* and *TUB1* were comparatively less stable. As the amplification efficiency of *RDN18* and *RDN28* was very high, they were excluded. Further, *EF1, ACT1* and the nextmost stable genes *GAPDH,* and *SDHA* were evaluated. (Error! Reference source not found.).The presence of fluconazole, noticeably increased the expression levels of azole resistance related genes, *CDR1, CDR2, MDR1, ERG1, ERG3,* and *ERG11* tested when normalized with *EF1*(2.1 to 9.7-fold) and *ACT1* (2.1 to 7.1-fold).[2–4] The expression levels of azole resistance related genes were comparatively lower when normalized with *GAPDH* (1.2 to 5.8-fold) and *SDHA* genes (1.1 to 3.3-fold). However, this variation was not significant (p>0.05) indicating that any of these genes may be utilized for normalization in inducible expression analysis of resistance related genes (Figure7.8.1.1b: Inducible expression levels of *CDR1, CDR2, MDR1, ERG1, ERG3,* and *ERG11* using *EF1, ACT1, GAPDH,* and *SDHA* as internal controls. To check the statistical significance one way ANOVA with multiple comparisons was performed.

7.8.1.1b).

Table 7.8.1.1a: List of candidate reference genes and details of primes used for stability analysis

Gene symbol	Gene name	Accession number	Sequence (5'->3') forward and reverse	Amplicon length (bp)	Ta (ºC)
ACT1	β-actin	XM_002549283.1	CGTCGGTAGACCAAGACACC CCCAGTTGGAGACAATACCGT	137	59
EF1	Elongation factor 1α	XM_002547480.1	GGTCAAACCAGAGAACACGC TTCTTCAAATCTGTTTTTGTCCCA	111	59
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	XM_002551322.1	TTACGAAGAAATTTGTGCTGCT AGCATCAAAGACAGAGGAGTAAGA	130	59
PGK1	Phosphoglycerate kinase	XM_002548594.1	GCTGACGCTGTCGGTAAAG GCAGAAGCAACACAGGCA	116	59
RDN5.8	5.8S ribosomal RNA	AB437083.1	GAGCAATCCTACCGCCAGAG TGCGAGAACCAAGAGATCCG	113	59
RDN18	18S ribosomal RNA	M55527.1	GTGCTGGCGATGGTTCATTC CGTTTCTCAGGCTCCCTCTC	125	59
RDN28	28S ribosomal RNA	KY106836.1	GTGAAGCGGCAAAAGCTCAA CACCCTCTGTGACGTTCTGT	123	59
SDHA	Succinate dehydrogenase complex	XM_002549452.1	TTCGTAACCAAATAAGAAGTTCCGC GCTCATGTATTTGGCAGCGTTA	119	59
TUB1	α-tubulin	XM_002546417.1	TTGACTGGTGTCCAACTGGT CAGCAATAGCGGTAGTGTTAGA	126	59
UBC13	Ubiquitin-conjugating enzyme E2 13	XM_002550926.1	AGTATTCAAGCTTTGTTAGGTGCTC GAGTTTAGTCCATTCTTGAGCCAT	120	59

Table 7.8.1.1b: Stability analysis of reference genes in *C. tropicalis* treated with fluconazole

	EF 1	SDHA	RDN 18	RDN 28	UBC 13	GAPDH	ACT 1	PGK 1	RDN 5.8	TUB1
CT Change	0.15	0.18	0.20	0.22	0.27	0.37	0.56	0.69	0.71	0.80
ΔΔCT	0.00	-0.03	-0.05	-0.08	-0.12	-0.23	-0.41	-0.54	-0.56	-0.65
2-ΔΔCT	1.00	1.02	1.04	1.06	1.09	1.17	1.33	1.45	1.47	1.57
Ranking	1	2	3	4	5	6	7	8	9	10

Table7.8.1.1c: Ranking of *C. tropicalis* reference gene with respect to expression stability as analyzed by six different approaches

Ranking	$2^{-\Delta\Delta CT}$	hkgFinder	geNorm	NormFinder	BestKeeper	RefFinder
1	*EF1*	*EF1*	*RDN18*	*ACT1*	*ACT1*	*RDN18*
2	*SDHA*	*RDN28*	*RDN28*	*RDN18*	*RDN18*	*RDN28*
3	*RDN18*	*RDN18*	*EF1*	*GAPDH*	*PGK1*	*ACT1*
4	*RDN28*	*SDHA*	*ACT1*	*RDN28*	*RDN28*	*EF1*
5	*UBC13*	*GAPDH*	*GAPDH*	*SDHA*	*RDN5.8*	*GAPDH*
6	*GAPDH*	*ACT1*	*SDHA*	*EF1*	*EF1*	*SDHA*
7	*ACT1*	*UBC13*	*RDN5.8*	*UBC13*	*GAPDH*	*RDN5.9*
8	*PGK1*	*RDN5.8*	*UBC13*	*RDN5.8*	*SDHA*	*UBC13*
9	*RDN5.8*	*PGK1*	*PGK1*	*PGK1*	*UBC13*	*PGK1*
10	*TUB1*	*TUB1*	*TUB1*	*TUB1*	*TUB1*	*TUB1*

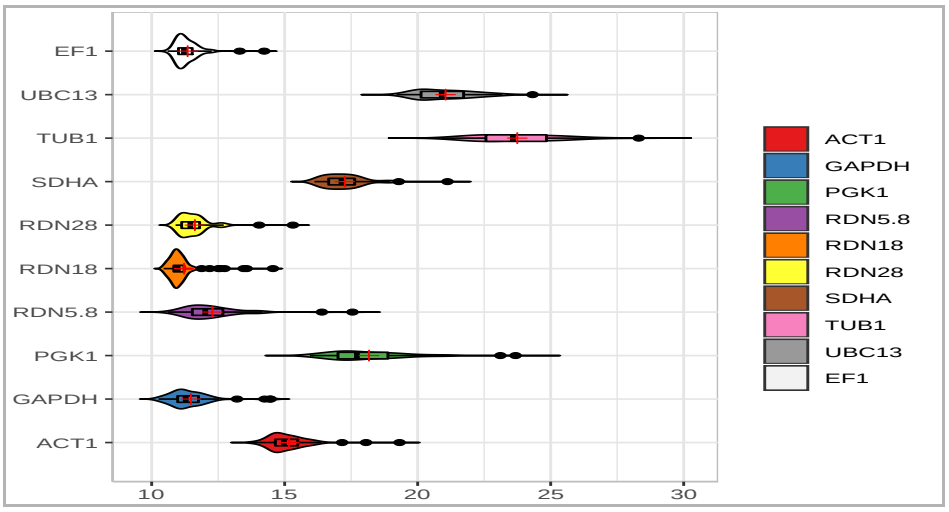

Figure 7.8.1.1a: Violin plot representing the distribution of the CT values obtained for 10 candidate reference genes form 60 samples (30 fluconazole treated and 30 untreated control). Violin plot representing minimum value to maximum value with probability density of the data.

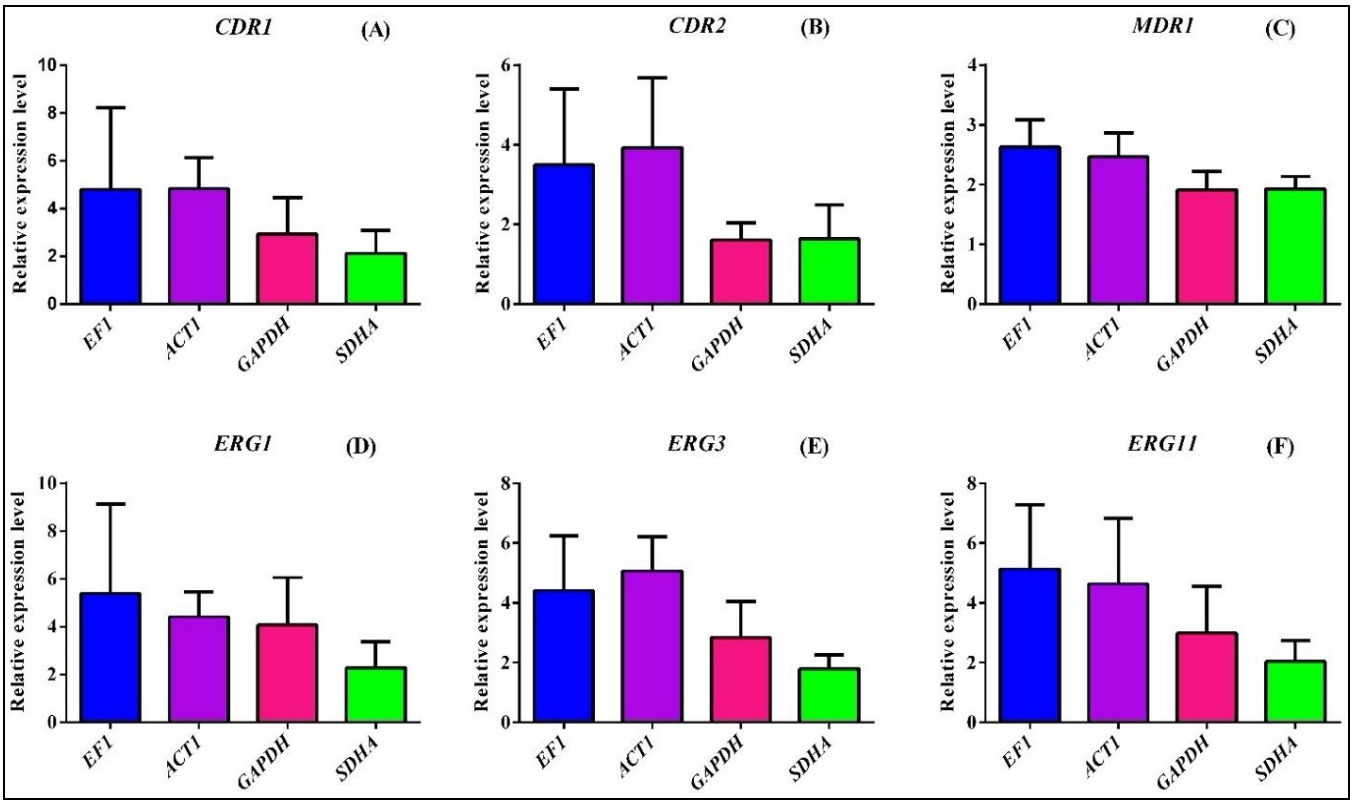

Figure7.8.1.1b: Inducible expression levels of *CDR1, CDR2, MDR1, ERG1, ERG3,* and *ERG11* using *EF1, ACT1, GAPDH,* and *SDHA* as internal controls. To check the statistical significance one way ANOVA with multiple comparisons was performed.

7.8.1.2: Sequencing of *ERG 11* gene among azole resistant *C. tropicalis*

Upon sequencing *ERG 11*gene we found, at 395 position adenine (A) was replaced by thymine (T), whereas at 461 position cytosine (C) was replaced by T (Error! Reference source not found.7.8.1.2a). Due to 'A' to 'T' alteration at 395 position and 'C' to 'T' conversion 461 position in *ERG11* gene, Tyrosine (Y) to Phenylalanine (F) substitution at 132 position and Serine (S) to F alteration at 154 position was noticed in the protein sequence of Lanosterol 14-alpha demethylase enzyme (*ERG11p*). No non synonymous mutations were noticed among the susceptible isolates. The mean inducible expression of *CDR1*, *CDR3*, and *TAC1* of resistant isolates without *ERG11* mutations (R-WTM)(4.9, 4.5, and 3.2 folds respectively) was significantly higher ($p<0.05$) than resistant isolates with *ERG11* mutations (R-WM)(1.8, 1.6, and 2 folds respectively) and susceptible (S)(0.3, 1, and 1.4 fold respectively) isolates. Whereas the expression of *CDR2* and *MRR1* in R-WTM (2.1 and 1.8) and R-WM (2.2 and 1) was significantly higher ($p<0.05$) than the S (0.1 and 0.02) isolates. No significant variation ($p>0.05$) in the *MDR1* expression was noted in these three types of isolates (

7.8.1.2b). The average fold overexpression of *ERG1, ERG2, ERG3, ERG11,* and *UPC2* in R-WTM (11, 3.4, 5, 6.1, and 4.6 respectively) was significantly ($p<0.05$) higher than the R-WM (1.5, 1.4, 1.6, 2.5, and 2 respectively) and S (1.3, 2, 2.2, 2.2, and 0.3 respectively) isolates (

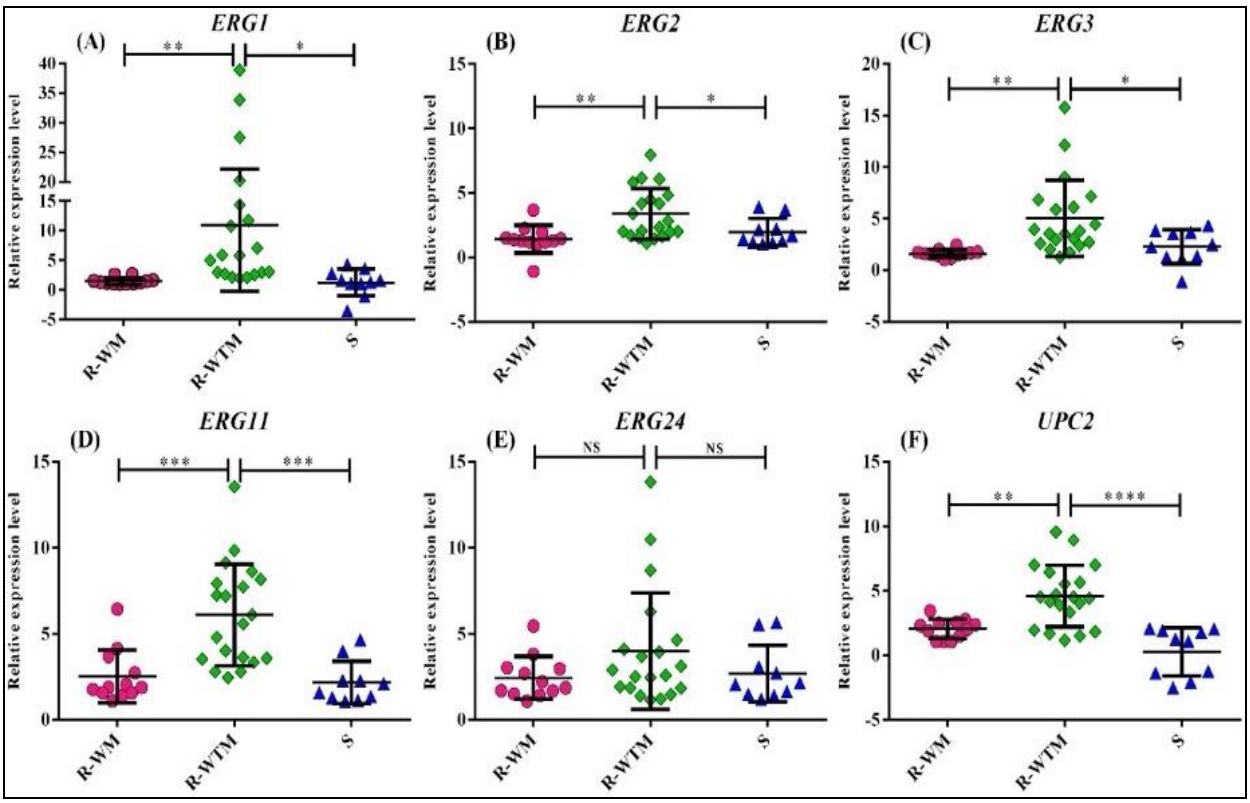

Figure7.8.1.2c). Though the mean expression *ERG24* was comparatively higher in resistant isolates compared to S isolates, no noticeable variation was seen in the mean *ERG24* and *HMG* expression among the R-WTM (4 and 0.1), R-WM (2.5 and 0.4), and S (2.7 and 0.5) isolates ($p>0.05$)

The average fold expression of *HSP90, HOG,* and *SOD1* were very less and no noticeable variation was seen among R-WTM, R-WM and S isolates ($p>0.05$). However, the *MKC1* expression was significantly higher in R-WTM (5.1 fold) compared to R-WM (0.2) and S (1.2) groups ($p<0.05$)

Structural superimposition of wild and mutant protein showed a significant root mean square deviation (RMSD) (2.22 Å). Moreover, RMSD within the mutated residues (amino acid positions 132 and 154) is considerably high (1.12 Å) reflecting significant structural deviation due to these mutations (Figure 7.8.1.2d7.8.1.2d). Gibbs free energy calculation (ΔΔG for Y132F= 1.34, ΔΔG for S154F= -2.87 and overall ΔΔG=1.27) suggested that the reported mutations are destabilizing the protein. Results of the docking study reflected that

the binding energy of both the ligand molecules was significantly low against the mutated protein. Binding energy of fluconazole against the wild protein versus the mutant protein was -6.24 kcal/mol and -3.34 kcal/mol, respectively (Figure 7.8.1.2e A1 and A2). Similarly, the binding energy of voriconazole against the wild protein versus mutant protein was -5.24 kcal/mol and -2.77 kcal/mol, respectively (Figure 7.8.1.2e B1 and B2). The potential binding site analysis revealed that Tyrosine 132 is highly crucial in forming hydrogen bonds between heme cofactor and the ligand molecules i.e. fluconazole and voriconazole in the native form. The substitution of Tyrosine 132 by Phenylalanine 132 negates the hydrogen bond both between the two molecules.

Table 7.8.1.2a: ERG11 gene sauce analysis among resistant and susceptible isolates

	Mutation in *ERG11* gene		**Amino acid alteration**		**No. of isolates**	**Population**
	395	**461**	**132**	**154**		
Reference sequence (MYA-3404)	A	C	Y	S	-	
Resistant isolates without mutation	A	C	Y	S	20	62.5%
Resistant isolates without mutation	T	T	F	F	12	37.5%
Susceptible isolates	A	C	Y	S	10	100%

A: Adenine; T: Thymine; C: Cytosine; Y: Tyrosine ; F: Phenylalanine; S: Serine

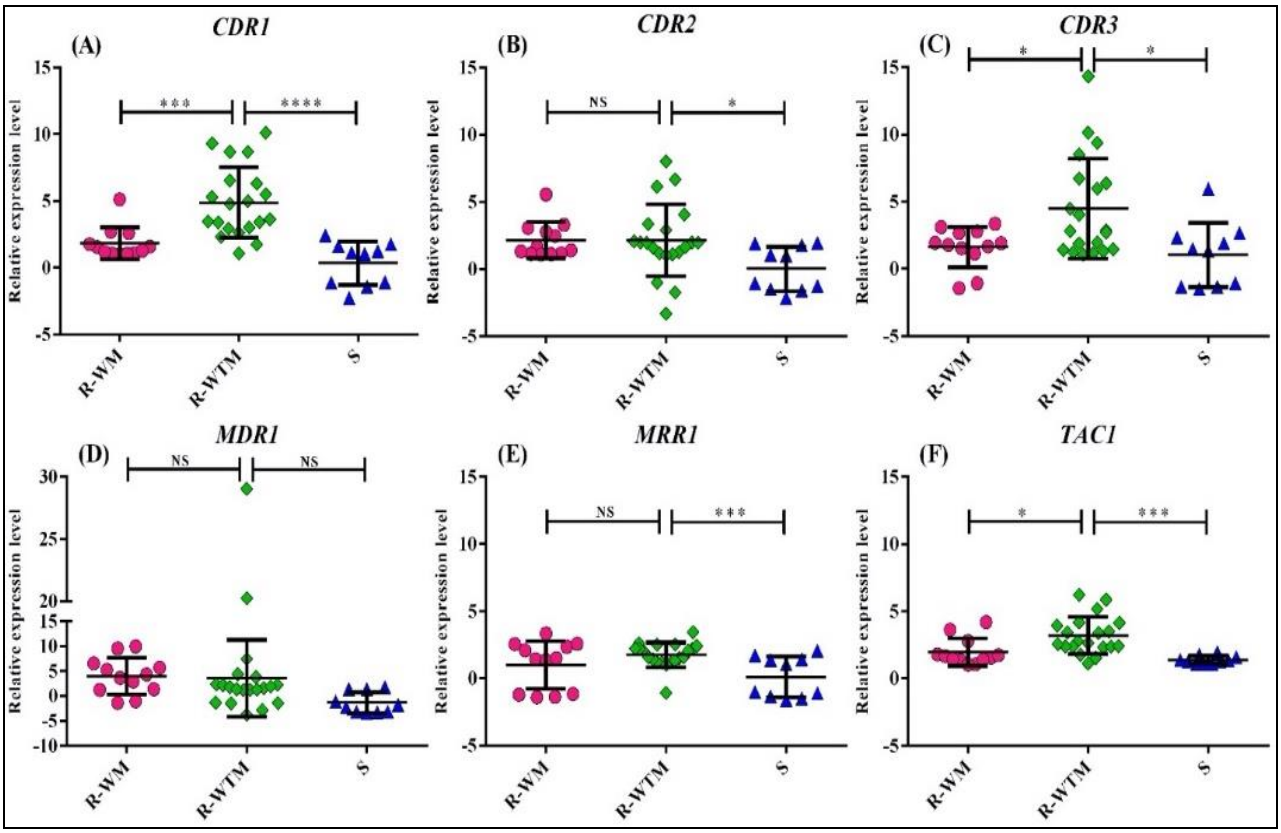

Figure 7.8.1.2b: Scatter dot plots depicting the inducible expression of different transporters (*CDR1, CDR2, CDR3* and *MDR1*) and their transcription factors (*MRR1* and *TAC1*) represented as fold change relative to untreated control. The level of expression was calculated using $2^{-\Delta\Delta CT}$ method. Unpaired t test was performed to determine the statistical significance. * Indicates $p<0.05$, *** Indicates $p<0.001$, **** Indicates $p<0.0001$, and NS indicates Non Significant

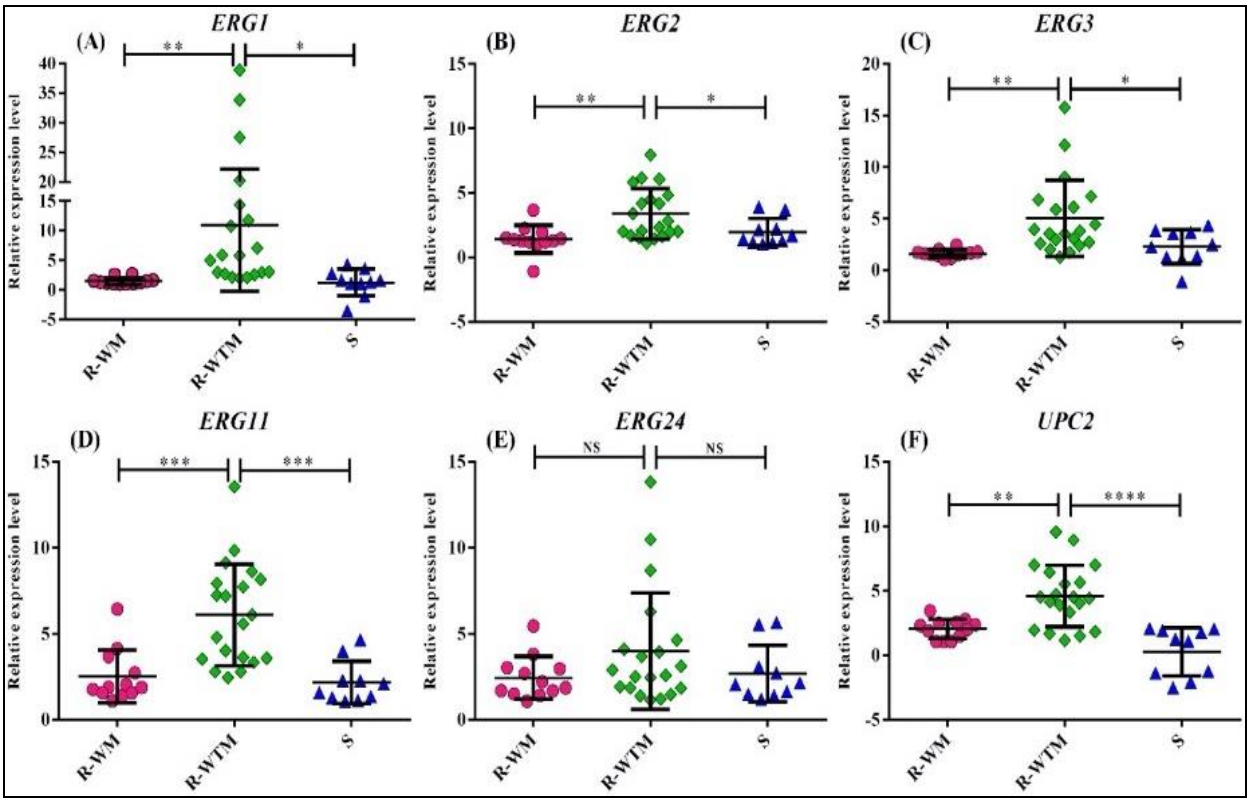

Figure 7.8.1.2c: Plot representing the inducible expression of ergosterol biosynthesis pathway genes (*ERG1, ERG2, ERG3, ERG11,* and *ERG24*) and transcription factor of *ERG11* (*UPC2*). Statistical significance was calculated by using unpaired t test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, and NS=Non significant

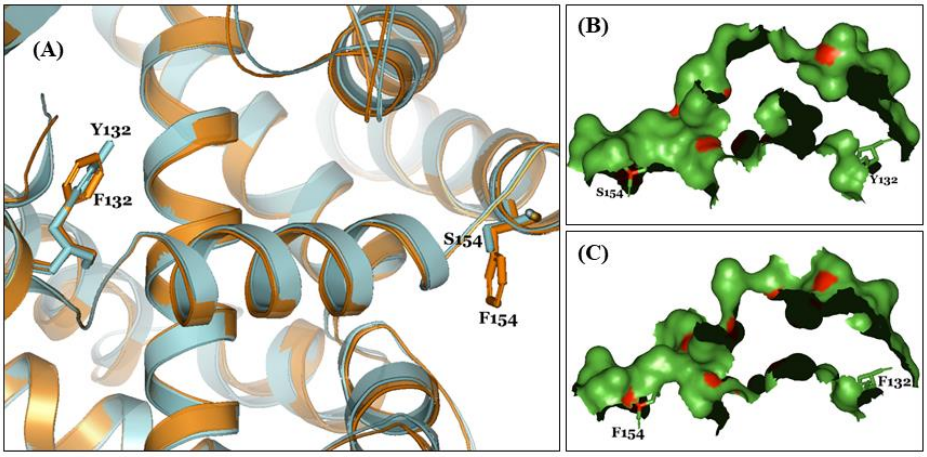

Figure 7.8.1.2d: Homology modelling of *ERG11p*. (A) Structural superimposition of both wild and mutant type. Wild type is colored in cyan and mutant is in brown. Mutated residues are shown in stick representation and labelled accordingly. (B) Surface representation of the wild type protein (C) Surface representation of the mutant type protein.

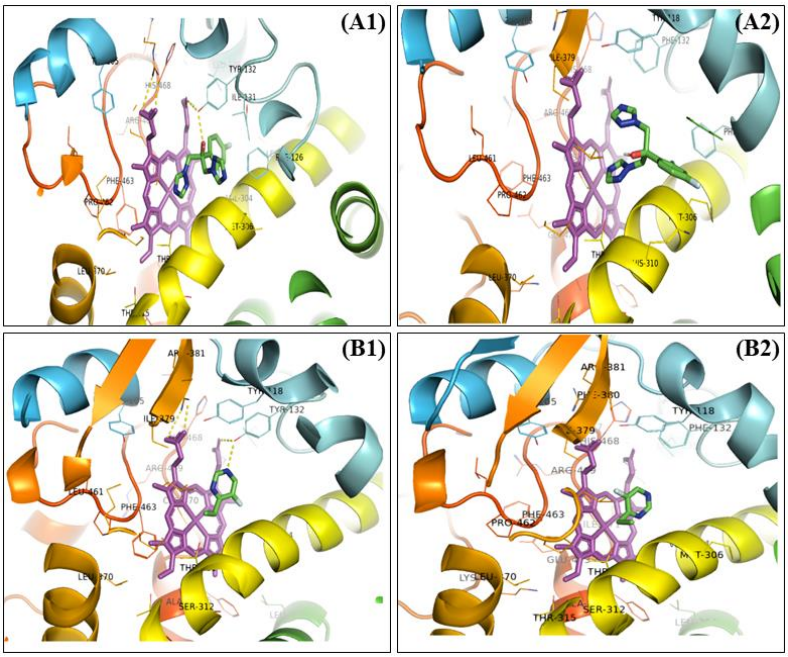

Figure 7.8.1.2e: Docked pose and interacting residues of (A1) wild protein (Y132 and S154) with fluconazole (A2) mutated protein (Y132F and S154F) with fluconazole (B1) wild protein with voriconazole (B2) mutated protein with voriconazole. For clarity, only selected binding site residues are shown. The 'heme' cofactor is shown in violet color (stick representation). Hydrogen bonds are presented as yellow dotted lines.

7.8.2: Molecular mechanism of resistance in *C. auris*

7.8.2.1: Fluconazole resistance

We sequenced the lanosterol 14-alpha demethylase (ERG11) gene for detecting fluconazole resistance in *C. auris* using in-house primers.It showed nucleotide variation at position A150G, A395T, T561C, C864T and T1428C. However, the protein sequence analysis showed only amino-acid substitution in Y132F (Tyrosine to Phenylalanine) conversion corresponding to nucleotide variation A395T. The other nucleotide variations were found to be synonymous in nature

7.8.2.2 Echinocandin resistance

A total of 199 *C. auris* isolates were obtained from 30 centres across country. Antifungal susceptibility (AFST) results demonstrated that anidulafungin exhibited potent efficacy with geometric mean (GM) MIC 0.18 mg/L compared to caspofungin (GM, 0.38 mg/L) and micafungin (GM, 0.22 mg/L), respectively. For evaluation of molecular mechanism of echinocandin resistance in 11 isolates, the primer pairs (*Cau*_HS1-F, 5-GCCATCTCGAAGTCTGCTCA-3; *Cau*_HS1-R 5-TGACAATGGCATT CCACACCT-3) were

designed to amplify hotspot region-1(HS1) of *FKS*1 gene (corresponding to GenBank accession number XM_018312389.1). On FKS sequencing nine isolates exhibiting MIC ≥2 mg/L for any of the three echinocandins carried an adaptive mutation in the HS1 region (Table 7.8.2.2 a). Among them, three harbored S639F mutation which has been previously implicated in echinocandin resistance in *C. auris*. However, a novel mutation, F635Y, was found in two isolates with caspofungin MICs of 4 and 16 mg/L. Of six sequential isolates from a single patient four had F635L substitution resulting in elevated MICs to anidulafungin. Based on the MICs and underlying FKS1 genotype, we categorized our C. auris isolates into four groups; a) echinocandin-resistant with non-wild type FKS1 genotype (RM), b) echinocandin-resistant with wild type FKS1 (RWT), c) intermediate susceptibility with WT FKS1 (IWT), and d) echinocandin susceptible wild type (SWT).Expression levels of *FKS*1 (XM_018312389.1) and two putative chitin synthase genes, *Chs*1 (XM_018310276.1) and *Chs*2 (XM_018313459.1) were evaluated after caspofungin treatment. The oligo sequences used were: *Chs1* (CGCCGTTTACAACCTTTGGA/TGAGAAGCAACGAGTGGGTTT); *Chs*2 (GGTGCCACGGAGTTAGACAA/AGTCAGCACGAGCTTTGACA); *FKS*1 (CGAAGAA CACGGTCAGGACA/CCTCAGGGGTCAAGACGTTC). The housekeeping gene, actin (CGCTGGTTTCTCGTTACCAC/CAGCAGTGGTAGAGAAGGTGT), was used as a reference for normalization of the Ct values. On induction with respective sub-MIC caspofungin concentration, IWT isolates demonstrated higher induction of Chs1 [6 (2.5-11.2)] compared to those in SWT comparator group [1(0.8-2.0), P=0.0005) (Figure 7.8.2.2 b A). Also, one among three isolates in RM group with S639F showed higher induction in Chs1 gene (8.3±1.8) compared to other two isolates (P=0.0001) (Figure 7.8.2.2 b B). All isolates of RM, RWT, and IWT group demonstrated higher upregulation of Chs2 compared to SWT category (P<0.0001) (Figure 7.8.2.2 b C). In addition, differential transcriptional upregulation of FKS1 gene was also observed across the four groups (P<0.0001) (Figure 7.8.2.2 b D).

A previously described flow cytometry-based method was used to measure the baseline and caspofungin-inducible cell wall chitin contents.[7] The baseline chitin contents [Staining Index (interquartile range)] did not vary across the four groups: RM, 10.2 (7.5-17.3); RWT, 24.6 (19-34); IWT, 12.2 (9.5-23); (SWT), 15.7 (13-22.3) (P>0.05) (Figure 7.8.2.2 c). However, exposure to caspofungin resulted in differential increase in chitin levels in 10 (10/17, 58.8%) isolates. The observations were in concordance with the transcript levels of *Chs*1 and *Chs*2.A significant rise in the chitin content was noted in the cell walls of RWT [70 (68.4-73)] and IWT [21 (19.6-35.7)] isolates compared to SWT [18 (14.-22.4)] isolates (RWT vs SWT, P<0.0001; IWT vs SWT, P =0.06). Possibly, one isolate in the RM category having higher basal level of chitin, could grow under combined calcofluor white-caspofungin (CFW-CSP) cell wall stress.

Amplified fragment length polymorphism analysis of these isolates was performed to assess the clonality of isolates as per an earlier described method.[8] AFLP analysis of all the 6 sequential isolates from a patient demonstrated that 3 isolates recovered from lower respiratory tract specimens (one BAL fluid and two from tracheal tube secretion) formed one cluster with fingerprint similarity of >98.5%. Two isolates recovered from urine samples formed second cluster (98% fingerprint similarity), while the blood isolate was different from the above two clusters (Figure 7.7.2.2 d).

Table 7.8.2.2 a: Echinocandin susceptibility profile and *FKS*1 genotype of 11 *C. auris* isolates with elevated echinocandin MICs along with 4 susceptible comparator isolates RM, resistant with non-wildtype *FKS*1; RWT, resistant with wild-type *FKS*1; IWT, isolates with intermediate echinocandin MICs and wild-type *FKS*1; SWT, susceptible isolates with wild-type *FKS*1; BALF, bronchoalveolar lavage fluid; TTS, tracheal tube secretion

					MIC (mg/L)		
Isolate ID	**Sample**	**Phenotype**	***FKS*1 genotype**	**Group**	**CAS**	**AFG**	**MFG**
NCCPF470197	Blood	Resistant	T1904A(F635Y)	RM	16	1	1
NCCPF470198	Urine	Resistant	C1916T(S639F)	RM	4	4	1
NCCPF470199	Urine	Resistant	C1916T(S639F)	RM	4	4	1
NCCPF470203	Blood	Resistant	C1916T(S639F)	RM	2	8	16
NCCPF470209	Blood	Resistant	T1904A(F635Y)	RM	4	2	1
NCCPF470237	Urine	Resistant	T1903C(F635L)	RM	1	2	1
NCCPF470238	Urine	Resistant	T1903C(F635L)	RM	1	2	1
NCCPF470240	Blood	Resistant	T1903C(F635L)	RM	1	2	0.5
NCCPF470241	TTS	Resistant	T1903C(F635L)	RM	1	2	1
NCCPF470200	Urine	Resistant	WT	RWT	16	2	2
NCCPF470201	Blood	Resistant	WT	RWT	2	1	2
NCCPF470239	BALF	Intermediate	WT	IWT	0.5	1	0.25
NCCPF470242	TTS	Intermediate	WT	IWT	0.5	1	0.25
NCCPF470202	Blood	Intermediate	WT	IWT	1	0.5	0.5
NCCPF470204	Blood	Intermediate	WT	IWT	1	0.5	0.5
NCCPF470206	Blood	Intermediate	WT	IWT	1	1	0.25
NCCPF470205	Blood	Intermediate	WT	IWT	1	0.5	0.5
NCCPF470196	Blood	Sensitive	WT	SWT	0.12	0.06	0.12
NCCPF470208	Blood	Sensitive	WT	SWT	0.12	0.25	0.25
NCCPF470210	Blood	Sensitive	WT	SWT	0.12	0.25	0.25
CBS12372	Blood	Sensitive	WT	SWT	0.12	0.12	0.25

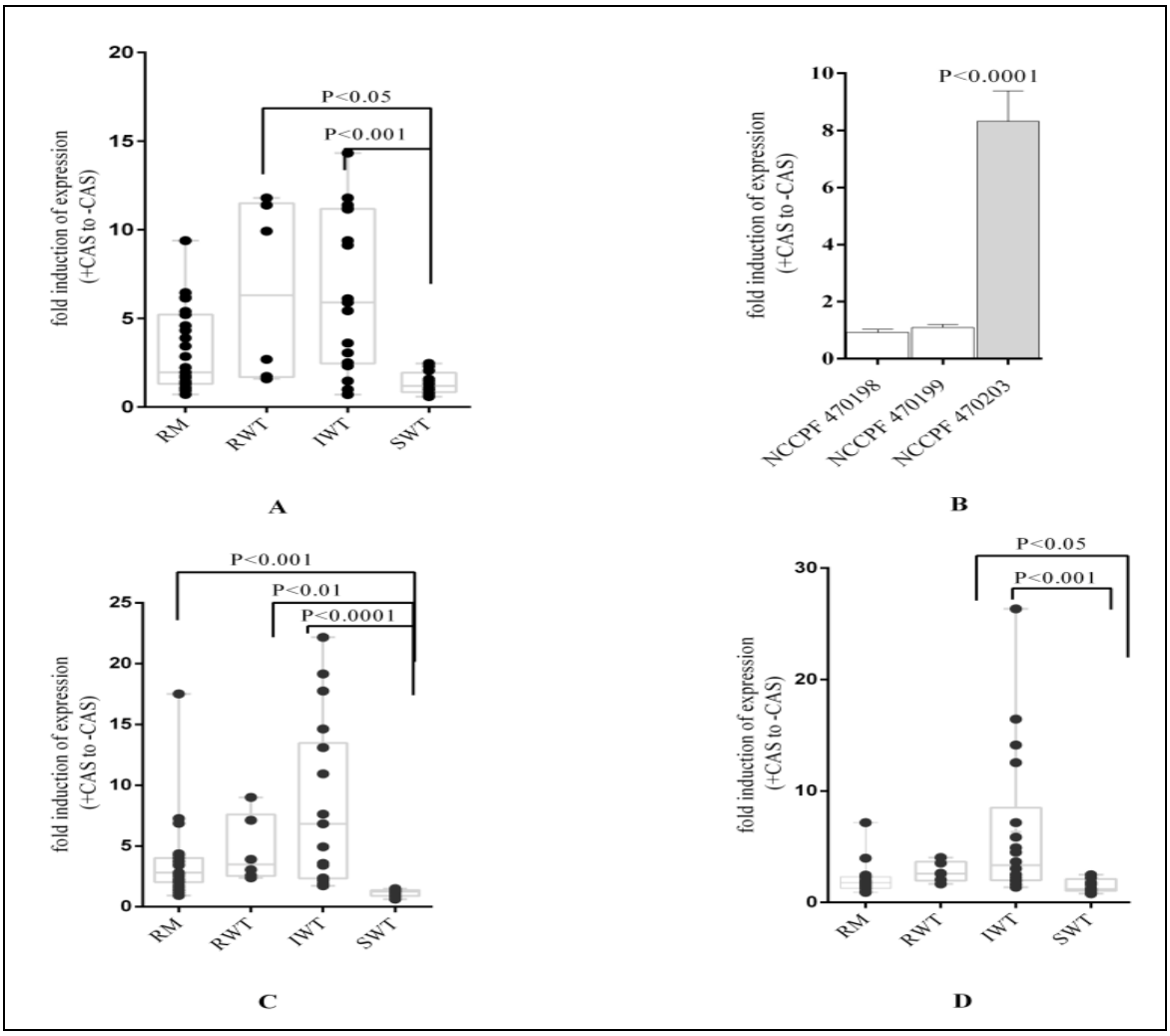

Figure 7.8.2 c**:** Fold-induction in expression of putative chitin synthase gene homologues, (A) *Chs*1 and (C) *Chs*2, and (D) *FKS*1. Three biological replicates with three technical replicates were used for each isolate to determine the transcript levels. The data was analysed using one-way Kruskal- Wallis test with Dunn's post-hoc test for multiple comparisons. Figure 4B represents fold-induction of *Chs*1 in three resistant isolates with S639F *FKS*1 mutation. The data was analysed using one way ANOVA with Bonferroni's test for multiple comparisons.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

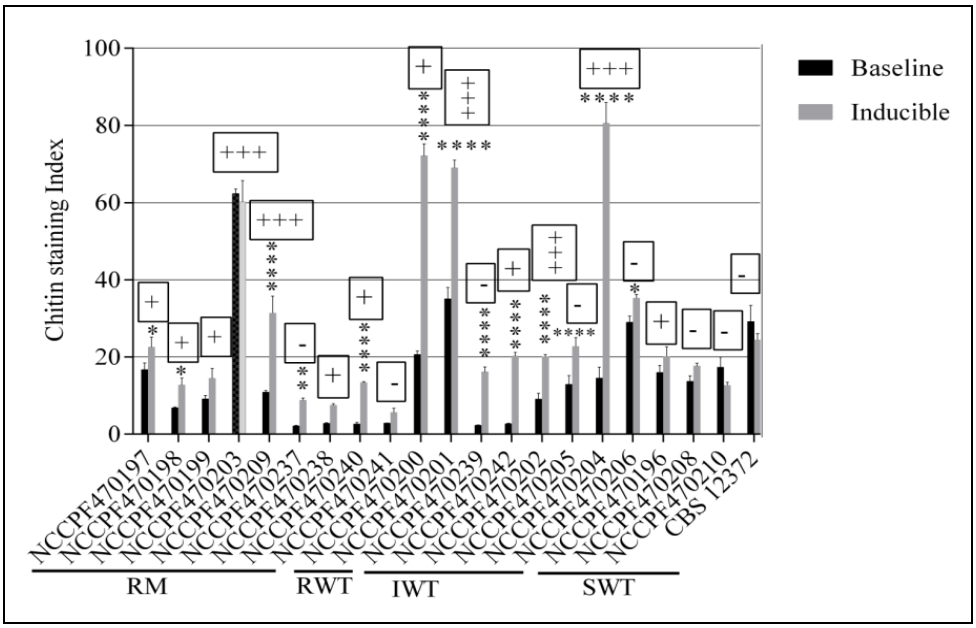

Figure 7.8.2.2 c: Baseline and caspofungin-induced differential chitin content represented as staining index. Three biological replicates were used for each isolate to determine the chitin levels. The data were analyzed using two-way ANOVA with isolates taken as row factor and induction with caspofungin as the column factor and multiple T tests. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

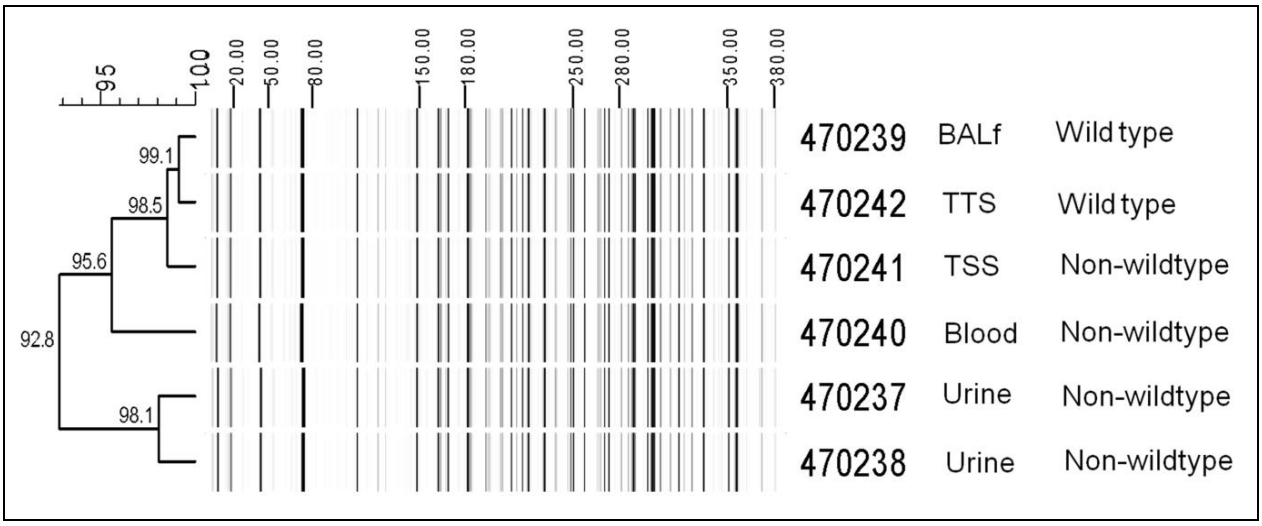

Figure 7.8.2.2 d: Amplified fragment length polymorphism analysis of 6 sequential isolates of *C. auris*. BALf, Bronchoalveolar lavage fluid; TTS, Tracheal tube secretion

7.8.3 Phenotypic and molecular responses of *C. auris* to various oxidative and osmotic stresses

Both fluconazole (FLU) resistant and susceptible isolates of clinical, environmental and patient colonizer *C. auris* were included. For checking osmotic tolerance sodium chloride (NaCl) were added to YPD (Yeast- peptone –dextrose) agar in a final concentration of 8% to 18% while H_2O_2 (30%, w/v) with a final concentration of 5mM to 50mM were added to YPD agar plates to induce oxidative stress. For metal stress, copper sulphate, ferrous sulphate and zinc sulphate were added to YPD agar to a final concentration of 0.5mM to 5mM, 1mM to 15mM and 0.5mM to 20mM respectively. Yeast cells ($\sim 10^6$ cells/ml and 10-fold diluted up to 1000 folds) from overnight cultures were spotted and plates were incubated at 30°C for 1-3 days. Cells were exposed to optimized concentrations of stress agents for 1hr. Total RNA was extracted using conventional TRIzol method, reversely transcribed into complementary DNA (cDNA) and quantitative real time PCR was performed using SYBR Green Master Mix. Comparative threshold (Ct) cycle method ($2^{-\Delta\Delta}$Ct) was used to calculate relative expression levels of genes (Hog1, Mkc1, Cek1, Sod1, Cta1, Sho1, Hsp82 and Hsp90 like protein).

C. auris FLU resistant isolates (of any group) could not tolerate salt concentration beyond 16% whereas FLU sensitive isolates had grown in presence of 18% sodium chloride. FLU sensitive strains tolerated up to 50mM H_2O_2 (30%, w/v) but FLU resistant strains could grow up to 30mM of H_2O_2 (30%, w/v). All FLU resistant isolates tolerated high concentration of iron, copper and zinc with an exception of the two clinical resistant isolates being inhibited by 12mM of copper concentration (Figure 7.8.3a). A significant increase in gene expression level upon H_2O_2 exposure in case of Hog-1 gene in *C. auris* clinical FLU susceptible isolates (Figure 7.8.3b and 7.8.3c) whereas resistant counterpart did not show any significant fold change. Gene expressions for other genes of interest demonstrated no significant pattern as the genes were upregulated in both resistant and susceptible isolates.

Fluconazole susceptible isolates are more tolerant to both hyper-osmotic and oxidative stress compared to the resistant group. Different stressful stimuli can act through different pathways and stress response may not be specific to any type of group. This tradeoff could be due to the consequences of their multidrug resistance adaptation eventually reducing their competitive fitness.

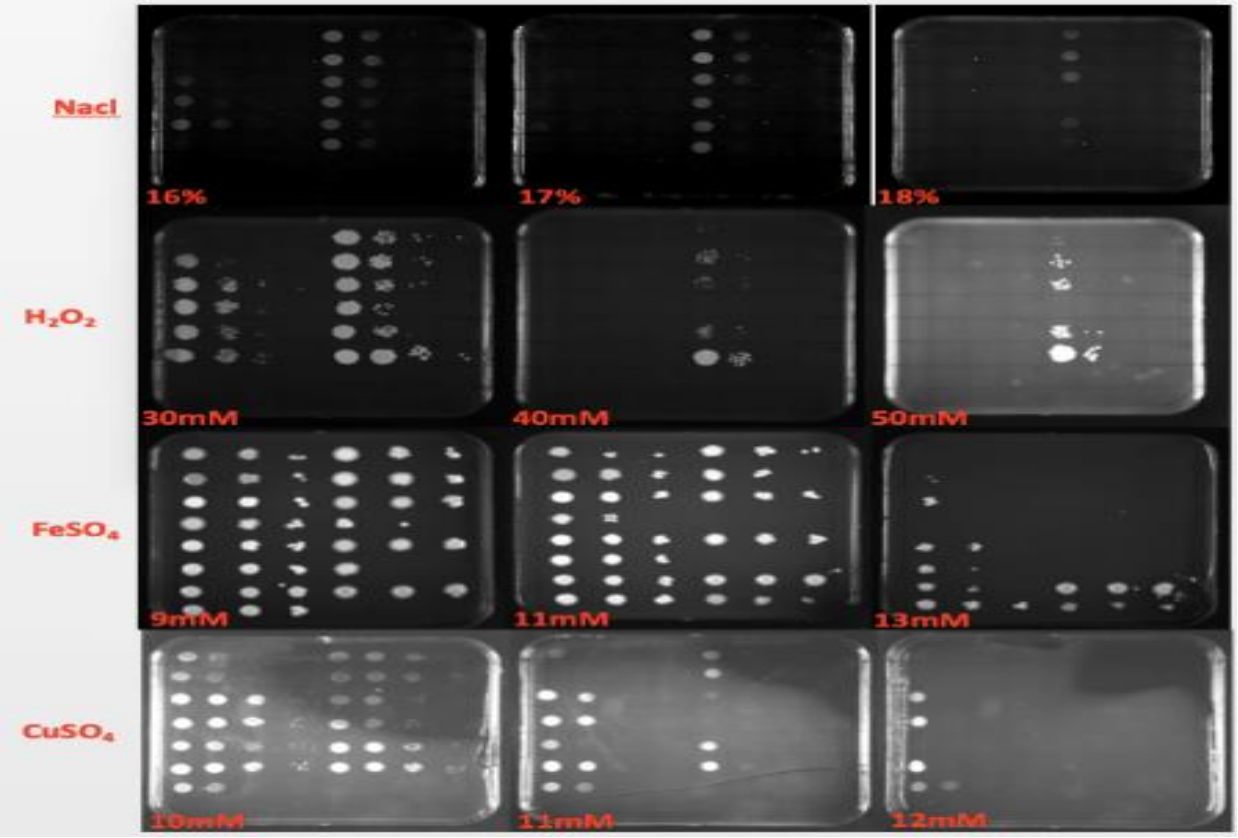

Figure 7.8.3.a: Stress response of *C. auris* against various oxidative stress.

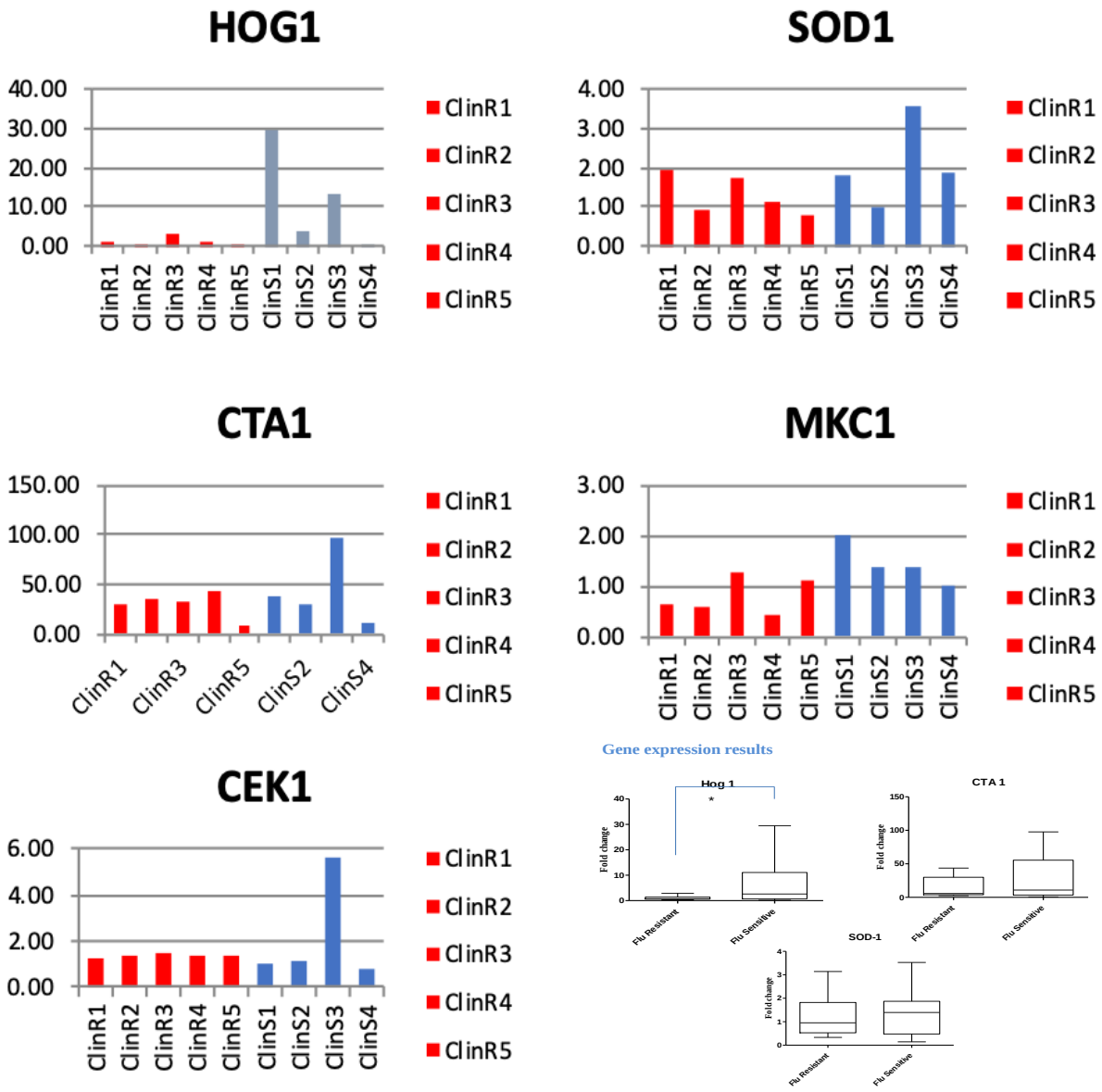

Figure 7.8.3 b: Expression of various genes during oxidative stress response among *C. auris* isolated from clinical samples.

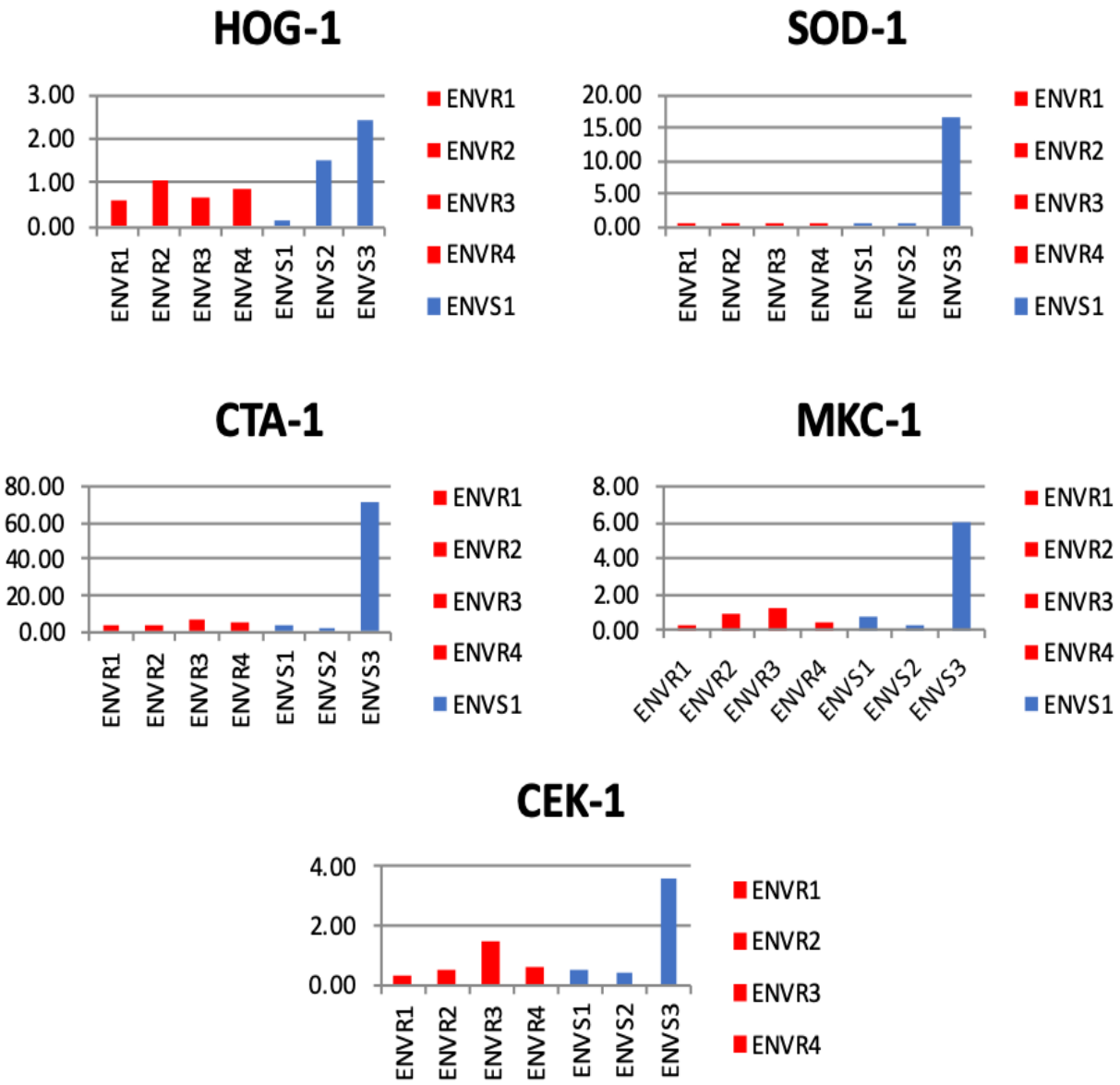

Figure 7.8.3 c: Expression of various genes during oxidative stress response among *C. auris* isolated from hospital environment.

7.8.4 Molecular analysis of outbreak investigation of *Candida krusei* in a pediatric unit[9]

A sudden rise of *Candida krusei* candidemia cases was noticed in our hospital within one year (2014) with maximum cases from paediatric unit. Therefore, we conducted a molecular typing of *C. krusei* isolates obtained from various sources (clinical, environmental and from hands of health care workers) to find the source of possible outbreak in paediatric unit. We compared the clonality of the *C. krusei* isolates obtained during outbreak with the *C. krusei* isolates obtained during subsequent years. Fluorescent Amplified Fragment Length Polymorphism (FAFLP) technique was carried out to evaluate the clonality.[10] FAFLP analysis of all *C. krusei* isolates from 2014 showed a similarity co-efficient of >90%exhibiting 4 major clusters (7.10a). The similarity of 'A', 'B', 'C' and 'D' clusters was >94% with inter-cluster difference of <1%. A total of 25 *C. krusei* isolates from blood along with an isolate from environment (isolate number = E55, wash basin), hand isolate from HCW (HCW61b) and control strain (*C. krusei* ATCC 6258) were tested. Cluster A consisted of 12 blood culture isolate and one environmental isolate obtained during January to June. Cluster B consists of 8 isolates obtained from Newborn unit of paediatric emergency (NUPE) and paediatric surgery ICU (NSICU). Cluster C consists of 6 blood isolates belonging to NSICU and one isolate from NUPE ward. Cluster D consists of 3 isolates of which two were from blood and one from hands of health care worker. FAFLP analysis of representative *C. krusei* isolates over different years (2014-2018) showed that isolates from 2014 were not genetically related to *C. krusei* from other years (Figure7.10b). Major cluster containing 4 isolates belonging to year 2016, 2017 and 2018 showed similarity of >96.4%. None of the other isolates tested showed any clonality with isolates from other years. Majority of the isolates tested belonged to NUPE ward, and one each from paediatric gastroenterology ward and paediatric hepatology ICU.

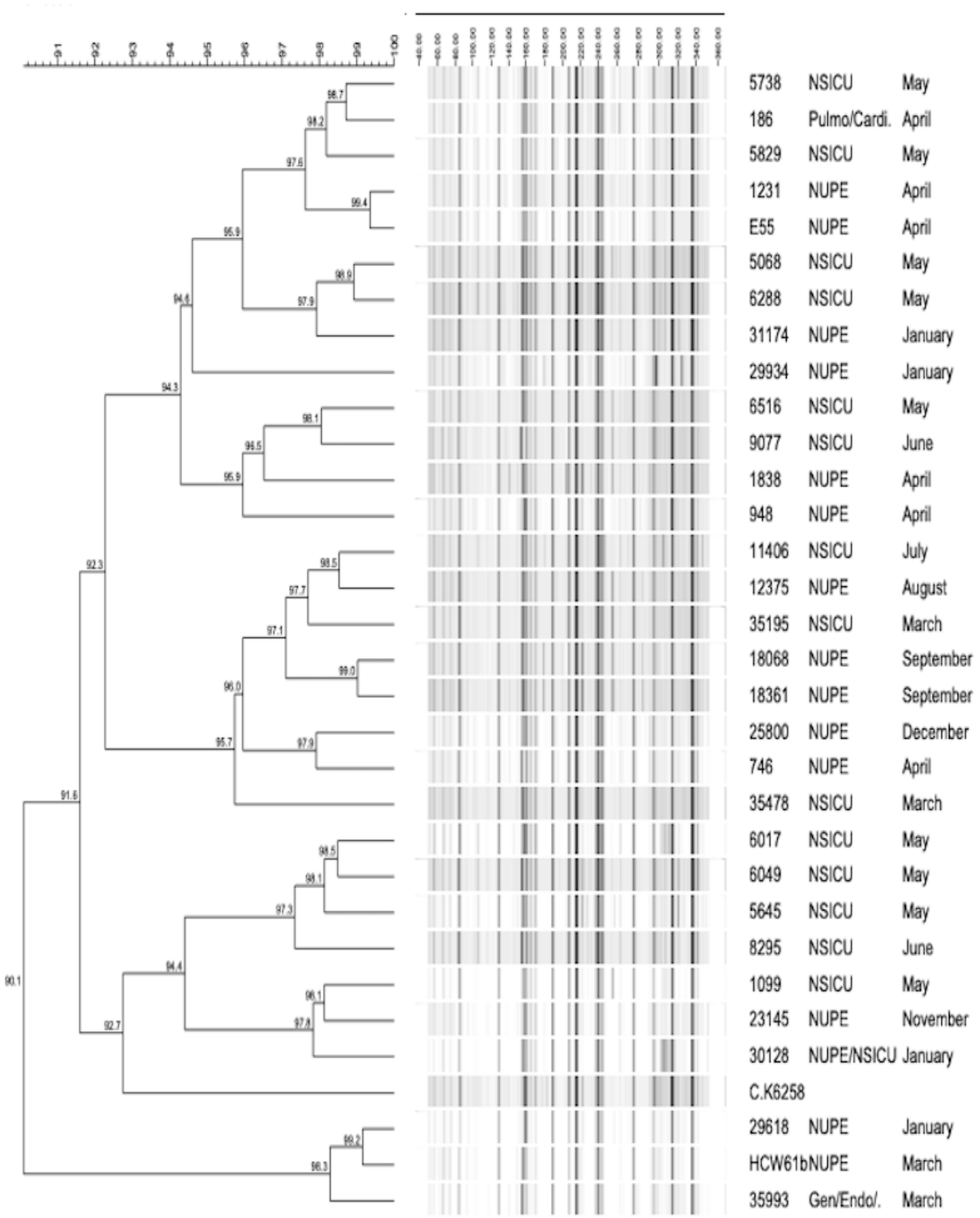

Figure 7.10 a: Dendrogram generated by FAFLP analysis of *C. krusei* isolates from blood and environment

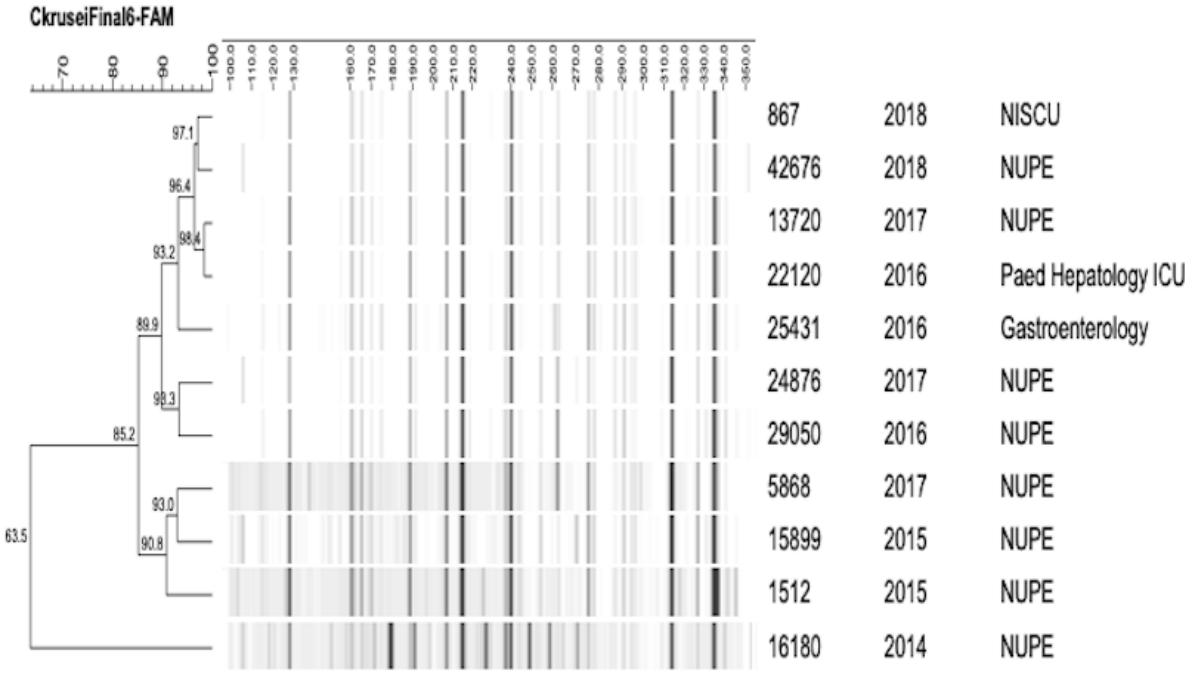

Figure7.10b: Dendrogram generated by FAFLP analysis of *C. krusei* isolates obtained over different years.

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Sir Ganga Ram Hospital, New Delhi

LTMGH Sion Hospital, Mumbai

NIMS, Hyderabad

RIMS, Imphal

ARTEMIS Hospital, Gurgaon

Dr Lal Path Lab, New Delhi

Fortis Escorts Heart Instiute, New Delhi

Fortis Memorial Research Institute, Gurgaon

Fortis Hospital, Noida

Fortis Hospital, Mohali

Max Super specialty hospital, New Delhi

SRL Lab, Mumbai