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STUDY OF ENTEROBACTERIACEAE GROUP OF ORGANISMS AND THEIR ANTIBIOTIC SUSEPTIBILITY PATTERN IN PATIENTS ATTENDING TERTIARY CARE HOSPITAL

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ABSTRACT

The emergence and spread of resistance in Enterobacteriaceae are complicating the treatment of serious nosocomial infections and threatening to create species resistant to all currently available agents. They are inhabitants of normal intestinal flora and are among the most common human pathogens, causing multiple infections . They are the source of community- and hospital-acquired infections.

An observational study was conducted over a period of six months (July 2023 – December 2023) in the microbiology laboratory of Swami Ramanand Teerth Government Medical College , Ambajogai , Maharashtra . A total of 468 bacterial isolates of Enterobacteriaceae from clinical specimens were processed for antimicrobial susceptibility testing

The rate of multidrug resistance and extended spectrum beta-lactamases production was 54.2% and 23.8% respectively. Of the total ESBL producers 92.4% were multidrug resistance. The rate of multidrug resistance and extended spectrum beta-lactamases production were higher in organisms isolated from clinical samples collected from inpatients. High rate of MDR and ESBL was seen in E. coli (54.7% & 21.4%), Klebsiella spp. (68% & 16.8%) and Citrobacter spp. (41.1% & 44.1%). The antimicrobial resistance rate was highest against ampicillin (80.3%) followed by cefixime (56.4%), ceftazidime (53.6%), ceftriaxone (53.0%), cotrimoxazole (51.2%), ciprofloxacin (45%) and ofloxacin (42.3%).

Multidrug resistance is common among Enterobacteriaceae. These bacteria have high rate of resistance against commonly used groups of antibiotics like cephalosporins and quinolones. Continuous monitoring, surveillance of antimicrobial resistance, proper infection control and practices are important to combat with these issues.

Keywords: Enterobacteriaceae; ESBL; MDR ; Infection ; Resistance

INTRODUCTION

Enterobacteriaceae family is a large, diverse group of facultative Gram negative rods that are common pathogens of healthcare and community-associated infections worldwide[(4)] .They are ubiquitously present in nature and can be found in the intestinal tract of humans and animals as commensal flora and are among the most common human pathogens, causing infections

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such as cystitis and pyelonephritis with fever, septicaemia, pneumonia, peritonitis, meningitis, and device-associated infections. They have the propensity to spread easily among humans (hand carriage, contaminated food and water) and to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons.[1] The *Enterobacteriaceae*, most notably *Escherichia coli* and *Klebsiella pneumoniae*, are the most important causes of serious hospital-acquired and community-onset bacterial infections in humans[2]. Emergence of multidrug resistance (MDR) in Enterobacteriaceae is a major public health threat which poses a great challenge to combat infections.[5]
They are increasingly reported and are a threat to public health implicating a need for accurate identification of the isolates to species level. [3]. Infections by extended spectrum β- lactamase (ESBL) producing Enterobacteriaceae are the most important among the causes of infections in the community and hospital in the recent years and are in rising trends.[6,7]

METHOD

An observational study was conducted over a period of six months (July 2023 – December 2023) in the Microbiology laboratory of Swami Ramanand Teerth Government Medical College , Ambajogai , Maharashtra. This research was approved by the Institutional Ethics committee (IRC) of Swami Ramanand Teerth Government Medical College , Ambajogai , Maharashtra. Letter of approval was obtained after submitting and presenting the proposal to the committee. Verbal consent was taken to all patients to include them as a sample source in the study. The study was done in 468 non-repeated bacterial isolates of Enterobacteriaceae from clinical specimens (pus, blood, urine, sputum and body fluids) from patients attending all OPD and IPD in the tertiary care centre.

Isolation and identification

All the clinical samples received in the Microbiology laboratory for culture and sensitivity were processed as a routine diagnostic process by standard microbiological techniques. In brief, the specimens were inoculated in culture plates (urine, pus , sputum and body fluids in blood agar, Mac-Conkey agar). All inoculated plates were incubated at 37°C for 24 hours aerobically. All received blood culture bottles were, incubated at 37°C and after 24 hours, sub-cultured in blood agar and Mac conkey agar every alternate day for seven days. Bacterial isolates of family Enterobacteriaceae were then identified further by studying colony characters, gram stain and biochemical tests.

Antimicrobial susceptibility Test

The antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method in Mueller Hinton agar (MHA) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines[9] by using the following commercially available antimicrobial discs from Hi-media, Laboratories, Mumbai, India. Ampicillin (10µg), ceftazidime (30µg), ceftriaxone (30µg), cefixime (30µg), amikacin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25µg /23.75µg), imipenem (10µg), meropenem (10µg), tigecycline (15 µg), piperacillin/ tazobactam (100µg/10 µg. For urinary isolates, nitrofurantoin (300µg) was also tested.

Screening of MDR and Potential ESBL Producers: In this study, the isolates that are resistant to at least one agent of three different classes of commonly used antimicrobial agents, were regarded as MDR.[10] The bacterial isolates with zone of inhibition (ZOI) ≤25mm for ceftriaxone, ≤22mm for ceftazidime, and/or ≤27mm for cefotaxime were considered as a potential ESBL producer as recommended by CLSI .[9]

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Phenotypic Confirmation of ESBL: Isolates that were considered as potential ESBL producers by initial screening were emulsified in nutrient broth to adjust the inoculum density equal to that of 0.5 McFarland turbidity standards. Combination disk test (CDT), as recommended by the CLSI, was performed in all isolates presumed to be ESBL producers. In this test, ceftazidime (30) disk alone and in combination with clavulanic acid (ceftazidime + clavulanicacid, 30/10µg) disk were applied onto a plate of MHA with the test strain and then incubated in ambient air for 18 hours of incubation at 37°C. Isolate that showed increase of ≥ 5 mm in the zone of inhibition of the combination disks in comparison to that of the ceftazidime disk alone was considered as ESBL producer.

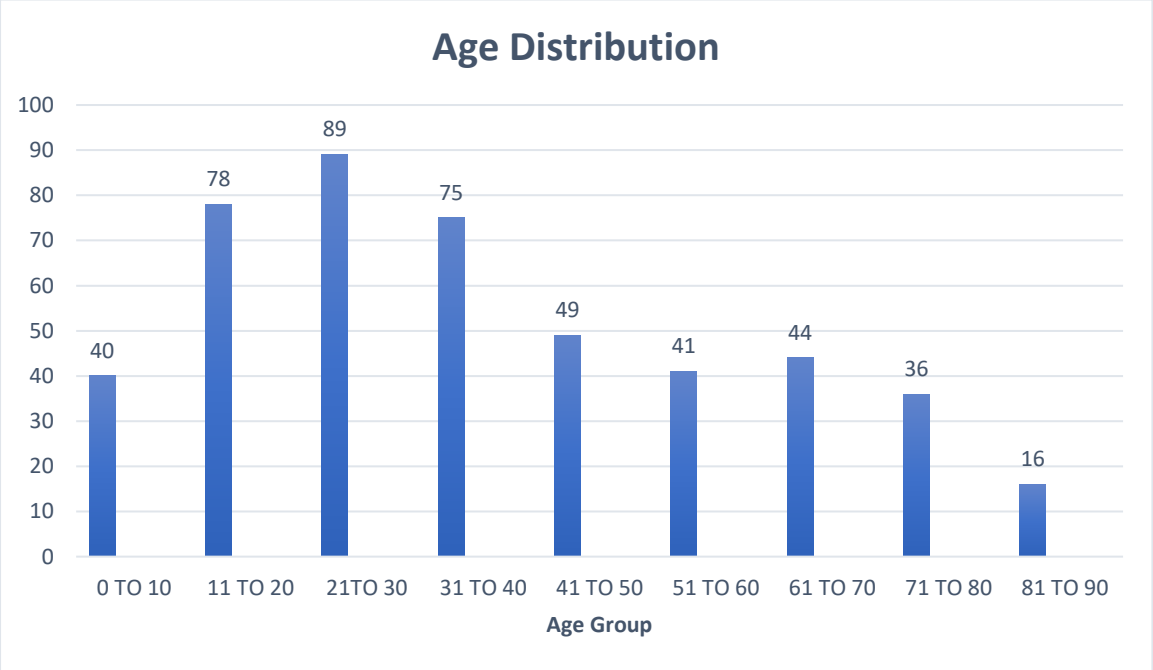
RESULT

A total of 5,338 clinical specimens (urine -2790, blood- 1138, sputum-644, pus-576 and body fluids-190) from both inpatients and outpatients of all age groups received for aerobic bacterial culture and antimicrobial susceptibility testing were included in the study.

Of the total specimens processed 475 clinical samples showed bacterial growth with growth positivity rate of 17.79 %. The prevalence rate of Enterobacteriaceae was 49.2% (n=468) among the total bacterial isolates and 8.76% among the total clinical specimen processed.
Of the total 468 (355 from OPD and 113 from IPD) bacterial isolates of family Enterobacteriaceae 179 were from male and 289 were from female.

The distribution of the isolates according to age group of patient is shown in figure 1.

Figure 1 : The distribution of the isolates according to age group of patient



Prevalence of Enterobacteriaceae isolates among different clinical samples is shown in table 1.

Table 1: Prevalence of Enterobacteriaceae isolates from different clinical samples

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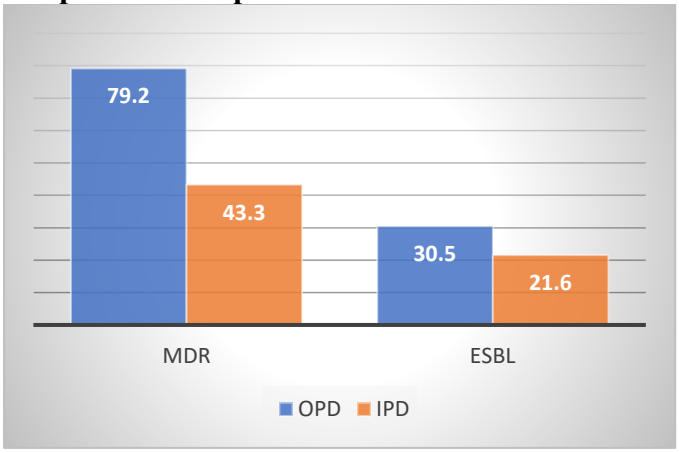
Organism	Urine	Blood	Pus	Sputum	Body fluids	Total
Escherichia coli.	246	5	51	15	10	327
Klebsiella spp	25	8	17	20	2	72
Salmonella Typhi	0	2	0	0	0	2
Citrobacter spp.	10	2	12	8	2	34
Enterobacter spp	9	2	3	6	0	20
Proteus spp.	4	1	4	3	1	13
Providencia Spp.	0	0	0	0	0	0
Total	294	20	87	52	15	468

Of the total Enterobacteriaceae isolates, 254 (54.2 %) were MDR and 110 (23.8%) were ESBL producers. Of the total ESBL producers 103 (92.4%) were MDR. Both the ESBL production and MDR was higher in
Enterobacteriaceae isolates among the clinical samples collected from inpatients (fig.2).

Figure 2: Rate of MDR and ESBL producing Enterobacteriaceae isolates in clinical samples from in-patients and OPD



The rate of ESBL production and MDR among the Enterobacteriaceae isolates is shown in table 2.

Organism	MDR n%	ESBL n%
Escherichia coli. (n=327)	179(54.7)	70 (21.4)
Klebsiella spp (n= 72)	49 (68)	12 (16.6)
Salmonella Typhi (n=2)	0	0
Citrobacter spp. (n=34)	14(41.1)	15(44.1)
Enterobacter spp (n=20)	5(25)	9(45)
Proteus spp. (n=13)	7(53.8)	4(30.7)
Providencia Spp (n=0)	0	0
Total (n= 468)	254	110

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The antimicrobial resistance pattern of Enterobacteriaceae isolates is shown in table 3

Antibiotics used	E. coli (n=327) No (%)	Klebsiella spp. (n=72) No (%)	S. Typhi (n=2) No (%)	Citrobacter spp. (n=34) No (%)	Enterobacter spp.(n=20) No (%)	Proteus spp .(n=13) No (%)	Providencia spp. (n=0) No (%)	Total (n=468) No (%)
Ampicillin	245 (74.9)	72(100)	1(50)	31 (92.2)	19(94.1)	8(58.8)	00(00)	376 (80.3)
Cefixime	173(53.0)	49(67.9)	00(0)	27(78.1)	12(61.8)	3(23.5)	00(00)	264(56.4)
Ceftazidime	167(51.0)	48(67.1)	0(00)	23(67.1)	11 (55.8)	2(17.6)	00(00)	251(53.6)
Ceftriaxone	163 (50.0)	47(65.6)	0 (00)	24(70.3)	12 (58.8)	2(17.6)	00(00)	248(53)
Piperacillin-Tazobactam	38(11.5)	20(27.4)	00 (00)	12(34.3)	5(23.5)	00 (00)	00 (00)	75 (16)
Amikacin	32 (9.9)	23 (32.0)	NT	16(48.4)	7 (35.2)	1(5.8)	00 (00)	79 (16.8)
Ciprofloxacin	148(45.2)	36 (50.3)	1(50)	15 (43.7)	9 (44.1)	2(17.6)	00 (00)	211(45)
Ofloxacin	135 (41.3)	36 (50.3)	2 (100)	15 (43.7)	8 (41.2)	2 (17.6)	00 (00)	198(42.3)
Cotromoxazole	159 (48.6)	46 (63.3)	1(50)	21 (60.9)	9 (44.1)	4(29.4)	00(00)	240 (51.2)
Imipenem	16(4.8)	9(12.2)	00(00)	10 (29.6)	2 (8.8)	1 (5.8)	00(00)	38(8.1)
Meropenem	15 (4.7)	9 (12.2)	00 (00)	10 (28.1)	2 (8.8)	1 (5.8)	00 (00)	37(7.9)
Tigecycline	1(0.3)	2(2.2)	0(00)	0(00)	0(00)	3(23.5)	00(00)	6(1.2)
Nitrofurantoin	26(8.1)	24(33.3)	NT	12(35.0)	11(55.5)	13(100)	0(00)	86(18.3)

DISCUSSION

Drug resistance among Enterobacteriaceae family has laid challenge by limiting their therapeutic options while
treating the diseases. This study has determines the frequency of different isolates of Enterobacteriaceae from clinical specimens and their antibiogram with special reference to MDR and ESBL production. The prevalence of ESBL production
among the Enterobacteriaceae in this study was 23.8% which is similar to the studies conducted in Nepal [11-16] and in India [22] Test for ESBL production among bacterial isolates by CLSI recommended phenotypic method may not detect ESBL production among the ESBL producing isolates that co-produce Amp- C beta lactamase[18]. The prevalence rate of ESBL
production among Enterobacteriaceae, ranging from 13.5% to 64.3% have been reported from different parts of the world[19,20] This could be due to the variations in their antibiotic prescribing policies, awareness and health education that determines the ESBL production by organisms[21-23] In the referral hospitals
and ICUs the rate of drug resistance and production of ESBL is high among the bacteria because the referred patients from the peripheral primary care centers already are laden with varieties of inappropriate antibiotics which leads to increase in drug resistance. This explains the reason for higher prevalence rate of ESBL in this study since our study centre is one of the referral hospital. This study showed higher rate of MDR and ESBL producing Enterobacteriaceae isolates in clinical samples from inpatient which is similar to the above statement. This explores the significant presence of resistant organisms in the community and the need for preventive measures to be applied to limit their spread not only in hospital set up but also in the community as well.

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MDR and ESBL production are commonly seen in *Klebsiella* spp. and *E. coli*[19,21.24] among the Enterobacteriaceae isolates. Similar pattern was seen in this study. Higher rate of MDR was seen in *Citrobacter* spp. however, it was not statistically significant. (P>0.05) Carbapenems, relatively expensive antibiotics are the choice of drug for ESBL producing organisms. This study showed almost 10 % of the isolates are resistant to carbapenems. This could be due to the production of carbapenemases and metallo B lactamases by the organism. Among the second line drugs, resistance to piperacillintazobactam, tigecycline and amikacin were found to be low amongst the Enterobacteriaceae isolates . More than
50% of the isolates showed resistance against the commonly used antibiotics like cephalosporins and quinolones. Resistance to β-lactams in Enterobacteriaceae is mainly due to the production of β-lactamases, which may be encoded either chromosomally or on plasmids. Several studies on Enterobacteriaceae isolates showed varieties of resistance rate against cephalosporins that ranges from 30 to 70%[19,24,25] Increased resistance to cephalosporins among Enterobacteriaceae in this study could be due to the excessive use of cephalosporins in our set up. Quinolones like ciprofloxacin and ofloxacin also are other commonly prescribed antibiotics to treat bacterial infections. The resistance rate against these groups of antibiotics is also rising among the Enterobacteriaceae Isolates[19,20,24] . Resistance to quinolones typically arises as a result of alterations in the target enzymes (DNA gyrases and topoisomerases IV) and of changes in drug entry and efflux.Wide spread use of fluoroquinolones has contributed to the rapid emergence of resistance worldwide.4 Over the counter availability
of all these antibiotics, open defecation system, lack of proper sewage system and lack of practice of isolation of patient infected with MDR organisms have resulted wide use of these antibiotics and spread of MDR organisms in our country that explains high rate of resistance against these groups of antibiotics.

CONCLUSION

Antimicrobial resistance among Enterobacteriaceae is a growing threat these days. Increased rate of resistance to the commonly used and relatively safer antibiotics like cephalosporins and quinolones explores the urgent need for alternatives to these groups of antibiotics. Low level of resistance against carbapenems and tigecycline were seen in this study. This does add little hope to fight against the infections by MDR bacteria; however these groups of antibiotics are the reserved drugs, expensive and have comparatively more adverse effects. It is therefore time to identify the causes and stop the spread of these resistant bacteria in hospital as well as in the community. Judicious selection of antimicrobial regimens, regular antimicrobial resistance surveillances are important to tackle these issues

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