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Corresponding Author
Jeswin Chandrasekhar,
Al-Azhar Medical College and Superspeciality Hospital, P.O Ezhalloor-685605, Thodupuzha, Dist-Idukki, Kerala, India
jeswin369@gmail.com

Author Designation
[1]Associate Professor
[2-4]Assistant Professor

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Understand the Baseline Prevalence Rate of ESBLs and Multidrug Resistance in Enterobacteriaceae from Different Clinical Sample in a Tertiary Care Hospital Located in Rural Part of Kerala

[1]Ami Chandrakant Patel, [2]Purvi Shantilal Khristi, [3] P.A. Remya and [4]Jeswin Chandrasekhar

[1,3,4]Department of Microbiology, Al-Azhar Medical College and Superspeciality Hospital, Kerala, India
[2]Department of Microbiology, Dr. N D Desai Faculty of Medical Science and Research, Nadiad, India

ABSTRACT
To overcome a problem of antimicrobial resistance and start effective Antimicrobial stewardship program we need to understand the resistance pattern of the organism. This study aimed to deliver the indication of different resistant profiles of clinically isolated Enterobacteriaceae from different source of samples from Al-Azhar Medical college and Super speciality hospital, prevalence of multidrug resistant (MDR), extensively drug-resistant (XDR) and pan-drug resistant (PDR) bacteria. A total of 432 Gram-negative bacteria were collected from different sources (urine, pus, sputum, ET aspirate, vaginal swab, catheter tip, ear swab, throat swab, aspirated body fluid). Out of all Gram negative bacteria Enterobacteriaceae accounted for 353 (81.71%). Samples were sub cultured and identified according to their cultural characteristics and biochemical tests. Antimicrobial susceptibility test was performed for 24 antibiotics from 11 categories against all isolated Enterobacteriaceae according to the recommendation of Clinical and Laboratory Standards Institute (CLSI). The result showed that out of 432 Gram negative isolates, Escherichia coli and Klebsiella species were predominant isolates with the percentage of 40.97-37.5% respectively. Rate of ESBL was highest among *E.coli* followed by Enterobacter species 52.54-50% respectively. The MDR rate was highest among Klebsiella species followed by *E.coli* 46.29-42.93% respectively.

INTRODUCTION

The World Health Organization recognized AMR as a global health problem and recommended that member states should strengthen the knowledge and evidence base through AMR surveillance and research in the global action plan on AMR. Lack of AMR surveillance data contributes to underestimating the magnitude of AMR problem and halting the implementation of AMR control measures[1]. Initiatives from the scientific community, mass media and political initiatives are creating awareness about antibiotic resistance. Despite of all the efforts, antibiotic resistance is continuously increasing worldwide[2]. Bacteria have evolved a diversity of resistance mechanisms to protect themselves from the biological effects of antimicrobial agents. Among these mechanisms are limiting or changing the cell wall structure, using molecular efflux pumps to eliminate drugs, production of enzymes that change the chemical structure of the drug and altering the target sites for antimicrobial action. Many of these resistance mechanisms are encoded on extrachromosomal elements called plasmids that may be transmitted from one bacterium to another within the same species and sometimes across bacterial families[3]. Globally, the antimicrobial resistance (AMR) reported that *Escherichia coli* and *Klebsiella* spp. showing high resistance in various parts of the world including resistant to third-generation cephalosporins and carbapenems of up to 54%[4,5].

Information of the resident bacterial flora and their resistance profile is essential. It helps in updating antibiogram and selecting empirical therapy[2,7]. Multidrug resistance (MDR) is defined as developed resistance to at least one agent in three or more antimicrobial groups.

Extensive drug resistance (XDR) is defined as a resistance to at least one agent in all but two or fewer antimicrobial groups, this means bacterial isolates remain sensitive to only one or two groups. Pandrug resistance (PDR) is a resistance to all antibiotics in all antimicrobial groups[8].

The surveillance data for MDR organisms helps Physicians to know the degree of antimicrobial resistance problem in the health care facilities. This surveillance data helps in explaining the importance of MDRO to public health and to encourage the wise use of antimicrobials[9-11].

In Al-Azhar Medical College and Superspeciality Hospital for implementing Antimicrobial Stewardship Program we need to analyze the prevalence rate of MDR, PDR and XDR. There for this study aimed to determine the prevalence of *Enterobacteriaceae* in clinical samples and screen the antibiotics profile to the most regularly used antimicrobials and to determine the occurrence rates of ESBL, multi drug resistance (MDR), extensive drug resistance (XDR) and pandrug resistance (PDR).

MATERIALS AND METHODS

Study population and sample size: This was a cross-sectional study conducted over 1 year from January to December 2020. Total specimen received for testing in a microbiology laboratory were 967. Out of this total positive sample were 580. A total of 432 isolated Gram-negative bacteria from different clinical sample sources (urine, pus, sputum, ET aspirate, vaginal swab, catheter tip, ear swab, throat swab, aspirated body fluid) were collected. All Enterobacteriaceae isolated in the microbiology laboratory were included in this study. All organisms other than Enterobacteriaceae, Gram Positive organisms were included as exclusion criteria.

Identification of bacterial isolates: All isolated Gram-negative bacteria were subcultured on blood agar and MacConkey agar and incubated aerobically at 37°C for 24 hrs and then identified according to the culture characteristics and biochemical tests[12].

Antibiotic susceptibility test: Different categories of antibiotics were used in this study included cephalosporins, fluoroquinolones, carbapenems, aminoglycosides, penicillins, monobactams, *β*-lactam/*β*-lactamase inhibitor complexes, folate metabolic pathway inhibitors, glycylcyclines, cephamycins and nitrofuran as recommended for Gram-negative bacteria[8]. The antibiotics used, namely, cefuroxime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), levofloxacin (5 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ampicillin (10 μg), aztreonam (30 μg), amoxicillin-clavulanic acid (30 μg), trimethoprim/sulfamethoxazole (5/250 μg), piperacillin-tazobactam (100/10 μg), nitrofurantoin (300 μg) were used according to the standard procedures (CLSI). The results were interpreted according to the recommendation of the Clinical Laboratory Standards Institute[13].

Quality control: For the reliability of study findings, quality control implements measures of performance checks during the entire procedure of the laboratory work. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC BAA700603 were used.

Detection of ESBL, MDR, XDR and PDR: The prevalence of extended-spectrum *β*-lactamase (ESBL) was recognized as the resistance rate against cefotaxime and ceftazidime. MDR, XDR and PDR were calculated in this study for each isolated bacteria as described by Magiorakos *et al.*[8].

Statistical analysis: Data were analyzed using Statistical Package for Social Sciences (SPSS), version 25 (IBM, SPSS Inc., Chicago, IL). Descriptive data were expressed as a percentage. A $p \leq 0.05$ for the association bacteria and source of samples was considered significant.

RESULTS

Out of 432 Gram-negative bacterial isolates number of Enterobacteriaceae were 353. Out of all Enterobacteriaceae *E. coli* and *Klebsiella species* were predominant with percentages 177 (40.97%) and 162 (37.5%), respectively. However, *Proteus mirabilis* was 7 (1.98%), *Citrobacter species* was 5 (1.41%), *Enterobacter species* was 2 (0.56%). Most of the isolated *Enterobacteriaceae* were from urine 215 (60.90%) and the highest number for *E.coli* followed by Klebsiella species with number of 128 (72.31%) and 83 (51.23%), respectively. There was a significant association between isolated bacteria and sources of the sample, with p = 0.001 (Table 1). Regarding the resistance rate of antibiotics, *E. coli* revealed the highest resistance rate to ampicillin and amoxicillin-clavulanic acid (92 and 74.01%, respectively), while antibiotics from the cephalosporins class exhibited a high resistance rate among *Enterobacteriaceae* bacteria, with the resistance rate for *E.coli* towards cefuroxime (51.42%) and cefotaxime and ceftazidime (52.54 and 44.63% respectively). However, among the drugs belonging to the fluoroquinolones classes the highest percentage of the resistant rate was obtained by *E. coli* for ciprofloxacin (42.37%) and levofloxacin (36.72%). The lowest resistant rate was obtained for carbapenems drugs with the *E. coli* resistant percentage of 1% for imipenem and meropenem. Resistant towards nitrofurantoin and aminoglycosides was 7.9 and 11.005% respectively. (Table 2). Note: CXM = cefuroxime, CTX = cefotaxime, CAZ = ceftazidime, CTR = ceftriaxone, CPM = cefepime, AMP = ampicillin, AMC = amoxicillin-clavulanate, PIT = piperacillin-tazobactam, AT = aztreonam, COT = trimethoprim-sulfamethoxazole, AK= amikacin, G = gentamicin, IPM = imipenem,MRP = meropenem, CIP = ciprofloxacin, LE = levofloxacin, NX = norfloxacin, OF = ofloxacin, BL-BLI-β lactam + β lactamase inhibitors Note MDR = multidrug-resistant bacteria, ESBL = extended-spectrum *β*- lactamase producer. There was a high prevalence of MDR bacteria and extended-spectrum *β*- lactamase producer ESBL among *E. coli*, *Klebsiella species and Enterobacteriaceae species* (Table 3). XDR and PDR was zero for all isolated bacteria.

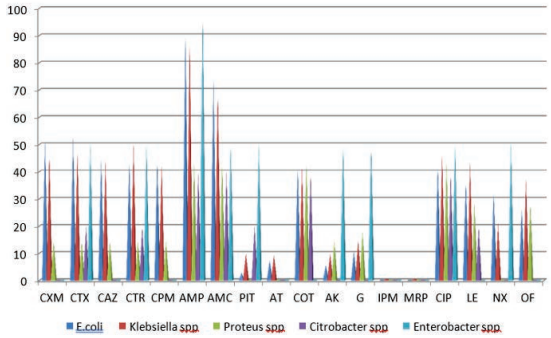

Fig. 1: Percentage of antimicrobial resistance patterns of *Enterobacteriaceae* isolated from different clinical samples

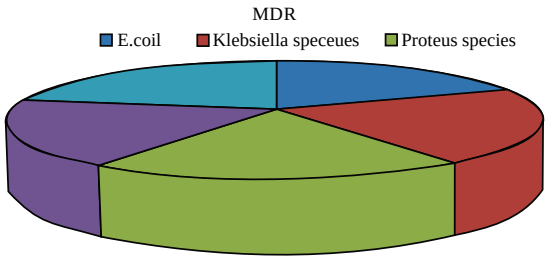

Fig. 2: The Prevalence of MDR among *Enterobacteriaceae*

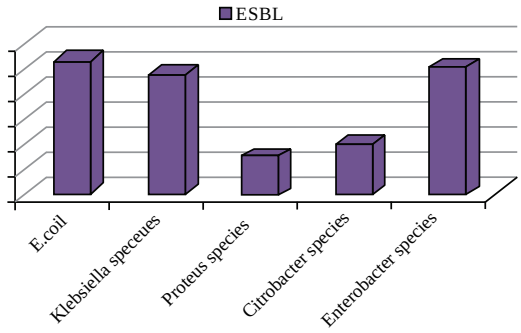

Fig. 3: The Prevalence of ESBL among *Enterobacteriaceae*

DISCUSSIONS

This study reported the prevalence of Gram-negative *Enterobacteriaceae* as a causative agent for infections, as well as the resistant rate towards various classes of antibiotics and identified the resistance mechanism and the frequency of ESBL, MDR, XDR and PDR. In the current study, 60.90% of Enterobacteriaceae isolated were from urine which accounted the highest number from all specimen followed by pus and sputum 25.49% and 9.34% respectively. In urine major pathogen were *E. coli* and *K. pneumonia* 72.31ESBL,*Enterobacterales*, *E. coli*, Klebsiella ,antimicrobials, multidrug resistance and 51.23% respectively. This data suggest that these organisms are the leading causative agent for urinary tract infection (UTI). These results entirely agreed with other studies which found that these bacteria were

Table 1: Distribution and relationship between isolates and source of sample (total No. of isolates n = 353)

Sample	*E.coli* (%)	Klebsiella spp(%)	Proteus spp(%)	Citrobacter spp(%)	Enterobacter spp(%)	Total (%)
Urine	128 (72.31)	83 (51.23)	2 (28.57)	1 (20)	1 (50)	215 (60.90)
Pus	33 (18.64)	48 (29.62)	5 (71.42)	4 (80)	0 (0)	90 (25.49)
Sputum	8 (4.51)	24 (14.81)	0 (0)	0 (0)	1 (50)	33 (9.34)
ET	1 (0.56)	1 (0.61)	0 (0)	0 (0)	0 (0)	2 (0.56)
Vaginal swab	3 (1.69)	2 (1.23)	0 (0)	0 (0)	0 (0)	5 (1.41)
Catheter tip	1 (0.56)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.28)
Ear discharge	1 (0.56)	3 (1.85)	0 (0)	0 (0)	0 (0)	4 (1.13)
Fluids	2 (1.12)	1 (0.61)	0 (0)	0 (0)	0 (0)	3 (0.84)
Blood	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	177 (50.14)	162 (45.89)	7 (1.98)	5 (1.41)	2 (0.56)	353 (100)

Table 2: Percentage of antimicrobial resistance patterns of *Enterobacteriaceae* isolated from different clinical samples

Antibiotics	*E.coli*	Klebsiella spp	Proteus spp	Citrobacter spp	Enterobacter spp
Penicillins					
AMP	92	89.5	42.85	40	100
Cephalosporins					
2nd gen					
CXM	51.42	46.9	14.28	0	0
3rd gen					
CTX	52.54	47.5	14.28	20	50
CAZ	44.63	43.82	14.28	0	0
CTR	44.06	50	14.28	20	50
4th gen					
CPM	44.63	42.59	14.28	0	0
BL-BLI					
AMC	74.01	70.3	42.85	40	50
PIT	2.8	9.87	0	20	50
Carbapenems					
IPM	0	0.61	0	0	0
MRP	0	0.61	0	0	0
Monobactams					
AT	7.34	9.25	0	0	0
Aminoglycosides					
AK	5.64	9.87	14.28	0	50
G	10.73	14.81	17.82	0	50
Fluoroquinolones					
1st gen					
NX	32.2	20.98	0	0	50
OF	26.55	37.03	28.5	0	0
CIP	42.37	46.29	42.85	40	50
2nd generation					
LE	36.72	43.2	28.5	20	0
Folic acid synthesis inhibition					
COT	41.8	41.35	42.85	40	0

Table 3: The Prevalence of MDR and ESBL among *Enterobacteriaceae*.

Organism	Number of isolated organism	MDR	ESBL
	No	No (%)	No (%)
E.coli	177	76 (42.93)	100 (52.54)
Klebsiella species	162	75 (46.29)	84 (47.5)
Proteus species	7	3 (42.85)	2 (14.28)
Citrobacter species	5	2 (40)	1 (20)
Enterobacter species	2	1 (50)	1 (50)
Total	353	157 (44.47)	188 (53.25)
p-value		0.001	0.001

also predominantly causing UTI[14-16]. The study finding stated that *E. coli* and *K. pneumonia* which shows a high resistant rate to cefotaxime and ceftazidime and were classified as extended-spectrum *β*-lactamase (ESBL) phenotypes. In the present study the ESBL rate was highest for *E.coli*, followed by Enterobacter species and Klebsiella species 52.54%, 50-47.5% respectively. This ESBL prevalence rate was near to the rate reported in the previous studies from New Delhi for *E. coli* and *K. pneumonia* (28.9-50%)[17] and in agreement with other studies reported the growing prevalence of extended-spectrum beta-lactamase (ESBL) producing isolates ranges from 40% up to50%[18-22].

In general, the increase in the resistance of isolated organisms to penicillin, BL-BLI and cephalosporins in this study might be due to the increase in the usage of these antibiotic's classes in the hospital. Also, our study presented the high MDR bacteria, which showed resistance to more than three groups of tested antibiotics. MDR rate was highest for Enterobacter species followed by Klebsiella species and E.coli 50%, 46.29-42.93 % respectively. This MDR rate entirely agrees with other studies which reported a high prevalence of MDR bacteria[23,24]. This MDR phenomenon may be due to acquiring of many resistant genes through R plasmid[3]. Furthermore, throughout the latest several decades the incidence of

MDR organisms in hospitals and health centers has increased gradually. So, this study reported the developments of multidrug resistance among *Enterobacteriaceae* and represents an alarming threat of appearance of multidrug-resistant pathogens.

The current study delivers the confirmation of high occurrence of bacterial infection in urine, pus and sputum samples. Also, there is a presence of high drug resistance to various antibiotics in *E. coli* and *K. pneumonia* isolates from different samples sources. Nevertheless, the study was conducted for the first time, however, this study will update the records of resistant rates of clinical isolates. Also, it will capture the responsiveness of all hospital's controlling team in making proper assessments and research to manage the progress of the resistant strain and also help to decrease the alarmingly increasing risk of drug resistance.

CONCLUSION

In conclusion, this study highlighted the antibiotics profile and prevalence of ESBL, MDR, XDR and PDR among *Enterobacteriaceae* from different clinical samples from Al-Azhar Medical College and Superspeciality Hospital, Thodupuzha. Therefore, the result of this study gave the baseline of multidrug resistant organism and it provides information regarding the urgent need for controlling and managing the development of MDR strain. Moreover, antibiotic stewardship procedures should be applied to limit the irrational use of antibiotics in Al-Azhar Medical College and Superspeciality Hospital, Thodupuzha.

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