Study of Drug Resistance in Enterobacteriaceae Isolates in a Tertiary Care Hospital

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Abstract: Background & objectives: Members of the family enterobacteriaceae comprise the most common gram-negative isolates in microbiology laboratories. Amongst these members, multiple antibiotic-resistant isolates including those producing extended spectrum β-lactamases (ESBL) and AmpC β-lactamases (AmpC) have increased steadily. Further, metallo-β-lactamase (MBL) producing strains are also recently detected. Methods: Three hundred enterobacteriaceae isolates were subjected to antibiotic susceptibility testing. ESBL production was tested by CLSI phenotypic confirmatory test and double disk synergy test using amoxiclav-cefotaxime and piperacillin-tazobactam - cefepime. AmpC was tested by cefoxitin-cefotaxime disk antagonism test. MBL was tested by combined disk test. Results: Amongst 300 enterobacteriaceae isolates, 111 were ESBL, 12 AmpC and 30 MBL producers. *K. pneumoniae* showed maximum drug resistance and β-lactamase production. Interpretation & conclusion: High drug resistance and β-lactamase production is observed in enterobacteriaceae isolates. It is necessary to keep vigilance for the resistant isolates. [Agrawal G et al NJIRM 2013; 4(2) : 113-117]

Key Words: β-lactamase, drug resistance, enterobacteriaceae

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Introduction: Members of family enterobacteriaceae are the most frequently encountered bacterial isolates recovered from clinical specimens[1]. β-lactamase production is the most common mechanism of β-lactam drug resistance in gram-negative bacteria[2]. AmpC β-lactamase (AmpC) and extended spectrum β-lactamase (ESBL) production amongst enterobacteriaceae isolates have been increasingly reported worldwide[3]**.** β-lactamases are typically associated with multiple antibiotic resistances, leaving few therapeutic options[2]. Further, metallo-β-lactamase (MBL) producers are increasing steadily. With the worldwide increase in the occurrence, types and rate of dissemination of MBLs, early detection is critical[4]**.** Hence, the study was undertaken to test drug resistance in enterobacteriaceae isolates.

Material and Methods: The study was conducted at Department of Microbiology, Indira Gandhi Government Medical College and Hospital, Nagpur (M.S.). Total 300 enterobacteriaceae strains were isolated from various specimens collected from different infections. They were subjected to antibiotic sensitivity testing as per Clinical and Laboratory Standards Institute (CLSI) guidelines[5].

ESBL production was tested in the enterobacteriaceae strains which were positive by initial screening suggested by CLSI[5]. The testing was done by CLSI phenotypic confirmatory test and double disk synergy test (DDST) using amoxiclav-cefotaxime and piperacillin-tazobactam - cefepime disks[3,5-8]. CLSI phenotypic confirmatory test (CAZ-CAC) was done by using ceftazidime (CAZ; 30 µg) and ceftazidime clavulanic acid (CAC; 30/10 µg) disks[5].

In DDST, Muller Hinton agar (MHA) plates were swabbed to form a lawn culture with 0.5 McFarland standard inoculum of the test strain. On the MHA plate, a disk of cefotaxime (30 µg) was placed 20 mm apart, centre to centre, from amoxiclav (20/10 µg) disk whereas piperacillin-tazobactam (100/10 µg) disk was placed 25 mm apart, centre to centre from cefepime (30 µg) disk. Plates were incubated at 37^{o}C overnight and were examined for enhancement of inhibition zone of cefotaxime and cefepime at the side facing amoxiclav and piperacillin-tazobactam disk respectively. Organisms that showed a clear extension of inhibition zone towards the disk of amoxiclav and piperacillin-tazobactam were considered ESBL positive[3,6-8].

AmpC production was tested by cefoxitin-cefotaxime disk antagonism test[8-9]. In this test, a lawn culture of 0.5 McFarland inoculum of the test strain was exposed to a disk of cefotaxime (30 µg) and cefoxitin (30 µg) placed at a distance of 15 mm from edge to edge. After overnight incubation,

there was flattening of radius of zone of inhibition produced by cefotaxime on the side nearest the cefoxitin disk in case of AmpC β-lactamase producer organism.

MBL production was tested by combined disk test (CDT)[10-11]. In this test, the lawn culture of 0.5 McFarland inoculum of the test strain was exposed to a disk of imipenem (10 µg) and imipenem-EDTA (10/750 µg). The difference of ≥ 7 mm in zones of inhibitions of two disks indicated MBL production.

Result: Three hundred enterobacteriaceae strains were isolated from various samples. Among these 180 (60%) were *Escherichia coli*, 81 (27%) *Klebsiella pneumoniae*, 12 (4%) *Citrobacter freundii*, 9 (3%) Salmonella Typhi, 6 (2%) *Citrobacter koseri* and *Proteus mirabilis* each, 3 (1%) *Proteus vulgaris* and *Providentia alcalifaciens* each.

Table 1: Antibiotic resistance (in percentage) in enterobacteriaceae isolates (n = 300)

Drugs	*E. coli*	*K. pneumoniae*	*C. freundii*	*C. koseri*	S. Typhi	*Pr. mirabilis*	*Pr. vulgaris*	*Pro. alcalifaciens*	Total
AMP	94.4	100	100	100	33.3	100	100	100	94.7
AMC	94.4	100	100	100	33.3	100	100	100	94.7
CEP	94.4	100	100	100	-	100	100	100	93.7
CXM	88. 9	100	100	83.3	-	100	66.7	100	89.7
CX	60.6	76.5	50.0	50.0	-	100	33.3	33.3	62.7
CTX	65.0	80.3	58.3	50.0	33.3	66.	33.3	33.3	67.0
CPM	65.0	80.3	58.3	50.0	-	66.7	33.3	33.3	66.0
PI	50.0	76.5	41.7	33.3	-	33.3	33.3	33.3	54.3
PIT	12.2	25.9	8.3	16.7	-	0	33.3	33.3	15.7
IPM	6.7	22.2	0	0	-	0	0	0	10.0
GEN	26.7	63	50.0	33.3	-	50.0	33.3	100	38.0
AK	13.3	35.8	50.0	16.7	-	16.7	0	33.3	20.7
TOB	26.7	63	50.0	33.3	-	50.0	33.3	100	38.0
NET	22.2	53.1	100	16.7	-	33.3	0	66.7	33.3
CIP	70.0	82.7	83.3	66.7	0	66.7	33.3	66.7	71.3
C	-	-	-	-	0	-	-	-	0
FO*	0	-	-	-	-	-	-	-	0
NIT*	8.3	11.1	16.7	16.7	-	33.3	0	66.7	10.3
NX*	85.0	85.2	100	66.7	-	83.3	66.7	100	82.7
COT*	91.7	79.0	91.7	83.3	66. 7	100	100	100	87.7
CB*	50.0	76.5	41.7	33.3	-	33.3	33.3	33.3	54.3

AMP – Ampicillin, AMC – Amoxiclav, CEP – Cephalothin, CXM – Cefuroxime, CX – Cefoxitin, CTX – Cefotaxime, CPM – Cefepime, PI – Piperacillin, PIT – Piperacillin-tazobactam, IPM – Imipenem, GEN – Gentamicin, AK – Amikacin, TOB – Tobramycin, NET – Netilmicin, CIP – Ciprofloxacin, C – Chloramphenicol, FO – Fosfomycin, NIT – Nitrofurantoin, NX – Norfloxacin, COT – Cotrimaxazole, CB – Carbenicillin,
*Urinary antibiotics

Table 1 shows antibiotic resistance of enterobacteriaceae isolates. All the isolates showed high resistance (83-100%) to ampicillin, amoxiclav, 1st and 2nd generation cephalosporins. The resistance to cephamycin, 3rd and 4th generation cephalosporins varied from 50-100 %. In addition to this, imipenem resistance was observed in *E. coli* (6.7%) and *K. pneumoniae* (22.2%) isolates in our settings. Among aminoglycosides, amikacin has shown better susceptibility (79.3%). All *E. coli* isolates were susceptible to fosfomycin. Among other urinary antibiotics viz. nitrofurantoin, norfloxacin, cotrimaxazole and carbenicillin; nitrofurantoin showed better susceptibility (89.7%). Drug resistance was found to be more in *K. pneumoniae*

Table 2: Testing of ESBL production in enterobacteriaceae isolates by different methods (n = 300)

Method	Phenotypic confirmatory (CAZ-CAC)	Double disk synergy	
		AMC-CTX	PIT-CPM
No. of strains showing ESBL production (%)	111 (37.0)	06 (2.0)	36 (12.0)

CAZ – Ceftazidime, CAC- Ceftazidime clavulanic acid, AMC – Amoxiclav, CTX – Cefotaxime, PIT- Piperacillin-tazobactam, CPM - Cefepime

than other enterobacteriaceae. S. Typhi showed 33.3% resistance to ampicillin, amoxiclav, cefotaxime each whereas complete susceptibility to chloramphenicol and ciprofloxacin (Table 1).

Of total 300 isolates, ESBL production was detected in 37% isolates by CAZ-CAC, 12% by PIT-CPM and 2% by AMC-CTX (Table 2). The strains found to be ESBL producer by either of the DDST method were found to be ESBL producer by phenotypic confirmatory method.

Amongst 300 enterobacteriaceae strains, 37% ESBL, 4% AmpC and 10% MBL producers were detected. There was no coproduction of β-lactamases. β-lactamase production was more in *K. pneumoniae* than other enterobacteriaceae. Overall, 51% enterobacteriaceae isolates were β-lactamase producers (Table 3).

Table 3: β-lactamase production in different enterobacteriaceae isolates (n = 300)

Enterobacteriaceae isolates (n1)	No. of strains (%) producing β-lactamase			
	ESBL	AmpC	MBL	Total β-lactamase
E. coli (180)	66 (36.7)	5 (2.8)	12 (6.7)	83 (46.1)
K. pneumoniae (81)	38 (46.9)	3 (3.7)	18 (22.2)	59 (72.8)
C. freundii(12)	4 (33.3)	1 (8.3)	-	5 (41.7)
C. koseri(6)	1 (33.3)	1 (16.7)	-	2 (33.3)
Pr. mirabilis (6)	1 (33.3)	-	-	1 (16.7)
Pr. vulgaris (3)	-	1 (33.3)	-	1 (33.3)
Pro. alcalifaciens (3)	1 (33.3)	1 (33.3)	-	2 (66.7)
Total (300)	111 (37)	12 (4)	30 (10)	153 (51)

n1 – Number of isolates, ESBL – Extended spectrum β-lactamase , AmpC - AmpC β-lactamase , MBL - Metallo-β-lactamase

Discussion: Antibiotic resistance in enterobacteriaceae isolates is increasing worldwide. It varies according to geographic locales and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of critical importance as the changing rate of antibiotic resistance has a large impact on the empirical therapy.

It is worrisome to observe that the enterobacteriaceae group which is a common infective bacterial agent had almost completely become resistant to ampicillin, amoxiclav, 1st and 2nd generation cephalosporins. Cephamycin, 3rd and 4th generation cephalosporins are also losing their shine. Carbapenems are considered as the last therapeutic option in highly resistant strains. Introduction of imipenem resistance in enterobacteriaceae isolates such as *E. coli*, *K. pneumoniae* in our settings is of great concern. The widespread and irrational use of the antibiotics might have led to the emergence of the drug resistance. Amikacin still appears to be a promising drug. In highly resistant uropathogenic *E. coli*, fosfomycin can be used. Nitrofurantoin can be helpful in resistant urinary enterobacteriaceae isolates.

In contrast to this, S. Typhi resistance appeared to be reduced. This is in accordance with the recent reports of decrease in multidrug resistant S. Typhi isolates[12].

Amongst different methods of ESBL testing, phenotypic confirmatory test (CAZ-CAC) recommended by CLSI was found to be better method as compared to either of the DDST methods in the present study (Table 2). High level expression of AmpC β-lactamases may mask recognition of ESBLs[3]. The unique combination of cefepime and piperacillin-tazobactam may be effective in detection of ESBL in such situation. High level expression of AmpC production has minimal effect on activity of cefepime[8]. Tazobactam and sulbactam are much less likely to induce AmpC β-lactamases and are, therefore, preferable inhibitors for ESBL detection[13]. In the present study, the coproduction of ESBL and AmpC was not detected. Hence, superiority of this test was not completely revealed. Some authors advocate inclusion of cefepime in differentiation of ESBL versus AmpC. However, it is important to remember that cefepime has low MIC to ESBLs, because it is a zwitterion and enters the periplasmic space efficiently. Hence it is recommended to use multiple tests like phenotypic confirmatory test and DDST using PIT-CPM for the detection of ESBL.

In present study, amongst 300 enterobacteriaceae isolates, 37% strains were ESBL producers. This type of resistance problem is endemic in several places worldwide, with rates exceeding 50% in some countries[14]. AmpC production was 4% in present study whereas Black et al[2] reported it as 31%. As high as 10% enterobacteriaceae isolates and 22.2% *K. pneumoniae* isolates were found to be MBL producer. In present study, β-lactamase production was found to be more in *K. pneumoniae* than other enterobacteriaceae. The so-called New Delhi Metallo-beta-lactamase (NDM-1) is a newer type of metallo-beta-lactamase first described in 2009 in *K. pneumoniae*[15]. The bla NDM-1 gene was isolated from *K. pneumoniae* and *E. coli* cultures from the same patient suffering from UTI; the organisms were found to be resistant to all antibiotic classes with the exception of colistin[16].

The antibiotic susceptibility pattern of enterobacteriaceae isolates should be carefully evaluated by clinical laboratories. The laboratories are still not fully aware of the importance of even ESBL and AmpC. The mechanisms of β-lactam resistance in the isolates need to be routinely assessed, so that appropriate medication can be given[8]. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures[9]. As per CLSI guidelines, routine ESBL testing is no longer necessary. However, ESBL testing may still be useful for epidemiological or infection control purposes[5]. Phenotypical methods of β-lactamase detection are simple methods which can be introduced in small scale peripheral microbiology laboratories. They require testing of few additional antibiotic disks and some approximation of distances amongst them.

Conclusion: High drug resistance and β-lactamase production is observed amongst enterobacteriaceae isolates. First and second generation cephalosporins no more remained useful in tertiary care setting. Piperacillin-tazobactam, imipenem and amikacin still hold the hope. Drug resistance in S. Typhi appears to be reduced. Close monitoring of drug resistance and β-lactamase production in enterobacteriaceae isolates is necessary.

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