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Extended spectrum β–lactamases (ESBLs): Rising antibiotic resistance among Escherechia coli and Klebsiella pneumoniae

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Abstract

Antibiotic resistance is becoming a serious challenge for clinicians particularly in critically ill patients. Bacteria of Enterobacteriaceae family showing resistance to 3rd generation Cephalosporins by producing Extended spectrum β-lactamases (ESBLs) was first noted in 1983. ESBLs are plasmid mediated enzymes that can hydrolyze a variety of β-lactams including oxyimino-cephalosporins and compromise the efficacy of all β-lactams, except cephamycins and carbapenems. In the worldwide, the ESBL group of enzymes are found widely and causes a severe infection on human health which leads to various diseases. In this study, Antibiotic susceptibility pattern of Escherechia coli and Klebsiella pneumoniae from various samples received at microbiology lab were noted. Varied degree of resistance was observed in these organisms starting from β –lactamases to extended spectrum β -lactamases (ESBLs) to resistance to Meropenem. This mandates judicious use of antibiotics as per sensitivity pattern prevailing locally.

Keywords: Antibiotic resistance, ESBL, Escherechia coli, Extended spectrum β-lactamases, Klebsiella pneumonia.

Introduction

Resistant bacteria are emerging worldwide as a serious challenge for clinicians particularly in critically ill patients. Cephalosporins are important component of empirical therapy in community as well as hospital settings. β -lactamase production by gram negative organisms is one of the most important mechanism of resistance to penicillins and cephalosporins. attack by β -lactamases. Third generation cephalosporins were introduced as a major breakthrough in the fight against bacterial resistance. It was surprising to find Klebsiella spp. resistant to third generation cephalosporins. The mechanism of this resistance was production of extended spectrum β -lactamases (ESBLs).[1,2]

Bacteria of Enterobacteriaceae family causing various diseases have also shown resistance to penicillins by producing penicillinase enzymes. These enzymes

(TEM1,TEM-2 and SHV-1) were produced by genetic mutation and are highly active against penicillins and 1st, 2nd generation of cephalosporins. The first report of plasmid encoded β lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983. Besides TEM-3 and SHV-2 these include CTX-Ms, PER, VEB and OXA. These ESBLs are remarkable for their diversity across the globe.[4,5] ESBLs now number >500 distinct enzymes and convey varying degree of resistance to penicillins, cephalosporins, β lactamase inhibitors and monobactams among gram negative organisms including pseudomonas.[6,7]

The ESBL are plasmid mediated β-lactamases that hydrolyze penicillins and cephalosporins, including the extended spectrum cephalosporins (cefoxime, ceftriazone, ceftizoxime, and ceftazidime) and monobactams and are inhibited by β-lactamases inhibitor like clavulanate, sulbactam and tazobactam. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options.[8] This study was carried out to determine the prevalence of ESBL among Escherechia coli and Klebsiella pneumoniae and to help determining the preliminary choice of antibiotic in serious clinical cases and formulate antibiotic policy in our hospital.

Material and Methods

After taking appropriate permission, this study was conducted at microbiology lab of JLN Medical College, Ajmer, Rajasthan between Aug 2019 to Feb 2020. Various clinical samples like Urine, Sputum, Pus and wound discharges, stool and Body fluids received in the lab during this period were processed as per standard bacteriological guidelines for aerobic culture. Samples, from which Escherechia coli and Klebsiella pneumoniae were isolated, were considered for this study.

Antibiotic susceptibility test: All isolated bacterial strains, including the standard Microbial Type Culture Collection strains of each bacterium, were subjected to antibiotic sensitivity tests by the modified Kirby–Bauer's method, using 4 mm thick Mueller–Hinton (MH) agar (HiMedia, Mumbai) medium following the standard antibiotic susceptibility-test chart of Clinical Laboratory Standard Institute (CLSI) guidelines.

Clinical and Laboratory Standards Institute has developed screening tests for identifying the ESBL-producing Escherechia coli, Klebsiella pneumonia, Klebsiella oxytoca and Proteus mirabilis. According to CLSI guidelines, strains showing resistance for ceftazidime cefotaxime, were selected for confirmation tests of ESBL.[9]

ESBL Confirmatory: Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL : ESBL production was confirmed among potential ESBL-producing isolates by phenotypic tests. Lawn culture of the organism was made and 3rd-generation cephalosporins ceftazidime (30 µg) disc and ceftazidime + clavulinic acid (30 µg + 10 µg) disc along with cefotaxime (30 µg) disc and cefotaxime + clavulinic acid (30 µg + 10 µg) disc were placed 25 mm apart. An increase of ≥5 mm in zone of inhibition for ceftazidime + clavulinic acid compared to ceftazidime and cefotaxime + clavulinic acid compared to cefotaxime were confirmed as ESBL producers.[9]

Results

From various clinical samples like Urine, Sputum, Pus and wound discharges, stool and Body fluids , a total of 246 isolates were Escherechia coli and 240 isolates were of Klebsiella pneumoniae were included in our study. Approximately 61 % of both Escherechia coli

and Klebsiella pneumoniae were found in males. This was similar to the sexual distribution of the samples received in the lab.

Table 1: Sex distribution of Escherechia coli and Klebsiella pneumoniae isolates

	Male	Female	Total
Escherechia coli	152 (61.79%)	94 (38.21%)	246
K. pneumoniae	145 (60.42%)	95 (39.58%)	240

Escherechia coli shows high degree of resistance to penicillin and cephalosporin Groups and maximum sensitivity was seen with Meropenem at 82.2 %. Higher sensitivity was also seen with netilmicin (67.6%) and tobramycin (64.7%). Addition of Clavulanic acid and Tazobactam to 3rd gen cephalosporins and Piperacilin increased the sensitivity against E.coli. Klebsiella pneumoniae shows similar patterns of sensitivity to all but with higher degree of resistance when compared to E coli.

Table 2: Antibiotic susceptibility pattern of Escherechia coli and Klebsiella pneumoniae

Antibiotics	Escherechia coli	Klebsiella pneumoniae
Ampicillin	17%	0%
Ampicillin+Sulbactam	32.3%	18.4%
Aztreonam	29.4%	11.4%
Cefepime	20.5%	10%
Cefixime	20.5%	8%
Cefotaxime	20.5%	8%
Cefotaxime+Clav	44.1%	26.3%
Cefpodoxime	26.4%	23.6%
Ceftazidime	17.6%	8%
Ceftazidime+Clav	44.1%	26.3%
Meropenem	88.2%	81.5%
Netilmicin	67.6%	52.6%
Ofloxacin	29.4%	31.5%
Piperacilin	17.6%	13.1%
Tobramycin	64.7%	44.7%
Piperacilin+Tazobactam	41.1%	28.9%

27.6% of E.coli and 22.9 percent of Klebsiella pneumoniae were ESBL positive i.e. these were resistant to Cefotaxime and Ceftazidime but sensitive to Clavulanic acid combinations of these antibiotics. There was another group of organisms which were resistant to both the cephalosporin (3rd Gen) and Clavulanic acid combinations and as well as Meropenem. These were the suspected Carbapenamases producers. They accounted for 9.8 % of E.coli and 17.5% of Klebsiella pneumoniae.

Table 3: Prevalence of ESBL amongst Escherechia coli and Klebsiella pneumoniae

	Total Number	ESBL Producers	Meropenam Resistant	Non-ESBL Producers
Escherechia coli	246	68 27.6%	24 9.8%	154 62.6%
Klebsiella pneumoniae	240	55 22.9%	42 17.5%	143 59.6%
TOTAL	486	123	66	297

Maximum E.coli isolates were from stool and sputum therefore the maximum number of ESBL producing E.coli were also from stool and sputum respectively. However the maximum number of Klebsiella pneumoniae were found from Pus and wound discharge followed by stool and sputum. But the maximum number of ESBL producing Klebsiella pneumoniae was from sputum followed by stool and then Pus and wound discharge.

Table 4: Clinical sample distribution amongst E.coli isolates

Clinical Sample	TOTAL Escherechia coli	ESBL Producing Escherechia coli	Meropenam resistant Escherechia coli
Pus and wound discharge	43	6	5
Sputum	49	13	7
Stool	98	31	8
Body fluids	18	2	2
Urine	38	16	2
Total	246	68	24

Table 5: Clinical sample distribution amongst Klebsiella pneumoniae isolates

S. No.	Clinical Sample	TOTAL Klebsiella pneumoniae	ESBL Producing Klebsiella pneumoniae	Meropenam resistant Klebsiella pneumoniae
1	Pus and wound discharge	96	11	14
2	Sputum	48	18	6
3	Stool	60	12	16
4	Body fluids	18	7	2
5	Urine	18	7	4
	Total	240	55	42

Discussion

ESBLs are most commonly produced by the Enterobacteriaceae, confer resistance to β lactam and monobactam antibiotics. These enzymes, often expressed by genes carried on large transferable plasmids constitute an important mechanism of resistance in nosocomial Gram negative pathogens. They are of greatest concern because the infections

caused by these are often multidrug resistant. Colonization with ESBL producing organisms leads to significantly longer hospital stay and higher cost to the patients. Therefore emphasis must be placed on controlling the spread of multi drug resistant organisms particularly within hospitals.[10]

This study demonstrates the prevalence of ESBL mediated drug resistance among Escherechia coli and Klebsiella pneumoniae to third generation cephalosporin In China, ESBL producers vary between 25 and 40%.[11] South East Asian countries reported presence of ESBLs in 5-8% of E. coli isolates from Japan, Korea, Malaysia and Singapore but in 12-24% of isolates from Thailand, Taiwan, Philippines and Indonesia.[12] India, the prevalence rate varies in different institutions from 28 to 84%.[13] A study from Coimbatore, Tamil Nadu, showed the presence of ESBLs to be 40% while a study from Nagpur showed it as 50% from the urinary isolates.[8,14,] Another study in 2005, from New Delhi, showed 68.78% of the strains of gram negative bacteria to be ESBL producers.[15] . In the present study, the prevalence of ESBL producers was found to be 27.6% amongst Escherechia coli and 22.9% amongst Klebsiella pneumoniae isolates. 9.8 % of E.coli and 17.5% of Klebsiella pneumoniae have shown resistance to Meropenem also. The alarming rate of resistance noted among these isolates in the present study, is of concern. This needs further evaluation to confirm carbapenamase production, their classification and mechanism of resistance.

ESBLs are harboured on plasmids that can easily be transmitted/transferred to other bacteria. Such plasmids may harbour resistance to different antibiotics such as aminoglycosides and fluoroquinolones. This may lead to multidrug-resistant (MDR) bacteria. In spite of this, antibiotics of these classes are often used. Higher prevalence of ESBL amongst sputum sample raises a concern of spreading of highly resistant bacteria via droplet infection particularly in hospital environment. This could be a serious condition particularly in post covid era where secondary infection with MDR bacteria can be life threatening. Spread of such MDR bacteria needs to be controlled within and between institutions.

Conclusion

In the present study, we found an alarming number of β -lactamase producing Escherechia coli and Klebsiella pneumoniae strains which were not just qualifying for ESBL but a significant number had evolved to even resist Meropenem. The Microbiology laboratories should be equipped to detect multiple beta lactamases including ESBLs and Carbapenamases, so that appropriate therapy can be chosen for patient management. [16,17] The high prevalence of ESBL in E.coli and Klebsiella pneumoniae is associated with a multitude of infections in hospitalized patients. Emphasis must be placed on the rational and judicious use of all antimicrobial agents. Clinicians should decide empirical therapy both in community as well as hospital settings based on local antibiotic susceptibility pattern. Restrictions should also be placed on irrational use of antibiotics, particularly by quacks in rural areas.

Furthermore, this paper will be helpful for the further investigations to develop quicker, cost effective, and reliable diagnostic strategies and new effective therapies.

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