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Phenotypic characterization and antibiotic suceptibility patterns of extended spectrum beta-lactamase producing enterobacteriaceae

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ABSTRACT

Background: The rise of Enterobacteriaceae strains that produce an extended spectrum b-lactamase (ESBL) has become a global concern for epidemiological surveillance and the prevention of nosocomial acquired infections in the modern era. The choice of appropriate antibiotics to be employed in the treatment of infections brought on by ESBL-producing bacteria relies greatly on the detection and identification of these ESBLs in the laboratory. Limitations in ESBL detection have aided in the unbridled emergence of bacterial resistance and constitute a major health concern.
Objectives: Isolation and identification of ESBL among Enterobacteriaceae by phenotypic methods with their Antibiogram.
Materials and Methods: Phenotypic techniques were used to identify ESBL-producing Enterobacteriaceae (ESBLs-E) isolates from diverse clinical samples. Kirby Baur disc diffusion technique was performed to determine antimicrobial's susceptibility.
Results: Among 212 Enterobacteriaceae isolates 124(58.3%) were positive for ESBL production. E.coli(74.5%) & K.pneumoniae (52.2%) two main isolates that produce ESBLs. Maximum ESBL producing Enterobacteriaceae isolates were obtained from blood samples 82% (41/50) followed by urine 59 % (62/105). Meropenem (96.7%), Amikacin (82.1%), and Cefoxitin were most susceptible antibiotics for ESBL-producing isolates while high resistance was observed in ceftazidime (62%), followed by Ciprofloxacin (60%).
Conclusion: The majority of ESBLs-E was mostly found in urine and blood samples.It was observed that E.coli produced the most ESBLs. There was a high prevalence of ESBLs-E in tertiary care hospital of central India. Therefore, strong infection control strategies must be implemented in hospital settings

1. Introduction

Enterobacteriaceae are large family of Gram-negative, non-sporing & facultative anaerobic bacteria. These bacteria are a significant contributor to both community- and hospital-acquired illnesses. Enterobacteriaceae are primarily responsible for septicaemia, wound, urinary & respiratory infections.[1,2]

Beta lactum antibiotics are primarily utilized for treatment of infections brought on by Enterobacteriaceae. Among them are a derivative of penicillin, cephalosporins, monobactams, carbapenems and many other antibiotics.

Increased usage of beta-lactam antibiotics has resulted in an increase in Enterobacteriaceae resistance. Their use, however, clashes with the concerning phenomenon of antimicrobial resistance among enterobacteriaceae strains due to production of beta-lactamase, an enzyme that attacks the -lactam ring of beta lactum antibiotics (particularly

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extended-spectrum beta-lactamases), which is a global health issue. So beta-lactam antibiotics can be used with lactamase inhibitors like Clavulanic acid, Sulbactam, and Tazobactam to mitigate this resistance.[3]

ESBLs are commonly found in Gram-negative bacteria particularly in enterobacteriacea and Pseudomonas aeruginosa.[4] Mutated versions of the TEM1, TEM2, and SHV genes on plasmids encode the most common type of beta-lactamases.[5]

Among gram negative bacterias Klebsiella & E. coli are most common strain that produces ESBL enzymes.[6,7]

The advent of ESBL-producing bacteria limits prescription of β -lactam antibiotics, has caused multiple illness outbreaks around the globe, and creates difficult infection control problems.

One of the key concerns in hospitals continues to be the emergence of microbes that produce Extended Spectrum Beta Lactamase (ESBL). A microbiological laboratory's ability to accurately detect these drug-resistant pathogens is crucial. One of the principle drug resistance mechanisms that significantly affect the available treatment option for infections by Gram negative bacteria, particularly strain of the family Enterobacteriaceae, is production of extended range beta-lactamases by bacterial strains.

In this scenario it is imperative to evaluate them to comprehend the epidemiology and disease burden of ESBL-producing Enterobacteriaceae in local setting, in addition to develop & put into practice infection control policies in hospitals to avoid the emergence and propogation of such bacteria. The purpose of this research was to ascertain the prevalence of ESBL producing Enterobacteriaceae in distinct clinical samples.

2. Material and Methods

Six-month cross-sectional research study was undertaken in the microbiology department of a teaching hospital.

The study included 212 non-repetitive Enterobacteriaceae strains isolated from clinical samples such as urine, blood, pus, and wound swabs, that were obtained in the previous six months for microbiological analysis at the Institute's Bacteriology Laboratory. Standard laboratory procedures were used to process the samples and identify the isolates.[8]

Sensitivity for different antibiotics was done using the Kirby-Bauer disc diffusion method as per the recommendation of the CLSI guidelines.[9] All the strains were tested for the antimicrobial susceptibility pattern. The isolates were tested for sensitivity pattern for first and second line of drugs. (Table 1)

The isolates were analysed for ESBLs generation by phenotypic confirmatory test by double disc synergy method on Muller Hinton agar (MHA) according to the CLSI guidelines along with routine antibiotic susceptibility testing for preliminary identification of ESBL generating Gram negative bacilli. E. coli ATCC 25922 was used as control strains.

Test isolates were inoculated on MHA with standard inoculum (0.5 McFarland). Ceftazidime (30μg) and ceftazidime - clavulanic acid (30 μg/10μ g) were examined. In the presence of clavulanic acid, a 5-mm increase in zone diameter over ceftazidime alone was considered as an ESBL producer.[9,10]

3. Result

Total of 212 non-repetitive series of Enterobacteriaceae strains were separated from various clinical samples.

Distribution of Enterobacteriaceae isolates in different samples depicted in Table 2.

90 (42.5%) of the isolates from the study's patient population were found in male patients, while 122 (57.5%) were found in female patients, indicating that males were more likely to contract the infection than females.

Age wise distribution of Enterobacteriaceae isolates presented in Table 3.

Out of 212 Enterobacteriaceae most prevalent isolates were E.coli 115 (54.2%), Klebsiella pneumoniae 60 (28.3%) and Citrobacter species 37 (17.4%).

As depicted in Table 4 E. coli, K. pneumoniae and citrobater spp. were most common isolates discovered in males & females.

All three strains of Enterobacteriaceae i.e. E. coli, K. pneumoniae & citrobacter isolates were predominantly isolated from urinary sample accompanied by blood, pus & wound swab. [Table 5]

Among 212 Enterobacteriaceae isolates, 124(58.3%) were positive for the ESBL production by using the Phenotypic confirmatory disc diffusion method. [Figure 1]

In our study various Enterobacteriaceae species exhibit various distributions of ESBL producers , E.coli produced ESBLs at the highest intra-species frequency, with a frequency of 74.5% (86/115), followed by K. pneumoniae and Citrobacter species, with of 52.2% (31/60) and 18.9% (7/37), respectively.[Figure 2]

Maximum ESBL producing Enterobacteriaceae isolates were obtained from blood samples 82% (41/50) followed by urine 59% (62/105) pus 40%(16/40) and wound swab29.4%(5/17).[Table 6]

In this study, maximum number of the ESBL-producing isolates were susceptible to Meropenem 117(94.3%), 106 Amikacin (85.4%), and Cefoxitin 88(71%), while Ceftazidime,Ciprofloxacin & Amoxyclav were most resistant drugs among these isolates with the susceptibility of 8(6.4%), 12(9.6%) and 34(27.4%) respectively.[Table 7]

4. Discussion

The vast majority of bacteria extracted from clinical samples belong to the family Enterobacteriaceae, rendering them

Table 1: Sensitivity reporting of antimicrobials depending on zone of inhibition for each drug used in the present study

Antibiotic	Disk Content	Zone Diameter		
		S	I	R
Meropenam (Mr)	10 μg	≥23	20-22	≤19
Amikacin (Ak)	30 μg	≥17	15-16	≤14
Cefoxitin (Cef)	30 μg	≥18	15-17	≤14
Gentamicin (G)	10 μg	≥15	13-14	≤12
Amoxyclav	20 μg /10 μg	≥18	14-17	≤13
Ciprofloxacin©	5μg	≥26	20-25	≤21
Ceftazidime (Cez)	30 μg	≥21	18-20	≤17

Table 2: Sample wise distribution of Enterobacteriaceae

Specimen	Number of Isolates (%)
Urine	105(49.5)
Blood	50(23.6)
Pus	40(18.9)
Wound swab	17(8)
Total	212

Table 3: Age wise distribution ofEnterobacteriaceae isolates

Age in years	No. of isolates
1-5	3(1.4%)
6-15	22(10.3%)
16-35	58(27.3%)
36-65	86(40.6%)
>65	43(20.4%)
Total	212

Table 4: Distribution of common organisms isolated amongEnterobacteriaceae

Isolate	E.coli	K.pneumoniae	Citrobacter
Male	68(59.1%)	34(56.6%)	20(54%)
Female	47(40.9%)	26(43.4%)	17(45.9%)
Total	115	60	37

Table 5: Distribution of Enterobacteriaceae isolates from various clinical samples

Isolates(212)	Clinical samples				
	Urine	Blood	Pus	Wound swab	Total
E.coli (115)	62(53.9%)	23(20%)	19(16.5%)	11(9.5%)	115
K.Pneumoniae (60)	27(45%)	18(30%)	13(21.7%)	02(3.3%)	60
Citrobacter (37)	16(59.2%)	09(33.3%)	08(29.6%)	04(14.9%)	27
Total					212

Table 6: Specimen wise distribution of ESBL producers

Specimen	Number of isolates	ESBL producers
Blood	50	41(82%)
Urine	105	62(59%)
Pus	40	16(40%)
Wound swab	17	5(29.4%)
Total	212	124

Table 7: Antibiotic susceptibility pattern of ESBL producing isolates

Antibiotic	Sensitive	Resistant
Meropenam	117(94.3%)	7(5.7%)
Amikacin	106(85.4%)	18(14.6%)
Cefoxitin	88(71%)	36(29%)
Gentamicin	40(32.2%)	84(67.8%)
Amoxyclav	34(27.4%)	90(72.6%)
Ciprofloxacin	12(9.6%)	112(90.4%)
Ceftazidime	8(6.4%)	116(93.6%)

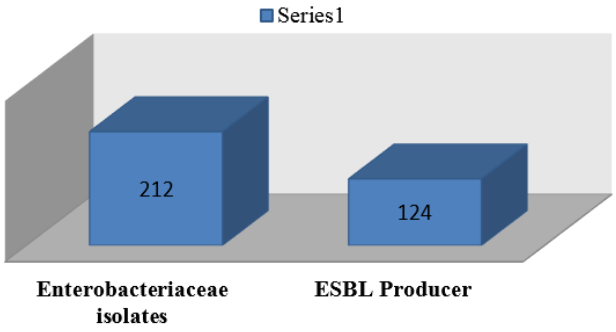

Fig. 1: Total no. of ESBL Producers among all Enterobacteriaceae isolates

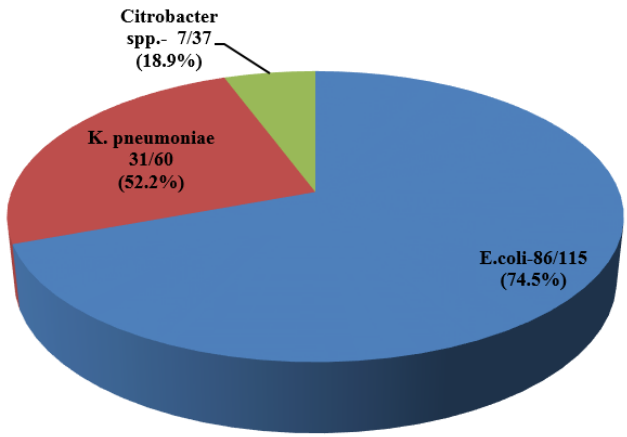

Fig. 2: ESBL producers among different isolates of family Enterobacteriaceae

one of the most significant human pathogens.[11] Extended spectrum cephalosporins, a member of the beta-lactam antibiotic class, are frequently used to treat these infections. ESBL has contributed to the significant increase in bacterial resistance to these antibiotics in recent years.[12,13]

Plasmid-coded versions of these enzymes may also carry gene that compromises the activity of other commonly prescribed non-beta-lactam antimicrobial agents, thereby decreasing number of other antimicrobial drugs for the treatment of these bacteria[14] & creating severe therapeutic challenges that have a big impact on patients' recovery

Thus sustained emergence of ESBLs poses diagnostic obstacles to medical microbiology laboratories.Therefore, precise and trustworthy ESBL detection in a microbiology laboratory is essential. In order to identify the ESBL among Enterobacteriaceae this study was carried out.

In six month study period, a total of 212 Enterobacteriaceae isolates were investigated. The majority were E. coli (54.2%) followed by K. pneumoniae (28.3%), Citrobacter species. (17.4%), Rao et al[15] also confirmed E. coli and K. pneumoniae as the most prominent species & this was quite comparable to the findings of our investigation. Shashwati et al[16] also found similar finding.

In our investigation, the majority of ESBL-producing Enterobacteriaceae were detected in blood specimens, accounting for 82% (41/50), followed by urine specimens, accounting for 59% (62/105).Blood was identified by several researchers also as a primary source of ESBL-producers.[17,18]

In our study, it was observed that meropenem (96.7%), amikacin (82.1%), and cefoxitin were the main drugs susceptible to ESBL-producing isolates. This was in close agreement with study conducted by Shashwati et al[16] and Yadav et al.[19]

In the present study, high resistance was documented in ceftazidime (62%), followed by Ciprofloxacin (60%), Amoxyclav (52%), Gentamycin (51%), Amikacin (48%),Cefoxitin (47%) and Meropenam (39%).However in other studies ceftazidime 83.2%, cefotaxime 74.7%, ciprofloxacin 61.1% were highly resistant antibiotics.[20]

5. Conclusion

It is concerning that Enterobacteriaceae members produce ESBLs at such high rates. This study shown that ESBL production can be accurately detected using the phenotypic confirmatory test. Phenotypic tests with routine lab tests for antibiotic sensitivity can help in detecting the development of ESBLs within 48 hours. To save time, this approach can be used consistently for all isolates of Enterobacteriaceae. This will guide medical professionals in choosing and recommending the best antibiotics to treat these infections. Meropenem, amikacin, and cefoxitin are the preferred options for treating Enterobacteriaceae that produce ESBLs. ESBL-producing isolates showed a increased prevalence of

resistance to Ceftazidime, Ciprofloxacin and Amoxyclav. Regional variations in the situation need the use of local patterns of susceptibility or institutional antibiograms, which aid in the development of the each institution's individual antibiotic policy. We recommend routinely checking for Enterobacteriaceae ESBL development in addition to effective infection control procedures.

6. Source of Funding

None.

7. Conflict of Interest

None.

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