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Prevalence of extended-spectrum beta-lactamases producing isolates obtained from patients of pediatric critical care unit in a tertiary care hospital

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ABSTRACT

Background: Over the past decades, antibiotic-resistant Gram-negative bacteria commonly *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* have increased significantly. These microorganisms have great clinical importance because they increase hospital stay of the patients in the intensive care unit (ICU) leading to high morbidity and mortality. Because of their role in increasing morbidity and mortality, this study was performed to isolate extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacilli screened by phenotypical method and further projected into molecular characterization by polymerase chain reaction. **Aims and Objectives:** The aims and objectives are to isolate the Gram-negative multidrug-resistant strains from clinically suspected bacterial infections in patients of neonatal, sick newborn, and pediatric ICU and to study antibiotic sensitivity pattern of isolated Gram-negative multidrug-resistant strains with special reference to molecular characterization. **Materials and Methods:** A total of 100 Gram-negative bacilli were isolated. Screening of ESBL positivity was done by double-disk synergy test (combined disc test method). Their antibiogram profile was interpreted. With the use of designed primers, 26 ESBL isolates each of *E. coli and Klebsiella* spp. were processed for molecular analysis of beta-lactamase family genes TEM and CTX-M. **Results:** Within the 100 samples, majority of the isolates (45%) were *Klebsiella* spp. and 40% was *E. coli* isolates. Highest ESBL-producing organisms were observed within *E. coli* (65%). Prevalence *bla*-TEM gene was highest followed by *bla*-CTX-M. These ESBL-producing organisms were found to be resistant to multiple classes of antibiotics. With extensive ESBL surveillance and proper usage of antibiotics, this threatening rise of antibiotic resistance can be mitigated. **Conclusion:** Gram-negative isolates showed high resistance to commonly used antibiotics. Significant proportions of them were MDR strains. Such high antibiotic resistance is associated with significant morbidity and mortality among pediatric population. MDR along with possession of ESBL associated resistance genes among Gram-negative bacilli pose a serious problem in therapeutic management of patients. Our study signifies that there is a high probability of Gram- negative bacilli to be multi-drug resistant and ESBL positive and earliest detection of such cases should be made.

Key words: Extended-spectrum beta-lactamase; *Enterobacteriaceae*; Antibiogram; Polymerase chain reaction

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INTRODUCTION

Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* have increased significantly. These pathogens which are normally found in human intestinal tract are found to be frequently associated with health-care-associated infections and also community-acquired infections. Globally, neonatal mortality accounts for more than one-third of deaths of children aged <5 years.[1] About 99% of these neonatal deaths take place in low and middle-income countries.[2] Beta-lactams are group of antibiotics that act on the bacterial cell wall. These include penicillin, cephalosporins, carbapenems, and monobactams. They inhibit the carboxypeptidases and transpeptidases (penicillin-binding protein) blocking the cross linking thus leading to the weakening of cell wall structure finally leading to cell lysis. Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria were isolated from the various specimens which contributed to significant morbidity and mortality in these pediatric populations.[2] *Enterobacteriaceae* is capable of producing beta-lactamase enzymes coded by genes predominantly present on plasmid. They are able to hydrolyze third and fourth-generation cephalosporins and monobactams, and these are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. Although most ESBLs are mutant of TEM, SHV enzymes, CTX-M type lactamases are also become important. Outbreak of these ESBL-producing organisms is now steadily increasing and control of these infections is of paramount importance in this situation.

Aims and objectives

To isolate the gram-negative multi-drug resistant strains from clinically suspected bacterial infections in patients of neonatal, sick new-born and pediatric intensive care unit. To study antibiotic sensitivity pattern of isolated gram-negative multi-drug resistant strains with special reference to molecular characterization.

MATERIALS AND METHODS

Relevant samples from patients of pediatric critical care unit having clinically suspected bacterial infection including blood, CSF, urine, wound swab, endotracheal tube tips, and venous catheter were collected from May 2020 to April 2021 in Burdwan Medical College and Hospital, West Bengal, India. Samples were transported and processed according to standard guidelines.

Antibiotic sensitivity testing

Mueller–Hinton agar was used and Kirby–Bauer disc diffusion method as recommended by CLSI was used to perform the antibiotic susceptibility testing. Isolates which showed reduced zone of inhibition to ceftriaxone (30 mcg), cefotaxime (30 mcg), and ceftazidime (30 mcg) were screened for ESBL production. Antibiotic disk and Agar - HiMedia laboratories, Mumbai, India.

ESBL detection

Done as per CLSI guideline.[3] Both ceftazidime and cefotaxime alone and combination with clavulanic acid were used. After the application of antibiotic discs, MHA agar was incubated for 16–18 h in 35°C. A >5 mm increase in a zone of diameter for either antimicrobial used in combination with clavulanate versus the zone of diameter of the agent used alone indicates ESBL production.

Molecular characterization - 26 isolates each of *E. coli* and *Klebsiella* spp. were processed for the detection of beta-lactamase producing genes *bla*-TEM and *bla*-CTX-M. Total DNA isolation was carried out.[4,5]

For polymerase chain reaction (PCR), amplification master mix was prepared containing - 20 µL of master mix was prepared with sterile double distilled water (Mili-Q grade) containing the primers blaTEM FP - 20 nmole, blaTEM RP - 20 nmole, blaCTX-M FP - 50 nmole, blaCTX-M RP - 50 nmole, 250 µM of each dNTP, 1.5 unit of Taq polymerase (Sibenzyme), 2.0 µL of 10X PCR buffer containing 1.5 mM $MgCl_2$, and 100 ng DNA template.

Primers designed-[6,7]
TEM-beta-lactamase.
Sequence- TEMF
5'-ATGAGTATTCAACATTTCCGT-3'
Sequence- TEMR
5'-TTACCAATGCTTAATCAGTGA-3'
CTX-M-beta-lactamase.
Sequence- CTX-MecF
5'-ATGYGCAGYACCAGTAAG-3'
Sequence- CTX-MecR
5'-ATATCRTTGGTGGTGCCRT-3'
Amplification was performed by Multiplex PCR. (Model-Veriti, Applied Biosystems, USA).
Initial Denaturation- 95°–5 min
Denaturation- 95°–30 s
Annealing- 52°–30 s
Elongation- 72°–1 min
Repeated over 30 cycles
Final Elongation- 72°–10 min.

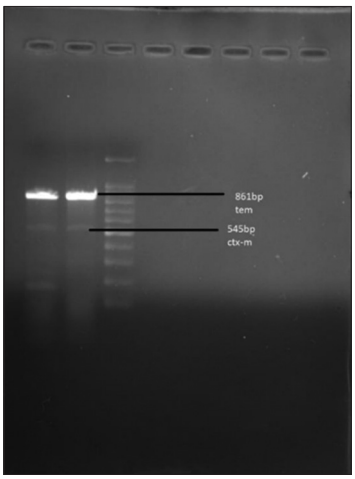
Agarose gel electrophoresis was done at 50 volts for 2.5 h. Gel was visualized on UV platform in gel documentation system (Bio-Rad, USA).

Table 1: Clinical diagnosis of patients from whom the isolates were obtained

Diagnosis	Frequency	Percentage
Pneumonia	35	35.0
UTI	33	33.0
Nephrotic syndrome	14	14.0
SSI	7	7.0
AES	6	6.0
Umbilical sepsis	5	5.0
Total	100	100.0

Table 2: Frequency distribution of clinical samples

Clinical sample	Frequency	Percentage
Blood	41	41.0
Urine	47	47.0
Wound swab	7	7.0
ET tube	5	5.0
Total	100	100



PCR products of *bla*-CTX-M and *bla*-tem.

Inclusion criteria

Samples from patients clinically suspected having bacterial infections from special newborn care unit, neonatal intensive care unit, pediatric intensive care unit after proper consent from legally accepted representative.

Exclusion criteria

Samples were found to be culture negative by standard laboratory methods. Gram-positive bacterial infection. Consent not obtained.

RESULTS

Out of 100 samples received, 35% patients had pneumonia, 33% had UTI, 14% had nephrotic syndrome, 7% had surgical site infection, 6% had acute encephalitis syndrome and 5% had umbilical sepsis. [Table-1]. Out of these 100 clinical samples 47% was urine sample, 41% was blood sample, 7% was wound swab sample and 5% was ET sample. [Table-2]. Within these Gram-negative isolates 45% was *Klebsiella spp*. followed by *Escherichia coli* (40%) and 15% was *Pseudomonas aeruginosa*. [Table-3]. Antibiotic susceptibility pattern of the isolated organisms was following *Klebsiella spp*. demonstrated maximum susceptibility to Polymyxin B (100%), Colistin (100%), while showed high resistance to Cefotaxime (86.7%) and Aztreonam (48.9%). *E. Coli* demonstrated maximum susceptibility to Polymyxin B (100%), Levofloxacin (75%) and Cefoperazone/sulbactam (72.5%); while showed high resistance to Cefotaxime (90%) and Aztreonam (60%). Pseudomonas aeruginosa demonstrated high susceptibility to Polymyxin B (100%), Colistin (100%), Cefoperazone/sulbactam (80%), Piperacillin-Tazobactam (Pip-Taz) (73.3%), Levofloxacin (73.3%) and Amikacin (73.3%); while showing high resistance to Cefotaxime (80%), Aztreonam (80%) and Ceftriaxone (66.7%). [Table-4]. 68 Multi drug resistant organisms found among 100 samples. [Table-5]. ESBL producing strains among found to be 57.8%. [Table-6]. ESBL producing strains among Escherichia coli found to be 65%. [Table-7]. Detection of ESBL producing genes among Klebsiella spp. found to be for bla-CTX-M is 61.5% and for bla-TEM 65.4%. [Table-8]. Among ESBL producing E. coli strains bla-CTX-M was 50% and bla-TEM was 53.8%. [Table-9]. Molecular studies showed preponderance of bla-tem gene followed by bla-CTX-M.

Table 3: Frequency distribution of the obtained Gram-negative bacterial isolates

Gram-negative isolates	Frequency	Percentage
Klebsiella spp.	45	45.0
Escherichia coli	40	40.0
Pseudomonas aeruginosa	15	15.0
Total	100	100.0

DISCUSSION

Gram-negative bacilli (GNB), particularly those in the bacterial family *Enterobacteriaceae* (e.g., *Klebsiella* spp. and *Enterobacter* spp.) and *Acinetobacter* spp., are common causes of serious community and hospital-acquired infections. These GNBs are also members of the ESKAPE group of pathogens. These are notoriously associated with antimicrobial resistance (AMR) and frequently carry genes that induce resistance to three or more classes of antimicrobials, making them multidrug resistant.

In the 1940s, the discovery of antibiotics was seen as one of medicine's major achievements that saved millions of lives.[8] However, in the past decade, AMR has significantly

Table 4: Resistance pattern of obtained Gram-negative isolates

Antibiotics	*Klebsiella* spp. (n=45)	*Escherichia coli* (n=40)	*Pseudomonas aeruginosa* (n=15)
Cefotaxime	39 (86.7)	36 (90.0)	12 (80.0)
Ceftazidime	26 (57.7)	22 (55.0)	6 (40.0)
Ceftriaxone	11 (24.4)	18 (45.0)	10 (66.7)
Cefoperazone/sulbactam	12 (26.7)	11 (27.5)	3 (20.0)
Piperacillin-tazobactam	5 (11.1)	3 (7.5)	4 (26.7)
Meropenem	20 (44.4)	26 (65.0)	8 (53.3)
Amikacin	10 (22.2)	12 (30.0)	4 (26.7)
Levofloxacin	12 (26.7)	10 (25.0)	4 (26.7)
Aztreonam	22 (48.9)	24 (60.0)	12 (80.0)
Polymyxin-B	0 (0.0)	0 (0.0)	0 (0.0)
Colistin	0 (0.0)	0 (0.0)	0 (0.0)

Table 5: MDR analysis

MDR	Frequency	Percentage
No	32	32
Yes	68	68

MDR: Multidrug resistant

Table 6: ESBL-producing *Klebsiella* spp. (n=45)

ESBL-producing *Klebsiella* spp.	Frequency	Percentage
Present	26	57.8
Absent	19	42.2
Total	45	100.0

ESBL: Extended spectrum beta-lactamase

Table 7: ESBL-producing *E. coli* (n=40)

ESBL-producing *E. coli*	Frequency	Percentage
Present	26	65.0
Absent	14	35.0
Total	40	100.0

ESBL: Extended-spectrum beta-lactamase, *E. coli*: *Escherichia coli*

Table 8: Detection of CTX-M and TEM gene in *Klebsiella* spp. isolates (among 26 samples)

Incidence of CTX-M and TEM gene	Frequency	Percentage
CTX-M	16	61.5
TEM	17	65.4

Table 9: Detection of CTX-M and TEM gene in *Escherichia coli* isolates (among 26 samples)

Incidence of CTX-M and TEM gene	Frequency	Percentage
CTX-M	13	50.0
TEM	14	53.8

increased in bacteria and reduced the effectiveness of many clinically important antibiotics.[9] GNBs are among the most common causative agents of infectious diseases.[10] Members of this family are ubiquitous, i.e., can be found in humans and animals' intestinal microflora but also in the environment.[11,12]

Nagvekar et al. in their study reported that *E. coli*, *Klebsiella*, *Acinetobacter,* and *Pseudomonas*/*Enterobacter* were the GNB which were the most prominent cause of infections in hospitalized patients; however, there was a greater incidence of *Acinetobacter* and *Klebsiella* multidrug-resistant (MDR) GNB among all other GNBs.[13]

Pokhrel et al. in their study reported Gram-negative organisms showed high susceptibility to colistin.[14] Their observation was consistent with the findings of Jasani et al.[15] *Klebsiella* and *Enterobacter*, the main Gram-negative isolates, showed maximum susceptibility to carbapenems, followed by colistin and tigecycline, respectively. Such high susceptibility toward carbapenem was also documented by Sheth et al. and Yusef et al.[16,17]

Manandhar et al. in their study reported for *E. coli*, *Enterobacter* spp., and *Klebsiella* spp., and the resistance genes most frequently detected were blaOXA, blaTEM, blaCTXM-1, and blaCTXM-8.[18]

This suggests that the detection of blaTEM or any gene of the blaCTXM or blaOXA family is an important index for multidrug-resistant phenotype, and as expected, the detection of a blaCTXM family gene or blaTEM indicates a higher odd of ESBL-positive phenotype. Collectively, this suggests that the detection of key AMR genes by molecular methods is an important index for ESBL positivity and MDR in bacterial isolates.

Limitations of the study

This study was conducted with relatively less samples and in a very limited time period. It covers patients only from a single Institution.

CONCLUSION

At the end of the study, we came to the conclusion that

Gram-negative isolates showed high resistance to commonly used antibiotics. Significant proportions of them were MDR strains. Such high antibiotic resistance is associated with significant morbidity and mortality among pediatric population. However, the use of broad-spectrum antibiotics as empirical therapy could be detrimental in the long run, and hence, they should be used judiciously and modified to narrow-spectrum antibiotics, as guided by the culture and susceptibility report at the earliest opportunity. Moreover, MDR along with possession of ESBL and carbapenemase-associated resistance genes among GNB pose a serious problem in the therapeutic management of patients. Further, our study signifies that there is a high probability of Gram-negative bacilli to be multidrug-resistant and ESBL-producing organisms and earliest detection of such cases should be made for better treatment outline ultimately giving an edge to fight against these emerging strains.

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SB- Data collection, data analysis, manuscript preparation; **MM**- Study design, Mentoring; **TB**- Clinical protocol; **MM**- Statistical analysis; **PG**- Editing, manuscript revision, submission of the article; **IS**- Literature survey, chart preparation; **SAZ**- Study protocol, review manuscript.

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