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Original Article

Antibiogram resistance pattern of extended-spectrum beta-lactamase-positive bacterial isolates

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ABSTRACT

Objectives: To identify the antibiogram resistance pattern of extended-spectrum beta-lactamase (ESBL)-positive bacterial isolates. **Materials and Methods:** This prospective, observational study was conducted in Rajarajeswari Medical College and Hospital, Bangalore, over a period of 12 months. The clinical samples were inoculated on the standard recommended media. Inoculated plates were incubated aerobically at 37°C for 24 h, and organisms were identified by culture and appropriate biochemical reactions. Antimicrobial sensitivity was performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar using commercially available antibiotic discs as per the Clinical and Laboratory Standards Institute Guidelines-2014. **Results**: *Escherichia coli* were the most common ESBL-producing organisms isolated from the study population accounting to 45% of the total cases. The next most common organisms were *Klebsiella* species and *Pseudomonas*. Ceftriaxone was the most commonly used empirical antibiotic (33.3% cases). Piperacillin and meropenem were used in combination with tazobactam or sulbactam, respectively, as per the culture and sensitivity reports. **Conclusion:** The association of change in antibiotic to mortality was found to have significance. The change in antibiotic in deterioration of the illness showed decrease in mortality.

Key words: *Antibiogram, Extended-spectrum Beta-lactamases, Escherichia coli*

Antimicrobial agents used for the last few decades have greatly reduced the illness and death from infectious diseases. Antibiotic use is beneficial when prescribed and taken correctly; their value in care of the patient is enormous. However, these drugs have been used so widely and for so long that the infectious organisms have adapted to the antibiotics which are designed to kill them, making these drugs less effective. Some microorganisms develop resistance to a single antimicrobial agent (or related class of agent), while others develop resistance to several antimicrobial agents or classes. The microorganisms have become so resistant in some cases that no available antibiotics are effective against them. In laboratory, an isolate is described as resistant to an antimicrobial when it is not inhibited by usual concentration of the agent with normal dosage schedule, and/or when it demonstrates that the fall in the range where specific resistance are likely, and the clinical efficacy of the agent against the isolate has not been reliably shown in the treatment studies (Clinical and Laboratory Standards Institute [CLSI]) [1].

Development of antimicrobial resistance may occur naturally via natural selection through random mutation or by programmed evolution. Programmed evolution is a regulated process in which the organism switches on altered levels (usually higher) to sensed non-mutagenic environmental cues. Three main mechanisms by which Gram-negative organisms exhibit resistance to antimicrobials are as follows: (1) Hydrolysis of drugs by enzymes, (2) decreased permeability of the antibiotic into the cell, and (3) decreased affinity of the target [2,3]. In the above mentioned, expression of β-lactamases is the major mechanism of resistance causing clinically significant infection in Gram-negative organisms [2]. β-lactamases are the enzymes capable of hydrolyzing β-lactam ring of penicillin, cephalosporin, and related antimicrobial drug rendering them inactive. β-lactamases vary in substrate and host range [4]. All *Enterobacteriaceae* produces intrinsic resistance of individual species to some antibiotics, except *Salmonella* species [5].

Recommended antimicrobial agents used in antimicrobial susceptibility test on Gram-negative rods include 2nd- and 3rd-generation cephalosporin, piperacillin, antipseudomonal penicillin, fluoroquinolone, trimethoprim-sulfamethoxazole, and aminoglycoside. Nitrofurantoin, quinolone, and trimethoprim are included in antimicrobial susceptibility test when the organism is isolated from pathological specimens [6]. Characteristic feature of extended-spectrum beta-lactamase (ESBL)-mediated resistance in *Enterobacteriaceae* is resistance to penicillin as well as to 2nd-generation and one or more of 3rd-generation and 4th-generation cephalosporin or aztreonam. However, transmission electron microscopy and (Sulphydryl variable) SHV type of ESBL are not able to hydrolyze extended-spectrum β-lactam antibiotics as efficiently as their parent enzyme [7].

Another feature of ESBL-mediated resistance is increased susceptibility to β-lactamase inhibitor particularly clavulanate [7]

due to the expansion of active site of ESBL producer which allows its increased activity against the extended-spectrum cephalosporin. This characteristic feature makes possible a synergy between cephalosporins and β-lactamase inhibitors. Clavulanic acid and tazobactam have better inhibitory activity than sulbactam. The objective of this study is to identify the antibiogram resistance pattern of ESBL-positive bacterial isolates.

MATERIALS AND METHODS

This prospective, observational study was conducted in Rajarajeswari Medical College and Hospital, Bangalore, over a period of 12 months from February 2015 to January 2016. Sample size is determined by equation $n \geq z^2 (pq)/d^2$ (n=sample size, z=a constant 1.96, p=prevalence, q=100−prevalence, d=relative error). In India, the prevalence of ESBL infection is highly variable, and as per the data available from the Department of Microbiology of our college, prevalence was 23.55%. Hence, in this study, p=23.5, q=76.5, d=11, and as per the formula, calculated sample size was 60.

All the Gram-negative bacterial isolates from the clinical samples such as sputum, pus, urine, ascitic fluid, colony-stimulating factor, pleural fluid, and miscellaneous samples received from the children aged 0-18 years admitted to our hospital were included in the study. Children in whom bacterial culture grew mixture of organisms or children treated with antibiotics before the collection of pathological specimen for culture were excluded from the study. Ethical clearance for the study was obtained from the Committee on Human Research, publication, and ethics of our institution.

Isolation

Samples were collected under aseptic precaution and processed. Antimicrobial sensitivity was performed by Kirby-Bauer disk diffusion method on Mueller-Hilton agar using commercially available antibiotic discs as per CLSI Guidelines-2014. ESBL was detected by the combined disc method using ceftazidime and clavulanic acid and cefotaxime and cefotaxime-clavulanic acid.

Case was defined as any person <18 years old presenting with fever with associated symptoms such as cough, expectoration, chest pain, wheeze, and shortness of breath; lower abdominal pain, frequency, dysuria, urgency, vomiting, and voiding difficulty; vomiting, reduced feeding, lethargy with or without fever, with features of shock pointing toward septicemia; or ear discharge. Laboratory investigations including complete hemogram, urine microscopy, liver function test, renal function test, arterial blood gas analysis, erythrocyte sedimentation rate, and C-reactive protein were done as per the protocol. A positive culture within 48 h of admission was considered as community-acquired infection, whereas those obtained after this period was defined as hospital-acquired infection. Patients were managed according to the standard protocols and blood investigations were repeated on day 0, day 3, and day 5 and on the day of discharge.

Antibiotic therapy including drugs, dosage, duration, and frequency was evaluated for each patient. Empirical treatment was defined as initial antibiotic regimen used before the organism was identified. Definitive therapy was defined as the regimen chosen after the isolation of organism and susceptibilities were available. Antibiotics were changed according to the clinical response.

Data of 60 patients were considered for analysis. Descriptive statistics were applied. Microsoft Excel 2010 and SPSS software were used for data analysis. Chi-square and Student's t-test were used to know the association of variables. Significant level was fixed at 0.05.

RESULTS

In our study, male:female ratio was 1:1 and there were 10% females and 12% males in the age group of 0-28 days as shown in Table 1. The most common symptom at the time of presentation was fever (27 cases), followed by vomiting (22 cases) and poor feeding (21 cases). The frequency distributions of clinical presentation of cases according to age group are shown in Table 2. The most common used antibiotic for empiric therapy was ceftriaxone (20 cases), followed by ampicillin and amoxicillin-clavulanic acid (12 cases each) as shown in Table 3.

Escherichia coli (45% cases) were the most common ESBL-producing organisms isolated from the study population, followed by *Klebsiella* spp. and *Pseudomonas* in 18.3% cases each. Organisms isolated from different body fluids are shown

Table 1: Age group – sex distribution

Age group	Number of patients		Total (%)
	Female (%)	Male (%)	
0-28 days	6 (10)	7 (12)	13 (22)
29 days-1 year	9 (15)	8 (13)	17 (28)
>1-6 years	7 (12)	4 (7)	11 (19)
>6-18 years	8 (13)	11 (18)	19 (31)
Total	30 (50)	30 (50)	60 (100)

Table 2: Distribution of clinical presentation according to age group

Age group	Fever	Dysuria	Loose stools	Constipation	Voiding difficulty	Vomiting	Pain abdomen	Cough	Reduced feeding
0-28 days	2	1	Nil	Nil	Nil	4	Nil	Nil	12
29 days-1 year	4	2	5	Nil	1	8	1	6	4
>1 year-6 years	10	2	2	1	3	2	4	2	3
>6 years-18 years	11	3	Nil	3	Nil	8	7	5	2
Grand total	27	8	7	4	4	22	12	13	21

in Table 4, and their sensitivity to different antibiotics is shown in Table 5.

In our study, antibiotics were given for following duration depending upon the clinical diagnosis and severity of infection: Culture positive urinary tract infection (UTI) was treated for 14 days, septicemia for 10 days, bacillary dysentery for 7 days, and others such as acute suppurative otitis media/chronic suppurative otitis media for 5 days. In pneumonia, of 12 patients, 11 were treated for 10 days while 1 was treated for 14 days due to associated acute respiratory distress syndrome (day 6).

Table 3: Empirical antibiotics used in the study is shown in the following table

Empirical antibiotics	Frequency (%)
Ampicillin	12 (20.0)
Ceftriaxone	20 (33.3)
Co-amoxiclav	12 (20.0)
Amikacin	4 (6.7)
Cefotaxime	3 (5.0)
Piperacillin/tazobactam	8 (13.3)
Meropenem	1 (1.7)
Total	60 (100.0)

DISCUSSION

The minimum number of patients (18.3%) belonged to the age between >1-6 years, and the maximum number of patients belonged to the age of >6-18 years (31.7% cases). There was equal incidence of infection with ESBL-producing organisms in both the sex. However, the study conducted by Sehgal et al. [8] showed male preponderance and premature babies as a major risk. Another study conducted by Patel et al. [9] showed female preponderance and age group of >1-5 years as the common age group affected.

E. coli was the most common ESBL-producing organism isolated from the study population which accounted for 45%.

Table 4: Organisms isolated in the culture samples

Culture sample	Organism isolated							Total (%)
	E. coli (%)	*Klebsiella* species (%)	*Pseudomonas aeruginosa* (%)	*Proteus* species (%)	*Enterobacter* species (%)	*Acinetobacter* species (%)	*Moraxella* species (%)	
Urine	16 (26.7)	1 (1.7)	0	4 (6.7)	0	0	0	21 (35)
Blood	6 (10)	4 (6.7)	0	0	2 (3.3)	1 (1.7)	0	13 (21.7)
ET secretion	2 (3.3)	0	8 (13.3)	0	1 (1.7)	0	1 (1.7)	12 (20)
Sputum	1 (1.7)	6 (10)	0	0	0	0	0	7 (11.7)
Pus	0	0	2 (3.3)	0	0	2 (3.3)	0	4 (6.7)
Stool	2 (3.3)	0	0	0	0	0	0	2 (3.3)
PICC line tip	0	0	1 (1.7)	0	0	0	0	1 (1.7)
Total	27 (45)	11 (18.3)	11 (18.3)	4 (6.7)	3 (5)	3 (5)	1 (1.7)	60 (100)

***E. coli*: *Escherichia coli*, PICC: Peripherally inserted central catheters**

Table 5: Antibiotic sensitivity pattern of different organisms isolated in culture specimen

Antibiotics	*E. coli* (n=27)	*Klebsiella pneumoniae* (n=5)	*Klebsiella oxytoca* (n=6)	*Pseudomonas aeruginosa* (n=11)	*Proteus mirabilis* (n=4)	*Enterobacter* species (n=3)	*Acinetobacter lowfii* (n=2)	*Acinetobacter bowmonii* (n=1)	*Moraxella* (n=1)
Amikacin	25	3	4	9	3	3	2	1	1
Ampicillin	6	1	1	2	1	-	-	-	-
Cefotaxime	5	2	1	3	1	-	-	-	-
Cefuroxime	4	2	1	1	1	-	-	-	-
Imipenem	26	4	4	8	4	2	1	-	1
Nitrofurantoin	23	2	2	6	3	2	1	-	1
Piperacillin/tazobactam	24	4	4	7	2	2	2	1	-
Cephalexin	5	1	1	3	1	-	-	-	-
Amoxicillin/clavulanic acid	3	1	2	2	1	-	-	-	-
Aztreonam	9	2	2	5	2	1	1	-	-
Ceftazidime	7	2	1	2	1	-	-	-	-
Ciprofloxacin	6	1	1	2	1	-	-	-	-
Meropenem	24	5	4	9	4	3	2	1	1
Norfloxacin	19	2	3	7	3	2	1	-	-
Trimethoprim/ sulfamethoxazole	18	3	4	7	3	2	1	1	1

E. coli*: *Escherichia coli

Table 6: Duration of antibiotic depending upon clinical diagnosis

Clinical diagnosis	Average days of antibiotic given			Total
	0-5 days	6-10 days	11-14 days	
UTI	0	0	20 (33.3%)	20 (33.3%)
Septicemia	0	21 (35%)	0	21 (35%)
Pneumonia	0	11 (18.3%)	1 (1.7%)	12 (20%)
Bacillary dysentery	0	3 (5%)	0	3 (5%)
Others	4 (6.7%)	0	0	4 (6.7%)
Total	4 (6.7%)	35 (58.3%)	21 (35%)	60 (100%)

UTI: Urinary tract infection

Table 7: Comparison between change in empirical antibiotic and outcome

Change in empirical antibiotic	Outcome		Total
	Frequency (%)		
	Death	Discharged	
No	2 (18)	9 (82)	11
Yes	3 (6)	46 (94)	49
Total	5 (8)	55 (92)	60
Pearson Chi-square	1.710		
p value	0.191 not significant		

The next common organisms were *Klebsiella* species and *Pseudomonas* accounting to 18.3% each. However, according to study conducted by Patel et al. [9], the most common isolate was *E. coli* (58.9%) followed by *Klebsiella* species (16.1%) and *Enterococcus* species (10.7%). Tumbrels et al shows that majority of infections caused urinary tract infections and lower tract infections [10].

Ceftriaxone was the most commonly used empirical antibiotic in our study group which accounted for 33.3%. This is according to an update by Paterson and Bonobo [9] due to the high rates of UTI in the study group. However, on the contrary to their recommendations [11] to start carbapenem as the 1st-line drug for ESBL infections other than UTI, piperacillin/tazobactam (47.3%) and meropenem (27%) were used as per the culture and sensitivity reports in our study population. Only 23% of the study population was subjected to therapy with amikacin, which was initiated in case of deterioration with the use of other antibiotic regimen. Majority of the patients had therapy for 6-10 weeks, which was reported to be nearly 58% of the total study population and 81.7% had to use 2 antibiotics. However, mean duration of antibiotic therapy was 12.5 days in study conducted by Sehgal et al. [8].

In our study, duration of antibiotics was based on clinical diagnosis and severity of infection as shown in Table 6.

In our study, 18% died and 82% got discharged in patients where no change of empirical antibiotic was done, while 6% died and 94% got discharged in those patients where change in empirical antibiotic was done as shown in Table 7. According to a study conducted by Sehgal et al. [8], mortality rate was 23.6%. The association of change in antibiotic to mortality was found to have significance. The change in antibiotic in cases of deterioration of the illness showed a non-significant decrease in mortality (p=0.191). Hence, it is recommended to change the antibiotics for getting better outcome.

CONCLUSION

The most common ESBL-producing organism isolated from the study population was *E. coli* followed by *Klebsiella* species and *Pseudomonas*. The most commonly used empirical antibiotic in our study group was ceftriaxone. Piperacillin/tazobactam and meropenem were used as per the culture and sensitivity reports. In case of deterioration with the use of other antibiotic regimen, amikacin was initiated.

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