ORIGINAL ARTICLE

THE CONTINUING MENACE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBLS) - A CENTRE BASED STUDY

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ABSTRACT

Background: Extended-spectrum β-lactamases (ESBLs) pose a major therapeutic challenge today in the management of hospitalized and community acquired infections. They belong to a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes. They can complicate the treatment strategies of a wide range of infections from uncomplicated urinary tract infections to life-threatening sepsis.

Objective: To determine the frequency of Extended-spectrum β-lactamase (ESBL) producing gram negative bacteria (GNB) and their sensitivity patterns in a tertiary care hospital.

Methods: In this cross-sectional study, conducted in the Microbiology section of Pathology laboratory, various specimens such as blood, body fluids, urine, and pus were included during a period starting from October 2020 till September 2021. The samples showing growth of gram negative bacteria were processed further for determination of ESBL production. After isolation, antimicrobial susceptibility pattern was determined for each isolate.

Results: GNR were isolated from 130 samples, among those 46 (35.4%) were labelled as ESBL. The commonest ESBL producing bacteria was *E. coli*, followed by *Pseudomonas aeruginosa* found in 17.39% samples. In *E. coli*, Imipenem (IPM) showed highest sensitivity of 84.8%, other sensitive drugs were Amikacin (AK), gentamicin (CN), and Tazobactam-piperacillin (TZP). For *Pseudomonas aeruginosa*, Imipenem and Tazobactam-piperacillin showed sensitivity of 75%, AK and CN were 50% sensitive. Against *Klebsiella pneumoniae*, IPM was 100% sensitive, AK and TZP were 85.7% sensitive. IPM, CN, and AK were 100% sensitive against *Morganella morganii*. AK and CN were 100% sensitive against *Citrobacter species*.

Conclusion: It was concluded that, there was significant prevalence of ESBL producing strains amongst Gram negative bacterial infections. *E. coli* and *Pseudomonas aeruginosa* were the commonest ESBL producers. IPM, AK, and TZP were sensitive against majority of the ESBL producing GNB.

Keywords: Gram negative bacteria, ESBL, Antimicrobial susceptibility.

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INTRODUCTION

The mainstay of treatment for managing bacterial infections is antibiotics. However, in recent years, upsurge due to injudicious use of antibiotics has caused the emergence of resistant bacterial strains.[1] This increase in antibiotic resistance is now a global health issue. Annually, 0.7 million

people die only because of being infected with drug resistant bacterial agents and this number will increase to 10 million by the year 2050.[2]

β-lactam antibiotics are widely used for managing bacterial infections and these account for more than 65% of the total antibiotic usage.[3] These β-lactam antibiotics are divided into six groups on the basis of chemical structure of β-lactam ring and they bind to Penicillin binding protein (PBP) and inhibit its synthesis. This PBP has very important role in bacterial cell wall synthesis, so inhibition ultimately leads to cell death.[4] Resistance against β-lactam antibiotics is very common and production of β-lactamase enzyme has gained great importance and is the matter of debate since last few years. β-lactamases are produced by gram negative as well as positive bacteria and functions by inactivating the β-lactam antibiotics by binding to their carbonyl portion and by hydrolyzing the β-lactam ring.[5]

Various classes of β-lactamases are produced by bacteria and amongst these, Extended-spectrum β-lactamase (ESBL) is very important, because it breaks down the β-lactam ring of many antibiotics such as Aztreonam, Penicillin, and all generation of Cephalosporins, while Carbapenems, Cephamycin and Moxalactam are spared.[6,7] Taghizadeh et al. reported that, ESBL producing bacteria are even capable of generating resistance to other classes of antibiotics as well.[8]

The infections caused by ESBL producing organisms are becoming common day by day and have become a major worldwide health threat. Most of these organisms are gram negative bacteria (GNB), which are responsible for several community and hospital acquired respiratory, blood stream, wound and urinary tract infections.[9,10] Therefore recognition of spectrum of these ESBL producing GNBs and their susceptibility pattern is necessary for selection of appropriate antibiotics. The objective of this study was to determine the prevalence of ESBL producing GNBs &their sensitivity patterns in a tertiary care hospital.

METHODS

In this cross-sectional study various specimens like blood, body fluids, urine and pus were included and processed in the microbiology section of a Azra Naheed Medical College and Sharif Medical College, Lahore. Specimens were processed according to standard guidelines and those having growth of GNB were separated for further processing. For microbial detection, the samples were inoculated on Blood and MacConkey agar at an incubation temperature of 37 °C for 24 hours. Growths were reported as positive if bacterial count was $>10^4$ cfu/ml. After that, gram staining and biochemical testing was performed for final diagnosis.

The Gram-negative bacteria were isolated and underwent further testing for determination of ESBL production using the Double Disc Synergy Test (DDST) method according to CLSI guidelines 2021.[11] All those strains having an increase in zone diameter of ≥5 mm in the clavulanic acid disc, in comparison to standard disc were labelled as ESBL producing strains.[12]

The ESBL producing strains were further processed for antimicrobial susceptibility testing. The antibiotics applied were Amoxicillin-Clavulanic acid (AMC), Piperacillin-Tazobactam (TZP), Cefuroxime (CXM), Cefixime (CFM), Ceftazidime (CAZ), Ceftriaxone (CRO), Imipenem (IPM), Amikacin (AK), Gentamycin (CN), Tetracycline (TE), Ciprofloxacin (CIP), Levofloxacin (LEV) and Trimethoprim/Sulfamethoxazole (SXT). Sensitivity of drugs was determined as per CLSI guidelines. All information was entered in Microsoft Excel. Frequency and percentage was used to represent the ESBL producing bacteria and their susceptibility pattern.

RESULTS

Out of 130 Gram negative bacteria, 46 (35.4%) turned out to be ESBL producers. The commonest ESBL producing bacteria was *E. coli*, followed by *Pseudomonas aeruginosa* found in 17.39% samples, *Klebsiella pneumoniae* in 7 (15.22%), *Morganella morganii* in 1 (2.17%) and *Citrobacter* in 1 (2.17%) sample (Figure: 1).

Regarding antibiotic sensitivity, IPM, AK, and TZP were sensitive in most of the bacteria. While TE, CIP, LEV and SXT were almost resistant to all organisms (Table: 1).

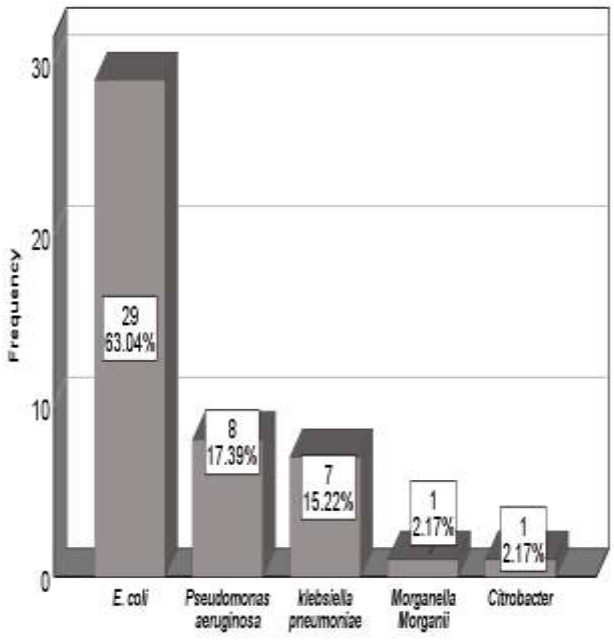

Figure 1: Frequency of ESBL Producing GNBs

Table 1: Antibiotic Susceptibility Pattern of Isolated Organisms

Antibiotics	*Overall Sensitivity*	*E. coli (N=29)*	*Pseudomonas aeruginosa (N=8)*	*Klebsiellapn eumoniae (N=7)*	*Morganellam organii (N=1)*	*Citrobacter* (N=1)
Co-amoxiclav (AMC)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tazobactam-piperacillin (TZP)	27 (58.7%)	15 (51.7%)	6 (75%)	6 (85.7%)	0 (0.0%)	0 (0.0%)
Ceftazidime (CAZ)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ceftriaxone (CRO)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cefuroxime (CXM)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cefixime (CFM)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Imipenem (IPM)	39 (84.8%)	25 (86.2%)	6 (75%)	7 (100%)	1 (100%)	0 (0.0%)
Amikacin (AK)	31 (67.4%)	19 (65.5%)	4 (50%)	6 (85.7%)	1 (100%)	1 (100%)
Gentamycin (CN)	23 (50%)	14 (48.3%)	4 (50%)	3 (42.9%)	1 (100%)	1 (100%)
Tetracycline (TE)	8 (17.4%)	3 (10.3%)	1 (12.5%)	4 (57.1%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin (CIP)	5 (10.9%)	2 (6.9%)	3 (37.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Levofloxacin (LEV)	10 (21.7%)	4 (13.8%)	4 (50%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
Co-trimoxazole (SXT)	9 (19.6%)	7 (24.1%)	0 (0.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)

DISCUSSION

The diagnosis of ESBL producing bacterial infections is of prime importance because of the narrow therapeutic options available for these infections. The prevalence of these organisms varies from region to region, being highly prevalent in Asia. In this study, the prevalence of ESBL producing organisms was 35.3% (46/130) among all Gram-negative bacterial infections. A study conducted in Iran by Gharavi et al. reported 28.28% ESBL amongst GNB from Urinary samples.[13] A study by Nanoty reported ESBL producing bacterial infections rate of 30.23%.[14] While a study from Bangladesh reported 16.07 % of ESBL producing GNB.[15] Whereas other studies from Bangladesh reported ESBL GNB prevalence rate ranging from 23 to 24.8%.[16,17] Another study from Yousef pour et al. reported 55.4%,[18] which is much higher than above mentioned figures. These reported differences can be due to varying geographic regions in these studies.

In current study, *E. Coli* was the commonest ESBL producing GNB (63.04%), followed by *pseudomonas aeruginosa* in 17.39% cases, *Klebsiella pneumonia* in 15.22% cases, while *Citrobacter* and *Morganella morganii* were found only in 2.17% cases. Similar results were reported by Gharavi et al who reported *E. Coli* in 35.7% cases, *Klebsiella* Species in 22.7% cases, *Citrobacter* species in 4.34% cases.[13] Other studies have also reported *E. Coli* to be the commonest ESBL producing organism[19] which is contrary to the results of present study.

On determination of antibiotic susceptibilities, *E. Coli* showed highest sensitivity of 84.8%, against IPM, other sensitive drugs were AK, CN, and TZP. For *Pseudomonas aeruginosa* IPM and TZP showed sensitivity of 75%, AK and CN were 50% sensitive. Against *Klebsiella pneumoniae* IPM was 100% sensitive, AK and TZP were 85.7% sensitive. IPM, CN and AK were 100% sensitive against *Morganella morganii*. AK and CN were 100% sensitive against *Citrobacter* species.

While a study by Shakibaie et al. reported 100% sensitivity of IPM and MEM against *E. Coli* and AK was 94.4% sensitive.[20] Another study showed 96.2% sensitivity of IPM, and 85.1% of AK against E. coli.[21] Jobayer et al. reported IMP and MEM are highly sensitive against *Pseudomonas aeruginosa*. And they reported 100% sensitivity of IPM, MEM, CIP and TZP against *Klebsiella* species.[15]

The major limitation of this study is sample size as we included that data of only 46 ESBL producing bacteria. And some of the bacteria such as *Morganella morganii* and *Citrobacter* species were only one in number, so their sensitivity to different antibiotics cannot be reported clearly.

CONCLUSION

This study concluded significant prevalence of ESBL producers amongst Gram negative bacterial infections. *E. Coli and Pseudomonas aeruginosa* were the commonest organisms among ESBL producing GNB. Considering the susceptibility profiles, IPM, AK, and TZP were sensitive against majority of the ESBL producing GNB and can be considered as empiric treatment options.

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AUTHORS' CONTRIBUTION

SI: Conception, planning of research, Data Collection, Manuscript writing.
KJ:SM: Critically revision
SI: References research, Discussion, Proof reading
SI: Reference research, Proof reading
SH: Data collection
MA: Data Collection, Proof reading