LAB RESEARCH ARTICLE

Determination of epidemiology and antimicrobial susceptibility of extended spectrum beta lactamase producing uropathogens

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Abstract

Objective: To find the most suitable antibiotic against urinary tract infection caused by Extended Spectrum Beta Lactamase producing uropathogens, and the epidemiology of Extended Spectrum Beta Lactamase producers.

Methods: The cross-sectional study was conducted at Pir Mehr Ali Shah University of Arid Agriculture, Rawalpindi, Pakistan, from July 2014 to July 2015, and comprised urine samples of patients suffering from urinary tract infection which were cultured on Cysteine Lactose Electrolyte Deficient agar medium. Analysis was done on Muller-Hintonagar plates and optical density was set as 0.1 at 530nm. Antimicrobial sensitivity was tested using Kirby-Bauer disc diffusion method. Further confirmation was done through gram staining and biochemical tests. Extended Spectrum Beta Lactamase production was confirmed through phenotypic methods, including phenotypic confirmatory disc diffusion test, double disc synergy test and Epsilometer test.

Results: Of the 150 samples, 98(65%) showed growth of a total of 114 pathogenic isolates. Escherichia coli was the commonest organism in 94(82%) samples. Piperacillin Tazocin was the most suitable antimicrobial drug in 88(90%) cases. Overall, 23(20%) isolates were producers of Extended Spectrum Beta Lactamase.

Conclusion: Piperacillin Tazocin was found to be the drug of choice for patient suffering from urinary tract infection.

Keyword: ESBL, UTI, Piperacillin Tazocin, PTZ,AGNB. (JPMA 69: 690; 2019)

Introduction

Urinary tract infections (UTIs) are ordinary and commonly-occurring serious morbidity, which affects not only all segments of the population, but also leads to rise in antimicrobial resistance with persistent and poor disease management.[1] UTI is caused almost exclusively by a wide range of bacteria. Examination of bacterial spectrum and antibiotics sensitivity is necessary for empirical treatment of nosocomial UTIs. Antibiotic resistance in bacteria is a dominant theme throughout the world, both in hospitals and in the community as well. Empirical treatment of nosocomial UTIs consists of examination of bacterial spectrum and antibiotics sensitivity testing.[1] All over the world, resistance against beta-lactam antibiotics is increasing due to Extended Spectrum Beta Lactamases (ESBLs) and Amp-c beta-lactamase production. Carbapenemases are plasmid-encoded and has reduced the activity of all penicillins, monobactams, cephalosporins and carbapenems. This resistance is not only for beta-lactam antibiotics, but also extends to other antibiotic classes through the same plasmid-carrying resistance genes and infection of these bacteria may result in therapeutic failure.[2] Beta lactamases cause resistance to beta-lactam agents and are produced by different aerobic gram-negative bacteria (AGNB).[3] Hospitals and nursing homes are found as the main reservoirs of ESBLs producing bacteria. ESBLs were discovered in 1980. Main reservoirs for these resistant organisms are hospital patients.[4] ESBLs producing uropathogens cause serious nosocomial infections and belong to the enterobacteriacae family. ESBLs producing organisms are not properly treated with cephalosporins and other classes of antibiotics and mostly result in treatment failure.[5,6] In 1980, when the third generation of cephalosporins was introduced, a relief in the fight of beta-lactamase resistance to antibiotics appeared. Third-generation cephalosporins were produced due to increased resistance by beta-lactamases and were found to be effective against gram-negative bacteria such as Escherichia (E.) coli, Klebsiella (K.) pneumoniae, Proteus Spp. and Pseudomonas (P.) aeruginosa. These organisms produce chromosomal or plasmid mediated β-lactamases. Cephalosporins contain beta-lactam ring in their outer structure which is cleaved by beta-lactamases. Frequent and unadvised use of third-generation cephalosporins resulted in mutations in β-lactamases.[6] Most common members of beta-lactamase family are TEM-1, SHV-1, and TEM-2. Commercially available beta-lactamase inhibitors i.e., calvlanic acid and sulbactam or tazobactam, inhibit the ESBLs (TEM and SHV family) to some extent.[7,8] Sulbactum or

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clavulanic acid are used as ESBL inhibitors. However, inhibitor-resistant ESBLs are produced due to porin loss and Amp-c lactamase overproduction.[9] Efficacy of antibiotics depends on the location and severity of infection, kidney function, presence of implants, liver function, and geographical resistance. It is also observed that lactation and age of pregnancy also affect the efficacy of antibiotics.[9,10] Since beta-lactam antibiotics are still widely used, the production of beta-lactamase producers have become a serious issue. Beta-lactamase production, efflux mechanisms, Amp-C lactamases and loss of porin by gram-negative bacteria is the cause of resistance and have become a serious concern.[10]

The current study was planned to find the most suitable antibiotic against UTI caused by ESBL-producing uropathogens, and to see the epidemiology of ESBL producers.

Materials and Methods

The cross-sectional study was conducted at the Microbiology and Biotechnology Laboratory, Department of Biochemistry and Biotechnology, Pir Mehr Ali Shah University of Arid Agriculture, Rawalpindi, Pakistan, from July 2014 to July 2015, and comprised urine samples of UTI patients. Conventional techniques were employed for the identification of urine samples symptomatic of UTI. The samples were collected from urine plastic bags, transurethral catheterisation, midstream clean catch or aspiration from supra-pubic region according to clinical status and age of patients. The samples included were of patients aged 15-60 years who were either indoor patients or those visiting the emergency department (ED) and the outdoor patients department (OPD) with pyuria on urine routine examination. The samples were processed for culture on Cysteine Lactose Electrolyte Deficient (CLED) agar by incubating at 37°C for 24 hours). Bacterial cultures showing growth were processed for sub-culturing, biochemical testing and gram staining for identification. Kirby-Bauer disc diffusion method was used for antimicrobial sensitivity testing and Double Disc Synergy Test (DDST), Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and Epsilometer (E) test was used for the detection of ESBL production.

Morphological characteristics, biochemical tests, gram staining, and specific disc pattern were used for Aerobic Gram-Negative Bacteria (AGNB) (E. coli, K. spp. P. aeruginosa, Proteus spp.) identification. Different biochemical tests were performed for the confirmation of AGNB colonies. Each biochemical test was done with known controls.[11]

Indole Production (IP) test was done in which bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme tryptophanase. Ehrlich reagent containing an aldehyde was used to detect indole production. Indole reacts with aldehyde to give a red colour. E.coli was indole-positive and P. aeruginosa was indole-negative.[11]

Methyl Red-Voges Proskauer (MR-VP) test was used for the identification of fermentive bacteria (e.g. Enterobactericeae).These bacteria ferment glucose into mixed acids or butylenes-glycol which is detected by the appearance of red colour by using methyl red as indicator. K. spp. was MR-negative and VP-positive while E.coli was MR-positive and VP-negative.[11]

Citrate utilisation test was performed to determine the ability of bacteria to utilise sodium citrate as its only carbon source. Negative control was E.coli, American Type Culture collection (ATCC) 25922 and positive control was K. pneumoniae.[11]

Oxidase test was done to identify bacteria that produce cytochrome c oxidase. Appearance of purple colour indicated oxidase production. Negative control was E. coli ATCC 25922 and P. aeruginosa ATCC 27853 was positive control.[11]

Analytical Profile Index (API) 20E was used for non-conclusive samples and API chart was used for interpretation. For the identification of Enterobacteriacae, API 20E provided the standardised identification system that has 20 biochemical parameters. A suspension equal to 0.5 McFarland Index of isolates was prepared and carefully emulsified for homogeneous bacterial suspension preparation. Bacterial suspension was charged into strip wells, and tubes were filled according to the test protocol. Incubation of hydrogen sulphide (H2S), arginine dihydrolase (ADH), lysine decarboxylase (LDC), urea and ornithine decarboxylase (ODC) tests were conducted after overlaying them with mineral oil. From the suspension, a purity plate was prepared to check the quality of suspension. Reagents were added in respective wells 18-24 hours after incubation at 35±2°C. Seven-digit numerical code was determined from positive results and was looked up in API code chart for organism identification.[12]

Isolates showing resistance to 3rd generation cephalosporins, namely ceftazidime, ceftriaxone and cefotaxime, were further screened by phenotypic methods for β-lactamase production. Clinical Laboratory

and Standards Institute (CLSI) guidelines were followed during the screening.[12]

PCDDT was done by using a combination of ceftazidime (30 mcg) and clavulanic acid (10 mcg). Muller-Hinton (MH) agar plates were inoculated by respective culture, and incubated at 37°C for 24 hours. ESBL production was confirmed by increase in zone diameter of more than 5mm of ceftazidime towards clavulanic acid.[12]

DDST was done by using five antimicrobials, namely amoxicillin-clavulanic acid having quantity of 20mcg and 10mcg respectively, ceftazaidime (30mcg), aztreonam (30mcg), ceftriaxone (30mcg) and cefotaxime (30mcg). A distance of 1.5cm was maintained between these discs having amoxicillin-clavulanic acid disc in the centre. Development of the zone of inhibition towards the clavulanate disc after 24 hours at 37°C incubation indicated ESBL-positive isolates.[13]

Further confirmation of ESBL production was done through E-Test by using Multi-Enzyme (Ezy) Strips. Conventional E-strips differ from these in that they contain a gradient of 3 antibiotics on either side with or without clavulanic acid respectively instead of one antibiotic. These multi-Ezystrips have cefotaxime, cefipime and ceftazidime noted as minimum inhibitory concentration (MIC) side on one side in a two-fold gradient and the same antibiotics with clavulanic acid noted as MIC+ on the other side. A ratio of inhibition zones of MIC and MIC+ for ≥8mm was considered positive E-test. E-test strips were applied on MH agar after inoculation with test organism. The plates were incubated for 16-18 hours at 37°C. K. pneumoniae ATCC 700603 was used as the positive control and E.coli ATCC 25922 was used as the negative control throughout the study.[12,13]

Disk diffusion using Kirby-Bauer method was used to study antimicrobial susceptibility testing (AST), as described by CLSI.[14] Antibiotic disc concentrations varied for AST: oxytetracycline (30 mcg), ticarcillin (85 mcg), cefipime (30 mcg), cefuroxime (30 mcg), ceftriaxone (30 mcg), norfloxacin (10 mcg), nalidixic acid (30 mcg), cefadroxil (30 mcg), amoxycillin (30 mcg), ceftazidime (30 mcg), polymixin-b (300 mcg) cefoperazone (75 mcg), co-trimoxazole (25 mcg), ampicillin (20 mcg), piperacillin (100 mcg), chloramphenicol (30 mcg), cefotaxime (30 mcg), ceftizoxime (30 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), tetracycline (30 mcg), amikacin (30 mcg), gatifloxacin (10 mcg) and gentamicin (10 mcg). Standard quality control strain E. coli ATCC 25922 was used.[14]

Results

Of the 150 samples, 98(65%) showed growth of a total of 114 pathogenic isolates. Female patients had more UTI as compared to males. Out of these 98 samples, 58(59%) samples were of female patients and 40(41%) were of male patients. There was more UTI in nosocomial infections 56(57%) than in outpatients 42(43%).

E. coli was the commonest organism in 94(82%) samples

Table: Overall sensitivity pattern.

Antibiotics	Sensitive	Intermediate	Resistant
Tazocin (TZP)	90%	05%	05%
Imepenem (IMI)	89%	8.5%	2.5%
Meropenem (MRP)	77 %	12%	11%
Amikacin (AK)	75%	10 %	15%
Nitrofurantoin (F)	74%	06%	20%
Meropenem (MRP)	72 %	18%	10%
Polymixin B (PB)	66%	10%	24%
Vancomycin (VAN)	62%	20%	18%
Chloramphenicol (C)	50%	30%	20%
Gentamycin (CN)	45%	05%	50%
Tobramycin (TOB)	26%	25%	49%
Fosfomycin (FOS)	25 %	10%	65%
Ceftazidime (CAZ)	22%	08%	70%
Ceftriaxone (CRO)	21%	10%	69%
Ciprofloxacin (CIP)	19%	11%	70%
Ampicillin (AMP)	18%	20%	62%
Sulphmethazole (SXT)	15%	5%	80%
Augmentin (AUG)	05%	2%	93%

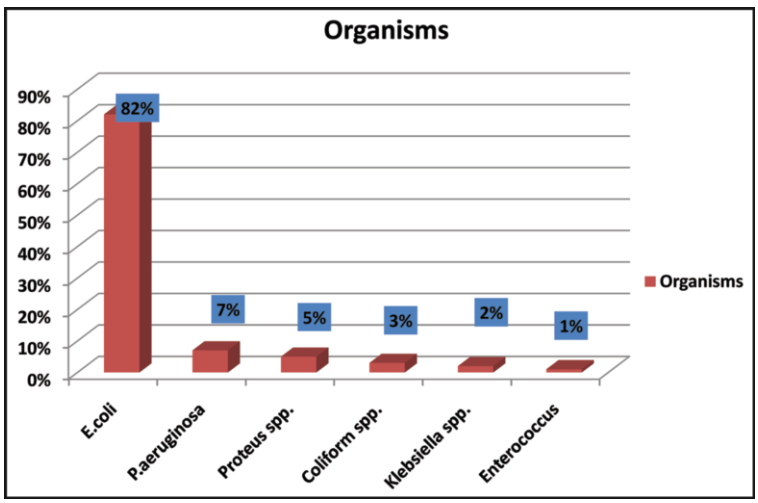

Figure: Distribution of Extended Spectrum Beta Lactamase (ESBL) Producing Isolates.

(Figure). Piperacillin Tazocin (PTZ) was the most suitable antimicrobial drug in 88(90%) cases (Table). Overall, 23(20%) isolates were found to be ESBL producers.

Discussion

One of the greatest achievements of the modern world was the development and discovery of antibiotics. Hospital stay of patients has increased due to bacterial resistance to antibiotics which has resulted in increased economic burden.[15] In the current study, most ESBL-producing organisms were obtained from in-patients. Nosocomial infection could be the reason of this increased ratio of ESBL-producing pathogens in in-patients than OPD and ED patients. Susceptibility to infection is increased in patients having prosthetic devices, continuous exposure to the hospital environment and the frequent use of antibiotics.[16]

A study demonstrated similar findings. E. coli was the commonest organism in the current study, followed by P. aeruginosa, Proteus spp., coliform, K. spp. (2%) and Enterococcus (1%). Micro-organism dominance varies from one environment to another. Standards of hygiene and geographical locations could be the possible cause of this attribute.[17] Frequent and inadvisable use of antibiotics in the current study population could be the possible cause of high level of resistance to co-amoxiclav. The use of these prescribed drugs in our settings is questionable because the prescription of these drugs is common.[18] Because these are more affordable than other antibiotics, therefore these are widely used. AGNB are mostly treated with fluoroquinolones, so the ciprofloxacin resistance is an early warning sign.[19] In our country, irrational and indiscriminate antibiotic usages and lack of antibiotic policy at all treatment levels are the main contributory factors towards growing antimicrobial resistance. Because of these resistant bacteria, morbidity, mortality and cost of treatment have considerably risen.[20]

In many cases, treatment failure is seen because some Microbiology laboratories sometimes erroneously detect microorganisms to be sensitive to any of the third-generation cephalosporins. Therefore, two more antibiotics are added in the ESBL detection pattern of PCDDT. Every laboratory where molecular methods of ESBL detection are not available should confirm ESBL detection through PCDDT, DDST and E-test.[21]

In UTI patients, ESBL production is a common phenomenon and to determine the ESBL producers in a certain setup, screening by PCDDT, DDST and E-test are good and valuable tools. Hygienic conditions and proper washing of hands can still reduce the spread of ESBL. Wherever possible, prolonged stay in hospitals should be avoided. As early as possible all prosthetics, cannulas, catheters and needles should be removed from the body. To avoid misuse and overuse of antibiotics, people should be educated about the dangerous effects of frequent and inadvisable use of antibiotics, nosocomial infections and proper hygienic conditions. A proper policy about antibiotics usage and infection control at hospitals should be formulated. Institutional studies will also help to improve understanding of the resistance pattern of AGNB in a certain geographical location which male to the formulation of an effective policy related antibiotics usage to treat these infections.[21]

Conclusion

Piperacillin Tazocin was found to be the drug of choice for patients suffering from UTI. E. coli was the most common ESBL producer.

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