ORIGINAL ARTICLE

Susceptibility Pattern of Extended Spectrum ß-Lactamase Producing Isolates in Various Clinical Specimens

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ABSTRACT

Objective: To determine the susceptibility pattern of extended spectrum ß-lactamase (ESBL) producing Gram negative isolates from various clinical specimens.

Study Design: Descriptive study.

Place and Duration of Study: Microbiology Department, Armed Forces Institue of Pathology, Rawalpindi, from January 2008 to January 2009.

Methodology: A total of 308 ESBL producing isolates from various clinical specimens sent to AFIP for culture and sensitivity were identified using standard microbiological techniques and tested for antimicrobial susceptibility. At the same time screening for ESBL production was also done. ESBL production was confirmed by combination disc synergy method. The susceptibility pattern of isolates was then recorded in frequency percentages.

Results: Out of the 308 ESBL producing isolates more than 99% were susceptible to carbapenems, 84% to tazobactam/piperacillin, 81% to sulbactam/cefoperazone, 12% to fluoroquinolones, 13% to cotrimoxazole, 59% to amikacin and 18% to gentamicin. Among the urinary isolates 49% were susceptible to Nitrofurontoin and only 5% to Pipemidic acid.

Conclusion: Antibiotic choices in case of ESBL producing isolates are limited and at present only carbapenems can be regarded as treatment of choice. As empirical agents, beta-lactam/beta lactamase inhibitor combinations should be used cautiously for serious infections. Fluoroquinolones showed very poor efficacy. Amikacin can be used alternatively in such cases. Nitrofurantoin is still a good oral agent for treating UTI.

Key words: *Antibiotic susceptibility. Combination disc synergy. ESBLs. Gram negative rods.*

INTRODUCTION

The development of antibiotic resistance limits the choice of antibiotics to be used. Widespread irrational antibiotic usage is leading to a greater trend towards antibiotic resistance. Lack of local antibiotic policy in most of the settings is further exerting a selective antibiotic pressure selecting out resistant strains. As a result resistant strains are now prevalent everywhere particularly in intensive care settings. One cannot continue to rely upon an alternative when resistance develops to one agent as risk of an outbreak by strains resistant to all known antibacterial agents is always there. Such multi-drug resistant strains have been consistently reported in recent years from several setups across the globe.[1]

Amongst antibiotics, ß-lactams are the safest and the most widely used antibiotics to date.[2] ß-lactam drug resistance is mainly mediated through ß-lactamases. These enzymes are chiefly produced by Gram negative bacteria. Gram negative bacterial resistance was countered by developing newer and higher generation ß-lactam antibiotics. In the mid 1980s, a new group of enzymes, extended spectrum ß-lactamases (ESBL) were discovered, conferring resistance against the extended spectrum cephalosporins and monobactams. The mechanism of resistance is plasmid mediated that are easily transferred among Enterobacteriaceae and thus contributing to ESBL dissemination.

The extended spectrum ß-lactams (Aminopenicillins and third generation Cephalosporins) are commonly used empirically for the treatment of Gram negative sepsis. But the emergence of ESBL producing organisms has posed a serious threat for their continuing use.[3] Nosocomial outbreaks caused by ESBL producing organisms have consistently been reported.[1] Multiple genes for ESBL in a single strain complicate detection as well as treatment. ESBL producing bacteria are also associated with multi-drug resistance i.e. resistance to other classes of drugs like quinolones, aminoglycosides and trimethioprim-sulphamethoxazole.

One of the methods to combat resistance is to have an effective local antibiotic policy in which different classes of antibiotics are used periodically to reduce selective pressure. When resistance emerges against one group it is to be changed. The prevalence of ESBL producing

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organisms usually remains unknown in our setup and guidelines for empirical therapy in nosocomial infections have not yet been developed. The susceptibility pattern of ESBL isolates changes from time to time and place.

The study was undertaken to find out the antimicrobial susceptibility pattern of ESBL producing isolates recovered from various clinical specimens in our setup.

METHODOLOGY

The descriptive study was conducted at Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. A total of 308 ESBL producing isolates from various clinical specimens sent to AFIP for culture and sensitivity were studied. Non-duplicate consecutive sampling was done from January 2008 to January 2009. Repeat samples and non-ESBL producing isolates were excluded from study. Clinical specimens like blood, urine, pus, sputum, CSF, pleural fluids etc. were inoculated on blood, chocolate, MacConkey and CLED agar according to type of specimen and following 24-48 hours incubation at $35^{o}C \pm 2^{o}C$ were examined for any growth. The isolates were identified on basis of colony morphology, Gram staining, certain rapid tests like catalase test, oxidase test, motility and biochemical reactions. They were simultaneously tested for anti-microbial susceptibility by modified Kirby Bauer disc diffusion technique by inoculating onto Mueller Hinton agar (Mast diagnostics UK) according to CLSI guidelines.[4] At the same time screening for ESBL production was also done by applying ceftazidime and ceftriaxone or cefpodoxime alone onto the agar plate. Isolates resistant to these agents were confirmed for ESBL production by combination disc synergy method according to CLSI guidelines.[4] The susceptibility pattern of all ESBL confirmed isolates were then recorded. Since there was no direct involvement of patients being laboratory specimens, informed consent was not an issue. However, study was presented to laboratory ethical committee and approved.

The numerical data was entered in SPSS version 16. Mean and standard deviation (SD) were calculated for quantitative variables. Frequencies and percentages were calculated for categorical variables like susceptibility of various antibiotics against ESBL producing isolates.

RESULTS

The 308 specimens yielding growth of ESBL producing isolates were obtained from subjects ranging in age from 1 to 95 years, with the greatest number around 50 years of age, mean age was 49 years with SD of 19.5. Two hundred and four (66%) isolates were recovered from samples of male patients and 104 (34%) from female patients. The specimen from which ESBL organisms were most frequently isolated was urine (45.1%) followed by pus (30.5%) and body fluids (7.5%). Other specimens yielding ESBL producing isolates with a lesser frequency included catheter tips, body tissues, stool, blood, sputum, HVS, endobronchial washings and throat swabs (Figure 1). *Escherichia (E.) coli* (61.7%) was the most common organism isolated followed by *Klebsiella pneumoniae* (21.1%) and *Pseudomonas aeruginosa* (5.5%) (Table I).

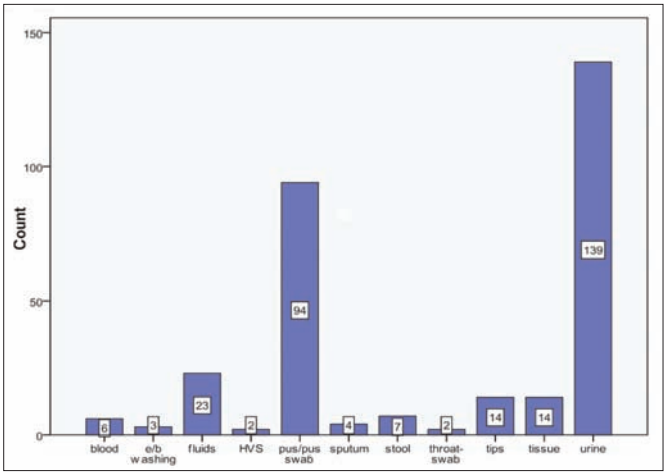

Figure 1: Distribution of ESBL producing isolates in various clinical specimens.

Table I: ESBL producing organisms isolated.

Species Isolated	Total number
Escherichia coli	190
Klebsiella pneumoniae	65
Pseudomonas aeruginosa	17
Citrobacter freundii	09
Proteus mirabilis	08
Enterobacter cloacae	06
Enterobacter agglomerans	01
Citrobacter diversus	02
Klebsiella oxytoca	04
Morganella morganii	02
Proteus vulgaris	01
Providentia alcalificians	01
Serratia marcescens	01
Serratia oderifera	01

Of the total 308 samples yielding ESBLs isolates, 123 samples were from OPD, 88 from surgical wards, 34 from medical wards and 27 from various ICUs. Other sample sources included Armed Forces Bone Marrow Transplant Centre (AFBMTC), oncology, urology and urology transplant, gynaecology and orthopaedic wards (Figure 2). Only 03 of 308 isolates were resistant to one of the carbapenems and 99% isolates were susceptible. Eighty four percent isolates were sensitive to tazobactam/piperacillin (tazocin), 81.2% to cefoperazone/sulbactam (sulzone), and 59.1% to amikacin. The rest of the antibiotics had very poor susceptibility

Table II: Antibiotic susceptibilities of ESBL producing isolates.

Antibiotics	Susceptible		Intermediate		Resistant		Not tested		Total
	No.	%	No.	%	No.	%	No.	%	
Carbapenems	305	(99.0%)	0		3	(1.0%)	0		308
Tazobactum/piperacillin	258	(83.8%)	15	(4.9%)	35	(11.4%)	0		308
Cefoperazone/sulbactam	250	(81.2%)	14	(4.5%)	44	(14.3%)	0		308
Amikacin	182	(59.1%)	13	(4.2%)	113	(36.7%)	0		308
Gentamicin	54	(17.5%)	0		254	(82.5%)	0		308
Fluoroquinolones	38	(12.3%)	3	(1.0%)	267	(86.7%)	0		308
Cotrimoxazole	37	(12.7%)	0		254	(87.3%)	17	(5.5%)	291
Nitrofurantoin	68	(48.9%)	8	(5.8%)	63	(45.3%)	169	(54.9%)	139
Pipemidic acid	7	(5.0%)	0		132	(95.0%)	169	(54.9%)	139

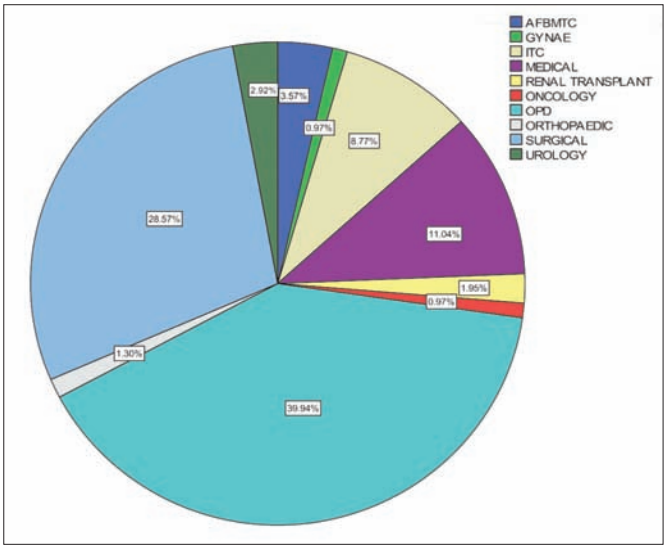

Figure 2: Percentage of sample sources of ESBL producing isolates.

profile. Only 17.5% isolates were sensitive to gentamicin, 12.7% to cotrimoxazole and only 12.3% isolates were sensitive to one of the fluoroquinolones. Antimicrobial discs of Nitrofurantoin and Pipemidic acid were additionally applied for urinary isolates. 49% of isolates were sensitive to Nitrofurantoin and only 5% to Pipemidic acid (Table II).

DISCUSSION

Multi-drug resistant (MDR) bacteria are a cause of great concern to clinicians. Not only these organisms are extremely resistant but they are also rapidly spreading. This ever growing bacterial resistance is a major hurdle in the successful treatment of both community as well as health care associated infections.[5] In our country irrational and indiscriminate antibiotic usages as well as lack of effective antibiotic policies at all levels are the main contributory factors towards growing anti-microbial resistance. Mortality, morbidity and cost of treatment have considerably risen because of these resistant isolates.[6] The ESBLs are quite prevalent in both community and nosocomial settings in our region and their incidence varies from 6.6 to 68%.[7-9] In this study, there was a male predominance as about 66% of the consecutively isolated isolates were from male patients and 34% from female patients.

ESBL producing *E. coli* was most frequently isolated followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* A high percentage of *E. coli* isolates being recovered from urine samples of OPD patients raises the suspicion that such isolates may be quite prevalent in community settings. In this regard the CTX-M type ESBL which is commonly encountered in *E. coli, K. pneumoniae* and *Salmonella* species from community needs to be looked into. Currently studies are lacking in our country regarding this aspect. A report from Pakistan also shows CTX-Mtype ESBL producing isolate.[10] Regional studies from China and India have shown a high prevalence of such CTX-M type ESBL producing *E. coli* in community setups.[11,12] Another diagnostic problem encountered with CTX-M type ESBL isolates is that they appear sensitive *in vitro* to ceftazidime, so if only this antimicrobial disc is used for screening they are likely to be missed on routine screening. It is, therefore, mandatory for all clinical laboratories screening for ESBLs to use both cefotaxime and ceftazidime or cefpodoxime disk alone to increase sensitivity of detection.

The mean age of patients in this study was 49 years. Majority of the isolates were from patients between 40 to 70 years. The data shows that ESBL isolates are encountered more frequently in the elderly. One study from Pakistan by Shah *et al.*[9] reported that majority of their isolates were from patients between 50 to 60 years of age. According to some other studies the mean age was 47, 58 and 83 years.[13-15]

Treatment options for ESBL producing isolates are available but they are limited. Patients with infections due to ESBL-producing isolates tend to have less satisfactory outcomes than those infected by pathogens that do not produce ESBLs.[16] Carbapenems are regarded as the treatment of choice but carbapenem resistant ESBL isolates have been reported from various parts of the world including United States, Greece, Korea, Israel, and China.[17] In this study, 03 isolates were carbapenems resistant. This study also showed excellent *in vitro* carbapenem susceptibility as

up to 99% of the isolates were susceptible to imipenem, meropenem and ertapenem. In a previous study reported from Pakistan all ESBL producing isolates were susceptible to carbapenems.[15] Similar susceptibility patterns were reported by other workers in which carbapenems susceptibility was above 95%.[18]

ESBL producing isolates are frequently susceptible *in vitro* to beta-lactam/beta-lactamase inhibitor combinations. Two such combinations, piperacillin/tazobactam (tazocin) and sulbactam/cefoperazone (sulzone) were tested in this study. Eighty four percent and 81% of the isolates were susceptible to the two compounds respectively. Similar results were also reported in the studies from Pakistan[15] and Saudi Arabia.[19] Nevertheless one must bear in mind the presence of chromosomal Amp-C enzymes that are resistant to in-activation by beta-lactam/beta-lactamase inhibitor combinations in some isolates. These combinations are, therefore, not recommended empirically in case of serious infections by ESBL producing isolates.[20]

Multiple genes for ESBL in a single strain complicate detection as well as treatment. ESBL producing bacteria are often associated with multi-drug resistance i.e. resistance to other classes of drugs like quinolones, aminoglycosides and cotrimoxazole. The plasmids that carry resistant genes for ESBL production also carry genes for quinolone resistance.[16] Even when such genes are not present there is a strong association between quinolone resistance and ESBL production.[21] Fluoroquinolones resistance was seen in 86% of isolates in this study. This shows a remarkable resistance rate as compared to other regional studies.[19] Therefore, these agents are not recommended for treating infections caused by ESBL producing isolates in this setup. Only 17.5% of our ESBL isolates were susceptible to gentamicin compared to 59% of amikacin justifying the use of amikacin as an empirical agent in gram negative infections. Cotrimoxazole is another agent that can be used alternatively in gram negative infections. This drug showed poor efficacy against ESBL producing isolates in our hands. The results in this regard are comparable with other studies from Pakistan and Saudi Arabia.[15,19]

Nitrofurantoin and Pipemidic acid are urinary antiseptics. Nitrofurantoin is not used very often in clinical settings in our country so there is little antibiotic pressure on this agent. Forty nine percent urinary isolates were susceptible to this agent and our results were comparable to another large study carried out in India in this regard.[22] Nitrofurantoin can, therefore, be considered as a reliable oral alternative agent for UTI but Pipemidic acid cannot be considered in this context.

Other available therapeutic options for treating ESBL infections are Tigecycline,[23] Fosfomycin[24] and Colistin which were not tested in this study. Additional studies in future regarding the efficacy of these agents in our set up are encouraged.

CONCLUSION

Antibiotic choices in case of ESBL producing isolates are limited. At present only carbapenems can be regarded as treatment of choice for ESBL producing isolates. Beta-lactam/beta-lactamase inhibitor combinations should be used cautiously as empirical agents for treating serious infections caused by such isolates. Fluoroquinolones which were previously considered as satisfactory alternative to carbapenems showed poor efficacy. Amikacin can be used alternatively in such cases. Nitrofurantoin is still a good oral agent for treating UTI caused by ESBL producing isolates. Local antibiotic policies must be made and followed to control spread of resistance among isolates and resistant isolates. Injudicious usage of antibiotics must be avoided.

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