Original Article

Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella spp.* isolated from Tertiary Level Hospitals

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Abstract:
Antibiotic resistance in Gram-negative rods is an increasing problem all over the world. Especially ESBL producing Esch. coli and Klebsiella spp. are of major concern since these pathogens are the most common causative Gram-negatives in both community and hospital-acquired infections. Extended spectrum β-lactamases (ESBLs) are the enzymes produced by the members of Enterobacteriaceae can confer resistance to all extended spectrum Cephalosporins, all Penicillins and Monobactams. In this study, total 200 ESBL producing strains were taken as study strain, of which 50 isolated from 308 samples collected from Sir Salimullah Medical College & Mitford Hospital (SSMC & MH) and 150 were known ESBL producing strains of BSMMU and BIRDEM hospital. Total 159 (51.62%) bacteria were isolated from these 308 samples collected from SSMC & MH. Among the isolates 139 (87.42%) were Gram negative bacteria (Esch. coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Acinetobacter spp.) and 20 (12.57%) were Gram positive bacteria (Staphylococcus aureus, Coagulase negative Staphylococcus). Out of 139 Gram negative bacteria 50 (36%) were found to be extended spectrum β-lactamases (ESBLs) producer. All ESBL-producing isolates were detected by screening test and double disc synergy test. Highest rate of ESBLs was observed in Klebsiella spp. (62.5%), followed by Esch. coli (39.13%) and no ESBLs was observed in Proteus spp., Pseudomonas spp. and Acinetobacter spp. All isolates were susceptible to imipenem. Of all isolates, 80% were susceptible to amikacin. The cephalosporins (1st to 4th generations) were almost 100% resistant. For nitrofurantoin, 77% were sensitive for E.coli and 52% for Klebsiella spp. High rate resistance was observed to Ciprofloxacin (86%), Nalidixic acid (92%), Tetracycline (87%), Co-trimoxazole (81%), Mecillinam (76%) and Azithromycin (66%) tested. Aztreonam, amoxicillin, were 100% resistant. This study shows that the frequency of ESBL producing strains of Esch. coli and Klebsiella spp. is high in both hospital and community levels and it has a significant implication for patients' management. Advance drug resistance surveillance and molecular characteristics of ESBL isolates is necessary to guide the appropriate and judicious antibiotic use.

Key words: Extended spectrum β-lactamases (ESBL), *Escherichia coli, Klebsiella spp.,* Antibiogram

Introduction:
The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases[1]. ESBLs are defined as β-lactamases capable of hydrolyzing oxyimino-cephalosporins and are inhibited by β-lactamase inhibitors[2]. An extensive use of β-lactam antibiotics in hospitals and community has created major resistance problem leading to increased morbidity, mortality and health-care costs[3].

The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options[4]. Microorganisms responsible for urinary tract infection (UTI) such as *Esch. coli* and *Klebsiella spp.* have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat[5].

Extended spectrum β-lactamases are a large, rapidly evolving group of plasmid mediated enzymes capable of hydrolyzing and inactivating Penicillins, Cephalosporins and Monobactams and are inhibited by β-lactamase inhibitors such as Clavulanate, Sulbactam and Tazobactam[6,7,8]. Since their description in the mid-1980s, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world[9]. They are also involved in nosocomial outbreaks conferring multiple drug resistant and resulting in limitation of therapeutic options[10,11].

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Specific risk factors that have led to spread of ESBL include prolonged hospitalization, severity of illness, intubations and mechanical ventilation, urinary or arterial catheterization and extensive use of broad spectrum antibiotics[12,13]. The aim of the present study was to identify the frequency of ESBL producing *Esch. coli* & *Klebsiella spp.* with their antibiogram.

Materials & Methods:
This cross-sectional study was carried out in the department of Microbiology, Sir Salimullah Medical College for a period of one year from January, 2009 to December, 2009.

Total 200 ESBL producing *Esch. coli* & *Klebsiella spp.* were taken as study strain, of which 50 isolated from 308 samples of wound swab, throat swab and urine were collected from in-patient and out-patient department of Sir Salimullah Medical College & Mitford Hospital (SSMC & MH) & 150 were known ESBL producing strains of BSMMU and BIRDEM hospital.

Samples from patients clinically suspected to have urinary tract infection, wound infection and respiratory tract infection were collected. Samples were collected aseptically in sterilized bottles or disposable sterile tubes and submitted to clinical Microbiology laboratory. The specimens received were inoculated on blood, MacConkey agar and Chocolate agar plates. Then all plates were incubated at 37°C for 24 hours. Significant isolates were identified as species level using conventional bacteriological methods. All ESBL producing isolates were phenotypically detected by screening test & double-disc synergy test.

Antimicrobial susceptibility testing: Isolates were screened initially using Kirby-Bauer method and all ESBL producing isolates were confirmed using the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS 2004) approved double disk synergy test[14]. Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI).

Screening test for ESBL producers:
Disc diffusion method
Screening test was done by disc diffusion method according to the NCCLS. Isolated Gram negative strains were treated as screening positive which showed specific zone diameter to any one of the following antimicrobials disc- Ceftazidime (≤22 mm), Cefotaxime (≤27 mm), Ceftriaxone (≤25 mm) and Aztreonam (≤27 mm)[15].

Double disc synergy test
ESBLs production was considered positive when the inhibition zone around the test antibiotic disc (Ceftazidime, Ceftriaxone, Cefotaxime and Aztreonam disc) was increased towards the Augmentin disc (20 µg Amoxicillin and 10 µg of Clavulanic acid) which was placed in the centre of the plate and 20 mm apart from other discs[16].

The following antibiotic disks were used for antimicrobial susceptibility: Amoxycillin, Cotrimoxazole, Gentamicin, Amikacin, Nalidixic acid, Nitrofurantoin, Netilmicin, Mecillinam, Cephradine, Ciprofloxacin, Chloramphenicol, Azithromycin, Tetracycline, Aztreonam, four generations of Cephalosporins (Ceftriaxone, Cefotaxime and Ceftazidime), Imipenem. According to the suggestion of CLSI, the results were interpreted. All data were analyzed using Statistical Package for Social Sciences (SPSS).

Results:
Among different samples ESBLs producing strains recovery were highest in wound swab 29 (44.61%) out of 65 Gram negative isolates, followed by urine 20 (27.39%) out of 73 isolates. In case of throat swab, only one ESBL positive *Klebsiella spp.* was isolated out of 1 isolates. But as the sample size was small, it cannot be taken as any conclusive evidence (Table-I).

Out of 139 Gram negative bacteria 50 (36%) were found to be extended spectrum β-lactamases (ESBLs) producer.
Highest rate of ESBLs was observed in *Klebsiella spp*. 5 (62.5%) out of 8, followed by *Escherichia coli* 45 (39.13%) out of 115 and no ESBLs was observed in case of Proteus, *Pseudomonas* and *Acinetobacter spp.* (Table-II).

Among total 200 ESBL producers, 171 were *Escherichia coli* & 29 were *Klebsiella spp.* Both *Escherichia coli* and *Klebsiella spp*. were 100% resistant to Amoxycillin, Cephradine, Cefuroxime, Ceftriaxone, Cefotaxime, Ceftazidime and Aztreonam.

Table I: Distribution of ESBL producing Gram negative bacteria based on different sample collected from SSMC & MH

Sample	Gram negative organism isolated	ESBL producing Gram negative bacteria
Urine	73	20 (27.39%)
Wound	65	29 (44.61%)
Throat swab	1	1 (100.0%)
Total	139	50 (36%)

Table II: Distribution of ESBL producing strains among the Gram negative organisms isolated from SSMC & MH (n=139)

Name of bacteria	Number of strains tested for ESBLs	Number of ESBLs producing strains
Escherichia coli	115	45 (39.13%)
Klebsiella spp.	8	5 (62.5%)
Proteus spp.	5	0 (0.0%)
Pseudomonas spp.	10	0 (0.0%)
Acinetobacter spp.	1	0 (0.0%)
Total	139	50 (36%)

Table III: Rate of antimicrobial drug resistance among the ESBL producing *Escherichia coli* & *Klebsiella spp.* (n=200)

Antimicrobial drugs	*Escherichia coli* N=171	*Klebsiella species* N=29
Amoxycillin	171 (100%)	29 (100%)
Cephradine	171 (100%)	29 (100%)
Cefuroxime	171 (100%)	29 (100%)
Ceftriaxone	171 (100%)	29 (100%)
Ceftazidime	171 (100%)	29 (100%)
Cefotaxime	171 (100%)	29 (100%)
Aztreonam	171 (100%)	29 (100%)
Nalidixic Acid	158 (92.39%)	25 (86.20%)
Ciprofloxacin	148 (86.54%)	24 (82.75%)
Tetracycline	149(87.13%)	25(86.20%)
Cotrimoxazole	140 (81.87%)	24 (82.75%)
Mecillinam	130 (76%)	18 (62%)
Azithromycin	114 (66.66%)	16 (55.17%)
Gentamicin	99 (57.89%)	20 (69%)
Chloramphenicol	85 (49.70%)	12(41.37%)
Netilmicin	55 (32.16%)	12 (41.37%)
Nitrofurantoin	39 (23%)	14 (48.27%)
Amikacin	40 (23.39%)	6(20.68%)
Imipenem	0 (0.0%)	0 (0.0%)

Discussion:

During the past decade, ESBL producing Gram-negative bacilli especially *Escherichia coli* and *Klebsiella spp*. have emerged as serious pathogens both in hospital and community acquired infections worldwide[17].

Our study demonstrated clear differences in susceptibility patterns with our 200 ESBL producing isolates, between *Klebsiella spp*. and *Escherichia coli* for different antimicrobial agent. Total 200 ESBL producing strains were taken as study strain, of which 50 isolated from 308 samples of SSMC&MH & 150 were known ESBL producing strains of BSMMU and BIRDEM hospital.

In this study, out of 308 different samples collected in SSMC&MH, total 159 (51.62%) bacterial strains were isolated; of which 139 (87.42%) were Gram-negative and 20 (12.57%) were Gram-positive bacteria. Among total 139 Gram-negative isolates 115 (82%) were *Escherichia. coli*, 8 (5.75%) were *Klebsiella spp*., 5 (3.59%) *Proteus spp*., 10 (7.19%) *Pseudomonas spp*., 1 (0.71%) *Acinetobacter spp*.

In 2007, a study by Islam carried out in SSMC&MH found 58.3% *Escherichia coli*, 13.46 % *Klebsiella spp.*, 12.56% *Proteus spp*. and 11.66% *Pseudomonas spp*[20]. In contrast to the findings of Islam, the percentage of *Escherichia coli* was more and that of *Klebsiella spp*. was found less in the present study. The reason of such difference might be due to the fact that, inclusion of more urine samples in this study and more burn wound samples in that study[18].

Among the 139 Gram-negative bacteria, ESBL was detected in 50 (36%) strains. Highest number of ESBL producers observed among *Klebsiella spp*. (62.5%), followed by *Escherichia coli* (39.13%). No ESBL producing strain was detected from *Proteus spp*., *Pseudomonas spp*. and *Acinetobacter spp*. in the present study.

In a study at BSMMU, ESBL was detected in 30.90% Gram negative bacteria, among them *Klebsiella spp.* was highest 43.47%, followed by *Escherichia coli* 35.38%, *Proteus spp.* 27.11%, *Acinetobacter spp*. 26.32% and less in *Pseudomonas spp*. 17.07% [21].

The results of the present study were similar to that of Rahman, (2007) except that ESBL producing *Proteus spp*., *Pseudomonas spp*. and *Acinetobacter spp*. were not detected in the present study[19]. This might be due to the fact that the numbers of such organisms were few. It is important to note that percentage of ESBL producer has been found increasing from 30% to 36% in 2 years among Gram negative bacteria.

Rate of ESBL producers in different strains varies from country to country and institution to institution. In India, in a study done at Jawaharlal Nehru institute, Pondicherry, it was reported that 58.06% *Escherichia coli* and 43.75% *Klebsiella spp*. were ESBL producers. In Europe, the incidence is 23-25% in *Klebsiella spp*. and 5.4% of *Escherichia coli* [20].

In this study, ESBL producing strains were isolated from urine samples, wounds and throat swabs. Highest percentage of ESBLs was found among the bacteria isolated from surgical & other wounds (44.61%) followed by urine samples (27.39%). In case of 5 throat swab samples, only one ESBL positive *Klebsiella spp.* strain was isolated. But as the sample size was small, it cannot be taken as any conclusive evidence.

All isolates were susceptible to Imipenem. Of all isolates, 80% were susceptible to Amikacin. The cephalosporins (1st to 4th generations) were almost 100% resistant. For Nitrofurantoin, 77% were sensitive for *Escherichia coli* and 52% for *Klebsiella spp*. High rate resistance was observed to Ciprofloxacin (86%), Nalidixic acid (92%), Tetracycline (87%), Co-trimoxazole (81%), Mecillinam (76%) and Azithromycin (66%) tested. Aztreonam, Amoxicillin, were 100% resistant.

The results of the present study was similar to that of Islam (2007) and Iraj Alipourfard (2010)[18,21]. There are very limited treatment options available for these pathogens. So, prevention remains a significant priority in controlling the development and spread of ESBL producing organisms.

Conclusion:

In conclusion, this study emphasizes the need for continued surveillance of ESBL producing bacteria as high prevalence of antibiotic resistance in ESBL positive *Escherichia coli* and *Klebsiella spp.* was observed. The control measure include judicious use of antibiotics, strict hygiene protocols and implementation of appropriate infection control measures in the hospital, especially while treating high risk patients.

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