Introduction

Extended spectrum β-lactamases (ESBLs) are β-lactamases capable of conferring bacterial resistance to the penicillins, all cephalosporins (excluding cephamyeins) and aztreonam and which are inhibited by β-lactamase inhibitors such as clavulanic acid[1].

ESBLs are most commonly found in *Escherichia coli*, *Klebsiella pneumonide*, *Proteus* species, *Salmonella*, *Shigella* spp, other members of *Enterobacteriaceae and Pseudomonas aeruginosa*[2]. Resistant organisms are now worldwide problems. Long term antibiotic exposure, prolonged ICU stay, severe illness, nursing home residents, catheterization or instrumentation are the major risk factor for colonization of ESBLs producing bacteria. ESBLs producing bacteria can cause both community and hospital acquired infection which can be very difficult to treat with common drugs[3-4].

This present study was undertaken to detect extended spectrum B-lactamases (ESBLs) among Gram negative bacteria isolated from hospitalized patients and community patients (OPD) by double disc synergy test and phenotypic confirmatory test.

Methodology

This cross-sectional, prospective study was carried out in the Department of Microbiology. Dhaka National Medical College, over a period of 1 (one) year 2016. Urine samples were collected from patients. Urine samples were from hospitalized patients and community patients. Samples were collected from in-patient and outpatient department of Dhaka National Medical College Hospital having clinical symptoms of microbial infection. Samples were collected from both sexes and different age groups.

Hospitalized Patients: Patients who have been hospitalized for at least 2 days or more received different antibiotics.

Community Patients: Patients who attended the outpatient departments for the first time were considered as community patients.

Data were collected as per pre-designed date collection form. Data were analyzed by Statistical Package for Social Science (SPSS). The results of the experiments were recorded and statistical analysis was done by using appropriate significance test. Using aseptic precautions all samples were collected. All urine samples were inoculated in Blood agar and MacConkey's agar media. All the pates were incubated at 37^0C aerobically for 24 to 48 hours. After incubation, plates were checked for presence of suspected pathogens.

ESBLs Detection Methods: The following tests were done for the detection of ESBLs from isolated Gram-negative organisms: **Double disc synergy test**[5]: By this method, synergy between a disc of augmentin (amoxicillin and elavulanie acid and) and 3rd generation cephalosporin was observed. The clavulanate in augmentin disc diffuses through the agar and inhibits the β-lactamase surrounding 3rd generation cephalosporin disc. Mueller Hinton agar plates were prepared and inculated with standardized inoculums (corresponding to 0.5 McF arland tube) with sterile cotton swab. Augmenin (20 µg amoxicillin & 10 mg of clavulanic acid) disc was placed in the center of the plate, 3rd generation cephalosporins such as ceftazidime, ceftraxone, cefotaxime and aztreonam disc were placed 20-30mm distance from augmentin disc. The plate was incubated overnight at 37^0C. ESBLs production was considered positive when the inhibition zone around the test antibiotic disc was increased towards the augmentin disc figure-1.

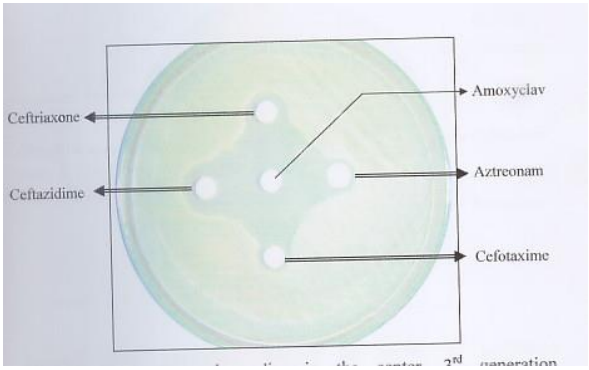

Figure I: Photograph of ESBL positive *Esch. Coli* strain by double disc diffusion method enhancement of zone of inhibition towards amoxyclav Disc.

Figure I shows amoxyclav. Disc in the center. 3rd generation cephalosporins (ceftriaxone, ceflazidime, cefotaxime) and aztronam disc in the periphery. Enhancement of zone of inhibition towards amoxyclav disc in the center.

Phenotypic Confirmatory Test[6]: Confirmation of ESBLs- producing isolates were done by inhibitor potentiated disc diffusion test according to NCCLS recommendation. Third generation cephalosporin i.e. cefotaxime (30µg) & ceftazidime (30 µg) disc alone and in combination with clavulanic acid (10 µg) were placed on inoculated plate. Mueller

Hinton places were inoculated with test bacteria (corresponding to 0.5 MeFarland tube). Ceftazidime, cefotaxime disc without clavulanic acid were placed on one side of inoculated plate and ceflazidime, cefotaxime disc combined with clavulamie acid placed on other side of plate. Then the plates were incubated at 36^0C overnight. After overnight incubation inhibition zone diameter was measured with scale.

It was observed whether there was an increase in zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid compared to its zone diameter for cefotaxime and ceftazidme tested alone (Figure 2). A difference of ≥5 mm between the zone diameter of the cephalosporin discs and their respective cephalosporin clavulanic acid disc was taken to be phenotypic conformation of ESBL producers[6].

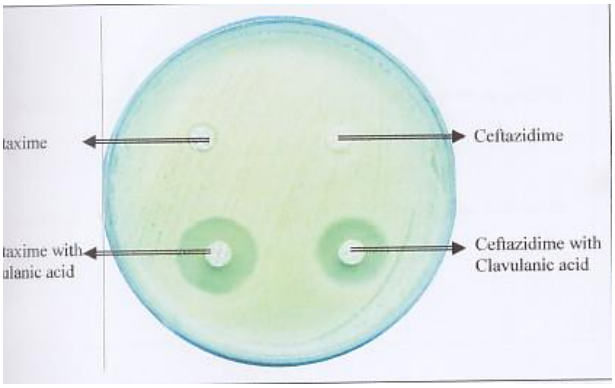

Figure II: Phenotypic confirmatory method showing 3rd generation cephalosporin with and without clavulanic acid.

Figure II shows 3rd generation cephalosporins without clavulantic acid (above) and 3rd generation cephalosporins with clavulanic acid (below). Increase in zone size seen in 3rd generation cephalosporins with clavulantic and confirmed as an ESBL producing organism.

Results

A total of 220 urine samples were collected from patients suspected to have urinary tract infections. Of them, 116 were from hospitalized patients and 104 were from community patients.

From the 220 samples, 132(60%) bacteria were isolated. Among 132(60%) isolated bacteria, 88(75.86%) bacteria were isolated from hospitalized patients and 44(42.31%) were isolated from community patients (Table 1).

Table 1: Distribution of Bacteria of Hospitalized Patients and Community Patients

Sample of the patients	Number to tested samples studied	Number of isolated bacteria
Hospitalized Patients	116	88 (75.86%)
Community Patients	104	44 (42.31%)
Total	**220**	**132(60.0%)**

Table 2 showed 28 (31.82%) trains out of 88 bacteria were ESBL producers in hospitalized patients. Among them, 21(31.34%) Escherichia coli. 4(40.00%) Klebsiella spp., 1(20.00) Proteus spp. and (33.33) Pseudomonas spp. were ESBL producers.

Table 2: Distribution of organism producing ESBL strains in hospitalized patients (n=88)

Name of bacteria	Tested for ESBLs	ESBLs Positive strains
Escherichia coli	67	21 (31.34%)
Klebsiella spp.	10	4 (40.00%)
Proteus spp.	5	1 (20.00%)
Pseudomonas spp.	6	2 (33.33%)
Total	**88**	**28 (31.82%)**

Table 3 showed 3 (6.82) strains out of 44 bacteria were ESBL producers in community patients. Among them. 2 (5.56%) *Esch. coli* and 1 (25%) *Klebsiella spp.*, were ESBL producers.

Table 3: Distribution of organism producing ESBL strains in community patients (n=44)

Name of bacteria	Tested for ESBLs	ESBLs Positive strains
Escherichia coli	36	2(5.56%)
Klebsiella spp.	4	1 (25.0%)
Proteus spp.	2	0 (0.0%)
Pseudomonas spp.	2	0 (0.0%)
Total	**44**	**3 (6.82%)**

Discussion

Antibiotic resistance is now a worldwide problem. ESBLs have become widespread throughout the world and are now found in a significant percentage of *Esch. coli* and *Klebsiella pneumoniae* strains in certain countires[7]. ESBLs are responsible for resistance to many classes of antibiotics resulting in treatment failure[8].

In this study, out of 220 urine samples, 132 (60%) bacterial strains were isolated. Of them, 88 (75.86%) were from hospitalized patients and 44 (42.31%) from community patients.

In the hospitalized patients, total 116 urine samples were studied. Of them, 88 (75.86%) bacterial strains were isolated and 28. (31.82%) strains were ESBL producers. In a study it was shown that 32.33% strains were ESBL producers in urine samples[5]. The findings of the present study were in accordance with the result of Rahman.

In the present study, the ESBL producers are more among the organisms isolated from hospitalized patients than the community patients. The reason might be due to hospitalized patients were recruited/ included after 2 days or more of hospitalization and all the patients received different antibiotics.

Among the community patients, total 104 urine samples were collected. Of them 44 (42.31%) bacterial strains were isolated and 3 (6.82%) strains were ESBL producers. This is very high frequency as ESBLs are rarely seen in the community patients. This is probably due to reason that in our society extended spectrum cephalosporins are used indiscriminately in community patients. In Pakistan a study done at Pakistan institute of Medical Services by Shah et al[9] it was observed that 7.39% strains were ESBL producers among the community patients.

Conclusion

Extended spectrum β-lactamaces continue to be the leading cause of resistance to β-lactam antibiotics among Gram negative bacteria. In the present study, it was also found considerable number of ESBLs producing bacteria responsible for urinary tract infection. Detection of ESBLs producing organisms will help for appropriate treatment of patients infected by these strains. This will reduce the duration of the hospital stay, cause less suffering of both the hospitalized patients and community patients.

References

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