Original Article

Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh

Rezwana Haque[1], M.A. Salam[2]

ABSTRACT

Objective: Extended spectrum ß-lactamases (ESBLs) represent a major group of lactamases currently being identified in large number worldwide mostly produced by gram-negative bacteria. The present study was done to see the frequency of ESBLs in gram-negative bacterial isolates causing nosocomial wound infections from a tertiary care hospital in Bangladesh.

Methodology: A total of 125 wound swabs were collected from surgical site infections and burn cases, admitted in Rajshahi Medical College Hospital (RMCH), during January to June, 2008. Swabs were cultured for aerobic bacteria and antimicrobial susceptibility testing was carried out using the Kirby-Bauer agar diffusion method. Gram-negative isolates were tested for ESBLs on Mueller Hinton agar by both modified double disc and phenotypic confirmatory methods.

Results: Culture yielded 71 (56.8%) bacterial growths with 60 (84.51%) gram-negative and 11 (15.49%) gram-positive bacteria (*Staph aureus*). Gram-negative isolates included 23 (32.39%) *E. coli*, 19 (26.76%) *Klebsiella* spp., 16 (22.54%) *Pseudomonas* spp., and 02 (2.82%) *Proteus* spp. The number of ESBL producing bacteria in modified double disc and phenotypic confirmatory methods were 28 (46.67%) and 25 (41.66%) respectively. Highest rate of ESBLs was observed in *Klebsiella* spp. (57.89%) followed by *Proteus* spp. (50.0%), *E. coli* (47.83%) and *Pseudomonas* spp. (31.25%), which showed significantly increasing resistance to 3rd generation cephalosporins, aminoglycoside, quinolone and trimethoprim-sulfamethoxazole.

Conclusion: Significant number of nosocomial wound infections is caused by ESBL bacteria; those are not detected by routine antimicrobial susceptibility testing. It is recommended that clinical microbiology laboratory should take urgent measure for ESBLs detection as routine to enhance hospital infection control programme.

KEY WORDS: Wound infections, Gram-negative bacteria, ESBLs, Antimicrobial susceptibility.

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INTRODUCTION

Extended spectrum ß-lactamases (ESBLs) produced mostly by members of Enterobacteriaceae have emerged as serious nosocomial pathogens globally.[1,2] The persistent exposure of bacterial strains to ß-lactams induces mutation and continuous production of ß-lactamases in these bacteria, expanding their activity even against the third and fourth generation cephalosporins such as ceftazidime, cefotaxime and cefepime and against monobactams e.g. aztreonam. Thus these new ß-lactamases are called extended spectrum ß-lactamases (ESBLs), which are mostly plasmid mediated enzymes.[3] Although ESBLs have been reported more frequently

from *Klebsiella pneumoniae* and *E. coli* but other members of Enterobacteriaceae and *Pseudomonas* spp. are also implicated for ESBL production.[4,5]

In recent years there has been an increased incidence and prevalence of ESBLs, majority are derived from the widespread broad-spectrum ß-lactamases TEM-1, TEM-2 and SHV-1. There are also new families of ESBLs, including the CTX-M and OXA-type enzymes as well as novel, unrelated ß-lactamases.[6]

Several different methods like disk approximation or double disk synergy, modified double disc test (MDDT), NCCLs phenotypic confirmatory method, E-test ESBL strips, three dimensional test, Vitek system etc. have been suggested for the detection of ESBLs in clinical isolates.[7] While each of the tests has merit, none of the tests is able to detect all of the ESBLs encountered. Disk approximation or double disk synergy is one of the currently available and widely practiced techniques for the detection of ESBLs. Phenotypic tests (double-disk synergy test, ESBL E-test, and the combination disk method) are based on clavulanate inhibition and extended spectrum of cephalosporin (ESC) susceptibility testing. They often need slight changes by either reducing the distance between the disks of ESC and clavulanate.[8,9] Up till now, there is no gold standard method for ESBL detection but NCCLS recommend the phenotypic method as confirmatory test.[10]

Until recently, only a few studies have been carried out to detect ESBL bacteria in Bangladesh and ESBL screening as a routine has not yet been practiced. Keeping in mind that this is going to be the first published report on ESBL from RMCH, a tertiary hospital in the Northern part of Bangladesh, we designed the present study to see the pattern of gram-negative bacterial isolates from nosocomial infection cases and their frequency of ß-lactamases production by two standard methods of ESBLs detection.

METHODOLOGY

Patients: The study included 125 patients of different age and sex suffering from hospital acquired infections, mostly surgical site infections and a few burn cases admitted in Rajshahi Medical College Hospital (RMCH), Bangladesh from January to June, 2008. Cases fulfilling the definition of hospital acquired infection were enrolled.

Culture and Antimicrobial susceptibility testing: Following aseptic collection, wound swabs were inoculated onto Blood agar and MacConkey agar media. The plates were incubated at 37°C aerobically and after overnight incubation, they were checked for bacterial growth. All organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard methods of identification. All gram-negative bacterial isolates were tested for antimicrobial susceptibility by using commercially available antimicrobial discs on Mueller Hinton agar.[10] *E. coli, Klebsiella* spp., and *Proteus* spp., were tested against ampicillin (30µg), cotrimoxazole (30µg), gentamicin ((30µg), ciprofloxacin (10µg), aztreonam (30µg), netilmycin (30µg), ceftriaxone (30µg), ceftazidime (30µg), and imipenem (30µg). For *Pseudomonas* spp., gentamicin (30µg), ciprofloxacin (10µg), aztreonam (30µg), netilmycin (30µg), ceftriaxone (30µg), ceftazidime (30µg) and imipenem (30µg) were used. Zone of inhibition was recorded as *Sensitive* or *Resistant* according to NCCLs chart.[11]

ESBLs detection

Modified double disc test (MDDT)[9]

Mueller Hinton agar was inoculated with standardized inoculum (corresponding to 0.5 McFarland tube) using sterile cotton swab. Augmentin (20µg amoxycillin and 10µg of clavulanic acid- AMC) disc was placed in the center of the plate and test discs of 3rd generation cephalosporins (ceftazidime- CAZ 30µg, ceftriaxone-CRO 30µg, cefotaxime-CTX 30µg) and aztreonam (ATM 30µg) discs were placed at 15 mm distance from the Augmentin disc. The plate was incubated overnight at 37°C. ESBL production was considered positive if the zone of inhibition around the test discs increased towards the Augmentin disc or neither disc were inhibitory alone but bacterial growth was inhibited where the two antibiotics diffuse together (Fig-1).

Phenotypic confirmatory test for ESBLs[10]

Confirmation of ESBL-producing isolates (MDDT-positive) was done by inhibitor potentiated disc diffusion test according to NCCLS recommendation. Combinations of ceftazidime and cefotaxime disc with clavulanic acid (10mg) were prepared an hour before their application to the Mueller Hinton plates inoculated with test bacteria (corresponding to 0.5 McFarland tube). Ceftazidime and cefotaxime discs without clavulanic acid were placed on one side of inoculated plate and ceftazidime, cefotaxime discs combined with clavulanic acid were placed on other side of plate. Diameter of zone of inhibition was measured after overnight incubation at 37°C. A ≥5mm increase in a zone diameter for cefotaxime and ceftazidime tested in combination with clavulanic acid versus its zone when cefotaxime and ceftazidime were tested alone confirmed an ESBL producing organism (Fig-2).

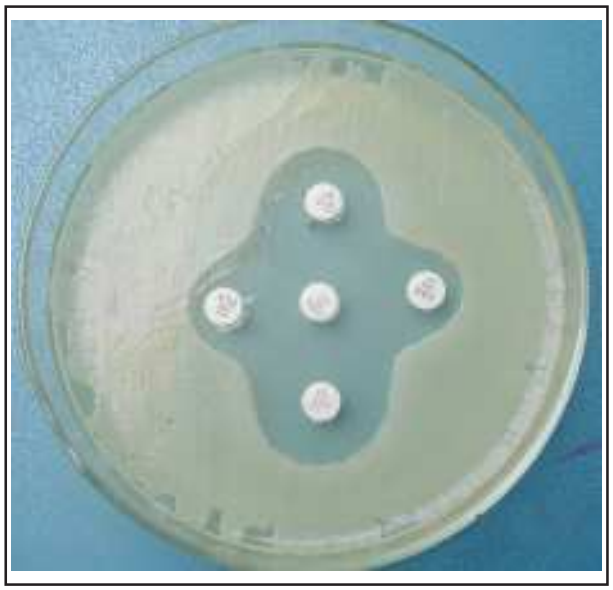

Figure-1: Enhancement of zone of inhibition produced by susceptible strain of *E. coli* to 3rd generation cephalosporins and aztreonam towards amoxyclav disc placed at the centre.

Figure-2: Resistance to 3rd generation cephalosporins without clavulanic acid (above) and sensitive to 3rd generation cephalosporins with clavulanic acid (below).

RESULTS

Pattern of bacterial isolates and frequency of ESBL gram-negative bacteria from wound culture is shown in Table-I. Culture of 125 wound swabs including 117 (93.60%) surgical site infections and 08 (6.40%) burn cases yielded 71 (56.80%) bacterial isolates. It was noted that *E. coli* was the leading bacteria (32.39%) followed by *Klebsiella* spp. (26.76%), *Pseudomonas* spp. (22.54%), *Staph. aureus* (15.49%) and *Proteus* spp. (2.82%). Out of 60 gram-negative bacteria, 28 (46.67%) were found to be ESBL-positive, with *Klebsiella* spp. (57.89%) as the leading organism followed by *Proteus* spp. (50.0%), *E. coli* (47.83%) and *Pseudomonas* spp. (31.25%).

Out of 60 gram-negative bacteria tested for ESBL, 28 strains were found MDDT- positive and 25 phenotypic confirmatory test-positive. One strain of *E. coli* and 2 strains of *Klebsiella* spp. were MDDT-positive but negative by phenotypic confirmatory test (Table-II).

Antimicrobial drug resistance patterns of ESBL bacteria are shown in Table-III. *E. coli* was 100% resistant to ampicillin and cotrimoxazole and variably resistant to ciprofloxacin (90.90%), ceftriaxone (81.82%), ceftazidime (72.73%), aztreonam (63.64%), netilmycin (54.55%) and gentamicin (45.45%). *Klebsiella* spp. was 100% resistant to ampicillin, aztreonam, ceftriaxone and ceftazidime, but variably resistant to netilmycin (90.91%), ciprofloxacin (81.82%), cotrimoxazole (72.73%) and gentamicin (63.64%). *Pseudomonas* spp. was found to be 100% resistant to ceftriaxone and aztreonam and 80% resistant to ciprofloxacin, gentamicin, ceftazidime and netilmycin. *Proteus* spp. was 100% resistant to

Table-I: Patterns of bacterial isolates and frequency of ESBL gram-negative bacteria from wound culture (n=125).

Bacteria	*Surgical site infections*	*Burn cases*	*Total*	*No. ESBL- positive*
E. coli	22 (33.85)	01 (16.67)	23 (32.39)	11 (47.83)
Klebsiella spp.	17 (26.15)	02 (33.33)	19 (26.76)	11 (57.89)
Pseudomonas spp.	14 (21.54)	02 (33.33)	16 (22.54)	05 (31.25)
Proteus spp.	02 (03.08)	00	02 (02.82)	01 (50.00)
Staph. aureus	10 (15.38)	01 (16.67)	11 (15.49)	Not done
Total	65 (100)	06 (100)	71 (100)	28 (46.67)

Figures in the parentheses indicate percentage

Table-II: Comparison of modified double disc test (MDDT) and phenotypic confirmatory method for ESBL detection.

Bacteria	*MDDT-positive*	*Phenotypic confirmatory test- positive*
E. coli (n=23)	11	10
Klebsiella spp. (n=19)	11	9
Pseudomonas spp.(n=16)	5	5
Proteus spp. (n=2)	1	1
Total	28	25

ampicillin, cotrimoxazole, ciprofloxacin, ceftriaxone, ceftazidime and aztreonam.

DISCUSSION

Extended spectrum ß-lactam antimicrobial drugs are commonly included in empirical antibiotic regimens for treatment of gram-negative sepsis but the emergence of ESBL producing bacteria poses a serious threat to the continued use of this family of antibiotics.[12] Therefore, infections caused by ESBL isolates need to be addressed with a general consensus in order to overcome the challenge of infection management worldwide.

As far as the rate of isolation and pattern of ESBL producing gram-negative bacteria are concerned, our findings are consistent with other investigators.[13-16] Detection rate of ESBL among gram-negative isolates by modified double disc test and phenotypic confirmatory method in our series (Table-II) correlates well with Kader et al. (2006). The slightly lower rate of detection in phenotypic confirmatory method may be correlated with the fact that the spectrum of modified double disc test is wider because organisms producing ESBL and AmpC enzymes (the chromosomally mediated lactamase production) is more difficult to differentiate by phenotypic confirmatory or standard NCCLS methods.[7,10] Currently, there are no standard phenotypic tests for the simultaneous detection of ESBL and AmpC, and therefore clinical laboratories need to use molecular testing to identify organisms producing both enzymes. *Klebsiella* spp. resistant to ceftazidime is a good marker of presence of ESBL but ideally the most sensitive ESBL screening agent is cefpodoxime for *Klebsiella* spp. and *E.coli.* Today it is commonplace for *Klebsiella* spp. to produce 3 to 6 types of lactamases and these changes in bacterial pathogens necessitate new and modified tests to provide accurate and clinically relevant susceptibility reports.[17,18]

We found, all ESBL-positive bacterial strains were 100% sensitive only to imipenem, while, they showed significantly increasing multi resistance *to all other antibiotics used* (Table-III), *which implies that ESBL producing organisms are multidrug resistance.* The prevalence of these multidrug resistant ESBL (MDR-ESBL) strains is also reported to be on the rise.[19]

In fact, routine antimicrobial susceptibility testing fails to detect ESBL resulting into treatment failure. The rate of isolation of ESBL gram-negative bacteria in the present study is really alarming for a tertiary care hospital like Bangladesh, where in most instances, empirical antibiotic therapy includes one of the 3rd generation cephalosporins and virtually all ESBL producing bacteria are resistant to them. It is very urgent to address the problem of hospital acquired infections caused by ESBL-producing bacteria, especially in a developing country like Bangladesh, where antibiotic abuse and irrational use is a common practice. We emphasize that more studies should be carried out with hospital infection cases in Bangladesh to actually reveal the over all ESBL situation of the country with the aim to formulate an antibiotic algorithm for empirical therapy.

Table-III: Patterns of antimicrobial drug resistance among the ESBLs producers.

Antimicrobial Drug	*E. coli(n=11)*	*Klebsiella (n=11)*	*Pseudomonas (n=5)*	*Proteus (n=1)*
Ampicillin	11 (100)	11 (100)	-	01(100)
Cotrimoxazole	11 (100)	08 (72.73)	-	01(100)
Ciprofloxacin	10 (90.91)	09 (81.82)	04 (80)	01(100)
Gentamicin	05 (45.45)	07 (63.64)	04 (80)	00
Imipenem	00	00	00	00
Ceftriaxone	09(81.82)	11 (100)	05 (100)	01(100)
Ceftazidime	08 (72.73)	11 (100)	04 (80)	01 (100)
Aztreonam	07 (63.64)	11 (100)	05 (100)	01(100)
Netilmycin	06 (54.55)	10 (90.91)	04 (80)	00

Figures in the parentheses indicate percentage

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