Original Paper

DETECTION OF EXTENDED SPECTRUM β-LACTAMASE PRODUCING BACTERIA IN COMBINED MILITARY HOSPITAL, DHAKA

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Abstract

***Introduction*:** Extended Spectrum Beta Lactamase (ESBL) are enzymes capable of hydrolyzing extended-spectrum cephalosporins, penicillins and monobactam but inactive against cephamycin and imipenem. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the emergence of the ESBLs. Usually they can be found in a variety of *Entrerobacteriaceae species*.

***Objective*:** This study was carried out to find out extended spectrum β-lactamase (ESBLs) producing Gram negative bacteria. Various clinical samples collected from Combined Military Hospital (CMH), Dhaka.

Materials and Methods: This cross sectional study was done by double disc diffusion method followed by Phenotypic confirmatory disc diffusion test (PCDDT)

Result: Total 411 samples were collected from patients with wound infections (burn and surgical wounds) .Urine samples were also collected. 250 (60.82%) bacterial strains were isolated. Among the isolates 225 (90%) were Gram negative bacteria (*E.coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Enterobactor spp., Acinetobactor spp.*) and 25 (10%) were Gram positive (*Staph.aureus, Enterococci spp. and Coagulaese Negative Staphylococcus(CNS*).

Out of 225 Gram negative bacteria 66 (29.33%) were found to be extended spectrum β- lactamases (ESBLs) producers. Highest rate of ESBLs was observed in Klebsiella spp. (38.46%), followed by *E. coli* (35.64%), *Enterobactor* (28.57%), *Acinetobactor* spp.(22.22%), *Pseudomonas* spp. (18.75%), *Proteus* spp. (9.09%). Among individual samples ESBLs positive strains were highest in urine sample (30.76%). followed by surgical & other wound (27.84%) and burn wound (25 %). Among isolated ESBL producing bacteria Klebsiella spp was highest in all types of samples; in burn wound 50%, in surgical wound 40% and in urine sample 35.71%.

Among ESBL producers all the strains were imipenem sensitive (100%) and sensitivity to cephamycin was as follows- *E.coli* (88.88%), *Klebsiella* spp. (90%), *Proteus* spp. (100%), *Pseudomonas* spp. (77.77%), *Enterobactor* spp. (83.33%) and *Acinetobactor* spp. (75%).

Conclusion: The routine susceptibility tests by clinical laboratories fail to detect ESBL producing strains. Treatment of ESBL producing organism can be done by Carbapenem group of drugs.National Committee for Clinical Laboratory Standards (NCCLS) recommends when ESBL production is confirmed, results should be reported as resistance to all penicillins, cephalosporins excluding cephamycins.

Key-words: β-lactamase,gram-negative bacteria,imipenem sensitive

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Introduction

Resistant bacteria are emerging world wide as a threat to the favorable outcome of common infections in community and hospital settings. β-lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by β-lactamases. It was surprising to find cephalosporin resistant Klebsiella spp. among the clinical isolates.The mechanism of this resistance was production of extended spectrum β -lactamases (ESBLs)[1].The ESBL enzymes are plasmid - mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-I enzymes. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early pencillins and low level resistance to first generation cephalosporins. Widespread use of third generation cephalosporinsandaztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs[2]. These enzymes mediate resistance to cefotaxime, ceftazidime and other broad spectrum cephalosporins and to monobactams such as aztreonam, but have no detectable activity against cephamycins and imipenem. Because of their greatly extended substrate range these enzymes were called extended spectrum β-lactamases[3]. The first ESBL isolates were discovered in Western Europe in mid 1980s and subsequently in the late 1980s in the US2[2] .

The resistant organisms are now a worldwide problem. They can be found in a variety of Entrerobacteriaceae species, however, the majority of ESBL producing strains are K. pneumoniae, K. oxytoca and E.coli.

Other organisms reported to harbor ESBLs include Enterobacter spp., Salmonella spp., Morganella morganii, Proteus mirabilis, Serratia marcescens and Pseudomonas aeruginosa.

However, the frequency of ESBL production in these organisms is low[2]. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporin use and instrumentation or catheterisation[3].

Developing countries have particular problems due to antimicrobial resistance. Crowding, poor sanitation and sexual contact lead to dissemination of resistant strains. Doctors working in developing countries prescribe antimicrobials to meet patient expectations and inappropriate use of them is a common phenomenon[4]. Rate of ESBL positivity of different strains varies from country to country. In India 58.06% E.coli and 43.75% Klebsiella spp. are ESBL producers. In Europe the rate of ESBL positivity is 23-25% in Klebsiellaspp. and 5.4% for E. coli. In Asia ESBL production in E.coli and K.pneumoniae varies, from 4.87% in Korea to 8.5% in Taiwan and 12% in Hong Kong[2].

Materials and Methods:

This is a cross sectional study in which a total of 411 samples (123 surgical & bum wound swab and 288 urine samples) were collected for isolation of bacteria from in-patient and out- patient department of Combined Military Hospital, Dhaka having clinical symptoms of microbial infection during the period of July, 2006 to June, 2007 . The samples were cultured on appropriate culture media and the isolates were identified based on colony morphology on Blood agar, MacConkey agar and CLED media and by standard biochemical tests.

Antimicrobial susceptibility testing: All bacterial isolates were tested for antimicrobial susceptibility by disc diffusion method on Mueller Hinton agar plates with commercially available discs[5]. For isolated Gram-negative organisms Ampicillin (10µg), Cotrimoxazole (1.25/23.75µg), Gentamycin (10µg), Amikacin (30µg), Nalidixic acid (30µg),Nitrofurantoin(30µg) and third generation Cephalosporins¬ ceftriaxone(30µg), ceftazidime(30µg) were used. The diameter of zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate susceptible or susceptible according to NCCLS criteria[6].

1. **Detection of ESBL:** Only Gram-negative bacteria which were resistant to first line antibiotics (Ampicillin, cotrimoxazole, Gentamycin, Amikacin, Nalidixic acid, Nitrofurantoin) and 3rd generation cephalosporins(ceftriaxone, ceftazidime) were tested for ESBL by following methods

2. **Double disc diffusion test for ESBL detection[7]:** By this method synergy between a disc of augmentin and 3rd generation cephalosporin was detected. Mueller Hinton agar plates were inoculated with inoculum. A augmentin disc (combination of 20ug amoxicillin and l0µg clavulanic acid) was placed on the center of inoculated plate and 30 µg disc of 3rd generation cephalosporins were placed on the agar at a distance of 20mm center to center from augmentin disc (amoxyclavulanic acid). Plates were incubated at 37°C overnight. ESBL production was interpreted as positive if inhibition zone around the test antibiotic disc increased towards augmentin disc or if neither disc were inhibitory alone but bacterial growth was inhibited where two antibiotics diffuse together.

3. ***Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL confirmation[8]:*** ESBL-producing bacteria detected by double disc diffusion method were confirmed by inhibitor potentiated disc diffusion test according to NCCLS recommendation. This test requires use of 3rd generation cephalosporin ie.cefotaxime (30 µg), ceftazidime(30 µg) disc alone and in combination with clavulanic acid (10 µg).Cefotaxime and Ceftazidime discs with clavulanic acid were prepared in the laboratory using stock solution of clavulanic acid at 1000 µg/ml.From this stock solution l0 µl of clavulanic acid solution was added to commercially available cefotaxime and ceftazidime discs within an hour before these were applied to the plates. Mueller Hinton plates were inoculated with test bacteria (corresponding to 0.5 McFarland tube). Ceftazidime, cefotaxime disc without clavulanic acid was placed on one side of inoculated plate and ceftazidime, cefotaxime disc combined with clavulanic acid was placed on other side of plate. Then the plates were incubated at 37°C overnight. After overnight incubation diameter of zone of inhibition was measured and observed whether there was an increase in zone diameter for cefotaxime and ceftazidime with clavulanic acid compared to zone diameter of cefotaxime and ceftazidime without clavulanic acid.

Interpretation of phenotypic test

An increase in zone diameter of 5 mm for cefotaxime and ceftazidime disc with clavulanic acid in comparison to cefotaxime and ceftazidime disc alone confirms ESBL producing organism[1].

Results

A total 411 samples were collected from patients with wound infections (swab from burn wound and surgical wound) and urine from suspected cases of urinary tract infection from Combined Military Hospital, Dhaka of which 288 were urine samples, 103 were wound samples and 20 were burn samples. Total 250 (60.82%) bacteria were isolated from these 411 samples (Table-I)

Out of 250 isolates 225 (90%) were Gram negative bacteria and 25 (10%) were Gram positive bacteria. Among the isolates majority were E.coli 101 (40.40%), followed by Pseudomonas 48 (19.20%), Klebsiella spp. 26 (10.40%), Enterobactor spp. 21 (8.40%),

Acinetobactor spp. 18 (7.20%), Enterococci spp 14 (5.60%), Proteus spp. 11 (4.40%), Staph. aureus 9 (3.60%), Coagulase Negative Staph. 2 (0.80%) (Table- II).

Out of 225 Gram negative bacteria 66 (29.33%) were found to be extended spectrum β- lactamases (ESBLs) producer. Highest rate of ESBLs was observed in Klebsiella spp. 10 (38.46%) out of 26, followed by E. coli 36 (35.64%) out of 101, Enterobactor spp. 6 (28.57%) out of 21, Acinetobactor spp. 4 (22.22%) out of 18, Pseudomonas spp. 9 (18.75%) out of 48, Proteus spp. 01 (9.09%) out of 11.

Among individual samples ESBLs positive strains were highest in urine sample, 40 (30.76%) out of 130 samples, followed by surgical & other wound samples, 22 (27.84%) out of 79 samples, burn wound 4 (25%) out of 16 samples (Table- IV).

Table-I: Rate of isolation of bacteria from different samples (N= 411).

Types of sample	Numberof samples studied	Number of positive sample (%)
Urine	288	146 (50.69%)
Wound	103	86 (83.49%)
Burn wound	20	18 (90.00%)
Total	411	250 (60.82%)

Note: Figures in parentheses represent percentage.

Table-II. Distribution of different bacterial species among the isolated bacteria from various sample (N=250)

Name of Bacterial species	Urine	Wound	Burn	Total % of isolated bacteria
E.coli (n=101)	63 (62.37)	34 (33.66)	04 (3.96)	40.40
Pseudomonas spp. (n=48)	15 (31.25)	25 (52.08)	08 (16.66)	19.20
Klebsiella spp. (n=26)	14 (53.84)	10 (38.46)	02 (07.69)	10.40
Enterobactor spp. (n=21)	20 (95.23)	1 (4.77)	0 (00)	8.40
Acinetobactor spp. (n=18)	13 (72.22)	5 (27.78)	0 (00)	7.20
Proteus spp. (n=11)	5 (45.45)	4 (36.36)	02 (18.18)	4.40
Total Gram (-ve) bacteria (n=225)	130	79	16	
Enterococci spp. (n=14)	14 (100)	00	00	5.60
Staph. aureus (n=9)	0 (0)	7 (88.89%)	2(11.11%)	3.60
Coa Neg Staph. (n=2)	2 (100%)	0 (0)	0 (0)	0.80
Total Gram (+ve) Bacteria (n=25)	16	7	2	100.00

Note: Figures in parentheses represent percentage.

Table-III. Rate of ESBLs positivity among the different strains of Gram negative bacteria tested (N=225).

Name of strain strains (%)	No of strains tested	No of ESBLs +ve
E.coli	101	36 (35.64)
Pseudomonas spp.	48	9 (18.75)
Klebsiella spp.	26	10 (38.46)
Enterobactor spp.	21	6 (28.57)
Acinetobactor spp.	18	4 (22.22)
Proteus spp.	11	1 (9.09)
Total	225	66 (29.33)

Note: Figures in parentheses represent percentage.

Table-IV. Distribution of ESBL strains among Gram –ve bacteria basing on type of sample (N=225)

Sample	No of Gram (–ve) organism isolated	No of ESBLs strains (%)
Urine	130	40 (30.76)
Wound	79	22 (27.84)
Burn	16	4 (25.0)
Total	225	66 (29.33)

Note: Figures in parentheses represent percentage.

Table-V. ESBLs producer among the different species of Gram (-ve) bacteria in different sample.

	Total Gm -ve Bacteria	No of ESBLs +ve Strain (%)	Total Gm -ve Bacteria)	No of ESBLs +ve Strain (%)	NoTotal Gm-ve Bacteria	No of ESBLs +ve Strain (%)
E.coli	63	23 (36.50)	34	12 (35.29)	04	1 (25.00)
Klebsiella spp.	14	5 (35.71)	10	4 (40.00)	2	1 (50.00)
Enterobactor spp.	20	6 (30.30)	1	0 (0)	0	0 (0)
Pseudomonas spp.	15	3 (20)	25	4 (16.0)	8	2 (25)
Acinetobactor spp.	13	3 (23.08)	5	1 (25.0)	0	0 (0)
Proteus spp.	5	0 (0)	4	1 (25.00)	2	00 (00)
Total	130	40 (30.76)	79	22 (27.84)	16	4 (25.0)

Note: Figures in parentheses represent percentage.

Among isolated ESBL producing bacteria Klebsiella spp was highest in all types of sample; in urine sample 5 (35.71%), in surgical wound 4 (40.00%) and in burn wound 1 (50%). ESBL positive E.coli in urine sample 23 (36.50%), in surgical wound 12 (35.29%) and in burn wound 4 (25%). ESBL positive Proteus spp. in surgical wound 1 (25%). ESBL positive Pseudomonas spp. in urine sample 3 (20%), in surgical wound 4 (16%) and in burn wound 2 (25%). ESBL positive Enterobactor spp. was found only in urine sample 6 (30.30%). ESBL positive Acinetobactor spp. in urine sample were 3 (23.08%) and in surgical wound 1 (20%), (Table-V).

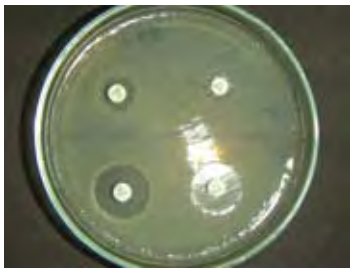

Fig-1: shows 3rd generation cephalosporins without clavulanic acid (above) and 3rd generation cephalosporins with clavulanic acid (below). Increase in zone size seen in 3rd generation cephalosporins with clavulanic acid confirmed an ESBL producing organism.

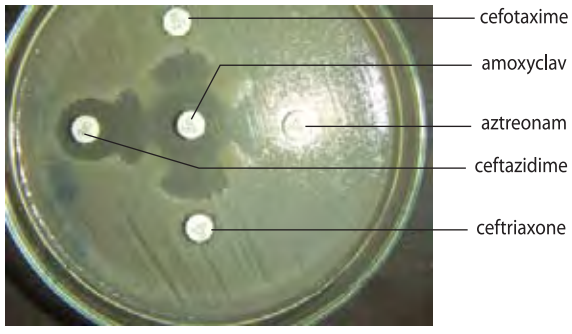

Fig-2: Double disc diffusion method

Discussion

Antiboitic resistance is now a global concern including Bangladesh. ESBLs are enzymes found in a variety of organisms like Enterobacteriaceae and Pseudomonas aeruginosa. These enzymes are responsible for resistance to many classes of antiboiotics resulting in treatment failures. Extended sprectrum -lactams are commonly considered in the empirical antibiotic choice for treatment of Gram-negative sepsis. Surveilance of resistance is essential to monitor & control the spread of antimicrobial resistance[9].

In this study out of 411 different samples total 250 (60.82%) bacterial strains were isolated; of which 225 (90%) were Gram- negative and 25 (10%) were Gram-positive bacteria.

Among total 250 isolates 101 (40.40%) were E.coli, 48 (19.20%) Pseudomonas spp., 26 (10.40%) Klebsiella spp., 21 (8.40%) Enterobacter spp., 18 (7.20%) Acinetobacter spp., 11 (4.40%) Proteus spp., 14 (5.60%) Enterococci spp., 9 (3.60%) Staphylococcus aureus and 2 (.80%) Coagulase Negative Staph. A study carried out in BSMMU hospital Dhaka revealed 42.39% E.coli, 22.28% Pseudomonus, 11.95% Klebsiella spp. 8.69% Proteus spp were ESBL positive[10].

In our study, isolation rate of different strains among burn wound patients showed highest case of Pseudomonas spp. (44.44%) followed by E.coli (22.22%) Proteus spp. (11.11%), Klebsiella spp. (11.11%) and Staph. aureus (11.11%).

Among surgical wound, highest rate of bacteria isolated were 88.89% Staph. aureus followed by 52.08% Pseudomonus, 38.46% Klebsiella spp., 36.36% Proteus spp. 33.66% E.coli and 27.78% Acineatobactor spp. A study at BSMMU Hospital Dhaka also observed high rate of Spaph.aureus (39.44%) followed by Klebsiella spp. (21.13%), E.coli (11.27%), Proteus spp. (8.45%), Acinetobacter spp. (7.04%), Pseudomonas spp. (5.63%) in surigcal wound sample[11].

Among urine samples, rate of isolation of Gram negative bacteria according to frequency were 62.37% E.coli, followed by 53.84% Klebsiella spp., 31.25% Pseudomonas spp. Whereas in case of Gram positive bacteria- 90.90% were Enterococcus spp. Among 21 isolated Enterobacter spp. 20 (95.23%) strains were detected in urine. The higher rate of E.coli 64.74% followed by Klebsiella 45.45% and Proteus 21.80% in urine sample were also observed in a tertiary hospital, Dhaka[10].

Among different samples, isolation rate of E.coli was highest in urine 63 (62.37%), followed by 34 (33.66%) in surgical wound and 4 (3.96%) in burn wound. Klebsiella spp. was highest in urine

14 (53.84%) followed by 10 (38.46%) in surgical wound and 02 (7.69%) in burn wound. Pseudomonas spp. was highest in surgical wound 25 (52.08%) followed by urine 15 (31.25%) and burn 08 (16.66%) Enterobacter spp. was isolated in urine 20 (95.23%). Acinetobacter spp. was found in urine 13 (72.22%) and in wound 5 (27.78%). Enterococci spp. in urine was 14 (100%). Staph aureus was found highest in surgical wound 7 (88.89%) followed by burn wound 2 (11.11%). Proteus spp. was highest in urine 5 (45.45%), followed by surgical wound 4 (36.36%) and 2 (18.18%) in burn.

Among the 225 Gram-negative bacteria ESBL was detected in 66 (29.33%) strains. All were resistant to first line of drugs. Although among the Gram-negative bacteria E.coli was isolated in maximum number of patients but the rate of ESBL positivity was highest in Klebsiella spp. 38.46% followed by E.coli 35.64% & then in Enterobactor (28.57%) & Pseudomonous 18.75%. A study at BSMMU, Dhaka showed Klebsiella spp. 40.90% Proteus spp. 40.62%, E.coli 26.92% & in Pseudomonous 4.87% as ESBL producers[10]. The reason of discripant rate of ESBL production in E.coli in our study in comparison to that might be the number of the strain which varies among institution to instution and geographic area.
Rate of ESBL positivity of different strains even varies from country to country. In an Indian study done at Jawaharlal Institute, Pondicherry observed 58.06% E.coli and 43.75% Klebsiella spp. as ESBL producers[12].

ESBL producing strains were isolated from urine samples, surgical wound and burn wound. Highest rate of ESBLs (30.76%) was found among the bacteria isolated from urinary strains, followed by (27.84%) in surgical wound and (25%) in burn wound. Among isolated ESBL producing bacteria Klebsiellaspp was highest in all types of sample. Increase in number of ESBLs producer is probably due to previous treatment with β-lactam drugs, extreme age, bed retention, immune suppression, association with other diseases and use of temporary or permanent urinary catheter.

In urine samples out of all ESBL positive strains, E.Coli (36.50%) and Klebsiella spp. (35.71%) are near about in equal frequency followed by Enterobactor spp. 30.30%, Acinetobactor spp. 23.08% and Pseudomonas spp. 20%. The higher rate in urine (30.76%) in the present study may be due to increased number of those patients were hospitalized and on urinary catheter.

Among surgical & other wound ESBL Positive strains were 35.29% E. coli, 40% Klebsiella spp., 25% Proteus spp., 16% Pseudomonas spp., 20% Acinetobactor spp. Among burn wound strains 25% were E. coli, 50% Klebsiella spp. and 25% Pseudomonas spp., Higher rate was also found in surgical wound (27.84%), followed by burn wound (25%). This may be due to the cause that most of the patients were in post-operative ward with improper handling of wound, overcrowding, understaffing or nursing workload with cross transmission of ESBL producing Enterobacteriaceae[13].

The isolation rate of ESBL producing Klebsiella spp. was highest among burn wound (50%), followed by surgical wound (40%) and urine (35.70%). Similar higher rate ESBL producing strains of Klebsiella spp. (44%) also observed in Singapore hospital[14]. They also found highest number of ESBL producing Klebsiella spp. in urine samples.

Among Pseudomonas spp. 9 (18.758%) ESBL positive strains were isolated out of total 48 isolates. Pseudomonas spp. exhibits multiple mechanism of drug resistance simultaneously other than ESBL, such as efflux pump, AmpC enzyme (β-lactamase) leads to resistance during course of treatment, these enzymes are resistant to clavulanic acid that is used to detect ESBL producing bacteria in double disc and phenotypic method. ESBL producing Pseudomonas spp. was highest in urine (75%) followed by throat swab (50%), pus (35%), aural swab (33%) and in sputum (25%) observed in a study in Bangladesh[15].

Treatment of ESBL producing organism can be done by carbapenem eg. imipenem, meropenem, ertapenem. ESBL producing organisms are sensitive to 2nd generation cephalosporins in vitro but not recommended for treatment according to NCCLS as may not be effective in vivo except cephamycin. NCCLS recommends that when ESBL production is confirmed, results be reported as resistance to all penicillins, cephalosporins excluding cephamycins[16].

Molecular characterization of the isolated ESBL was not possible in this study due to lack of facilities. Characterization is required to determine which type is prevalent in Bangladesh. Despite the above-mentioned limitations, this study has for the first time in a selective Armed Forces Hospital(Combined Military Hospital, Dhaka) revealed the extent of ESBL producing organisms responsible for various infections.

Conclusion

The routine susceptibility tests by clinical laboratories fail to detect ESBL producing strains and many ESBLs producing organisms do not appear to be resistant to newer cephalosporins or aztreonam in routine in-vitro susceptibility tests. The identification of such resistant organisms in particularly is important especially in developing countries where there is no good control of antibiotic use. Detection of ESBL producing strains will result in appropriate treatment of patients harboring these strains. This will reduce the duration of hospital stay, cause less suffering of patients due to adverse effects of different antibiotics and reduce the cost of both the patient and community in treating nosocomial infections. Early detection and prompt containment can limit the spread of these multiresistant bacteria. Knowledge of resistance pattern of bacterial strains will help to guide the appropriate and judicious antibiotic use. There will be withdrawal of selective pressure exerted by antibiotics and resistant bacteria will no longer have survival advantage.

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