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Status of ESBL Producing Bacteria Isolated from Skin Wound at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: ESBL producing bacteria are increasing with an alarming rate with a wide range of infections. Objective: The purpose of the present study was to see the status of ESBL producing bacteria isolated from skin wounds. Methodology: This cross sectional study was conducted in the Department of Microbiology at Mymensingh Medical College, Bangladesh from January 2011 to June 2011 for a period of 6 months. All the patients, at any age with both sexes presented with skin wound infection, were taken as study population. Wound swab was taken from all patients. Specimens were processed and bacteria were isolated and identified according to standard procedure. The ESBL status was confirmed by double disc diffusion test (DDDT) and minimum inhibitory concentration (MIC) by agar dilution method by standard procedure according to Clinical Laboratory Standard Institute (CLSI). Antimicrobial resistance was done by disc diffusion method. Result: A total number of 84 wound swabs were taken of which the most common ESBL producing bacteria were *Esch. coli* (61.5%), *Proteus* species (78.3%) and *Klebsiella* species (88.9%). All the isolates were sensitive to imipenem and nitrofurantoin followed by amikacin (92.9%). Conclusion: In conclusion, ESBL producing *E. coli* is the most common bacteria causing skin wound infection followed by *Proteus* species with a reduced sensitivity towards antibiotics.

Keywords

Extended Spectrum *β*-Lactamases, *Escherichia coli*, Klebsiella Species, Gram Negative Bacilli

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1. Introduction

Skin infections are very frequently encountered in clinical practice and are one of the most common sites of bacterial infections [1]. These infections are also among the most common indications for antibiotic therapy and hospital admissions [2]. However, increased use of antibiotics, particularly the third generation cephalosporin, has been associated with the emergence of *β*-lactamases [2]. These enzymes have serine at their active site and attack the amide bond in the beta lactam ring [3]. ESBLs are enzymes that mediate resistance to third generation cephalosporin as well as monobactams. Furthermore, these are inhibited *in vitro* by *β*-lactamase inhibitors such as clavulanic acid and tazobactam [4]-[6].

ESBLs have been reported worldwide in many different genera of *Enterobactericeae* and *Pseudomonas* species [7]-[9]. However, these are the most common in *Klebsiella pneumoniae* and *Escherichia coli* [10]-[12]. TEM-1 is the first plasmid mediated *β*-lactamase in Gram-negative bacteria [13]. Afterwards it was detected from *Klebsiella* species in Germany and France [5]. Later, ESBLs have been reported from all over the world. The true incidence is difficult to determine because of the difficulty and inconsistencies [13]. The high prevalence of ESBL genes indicates that the empirical treatment of serious infections and *β*-lactamase antibiotics except carbapenems is seriously compromised [14].

In Bangladesh Rahman *et al*. [15] reported 43.2% and 39.5% ESBL producing *Esch. coli* and *K. pneumoniae* respectively from urine, wound swab and pus. Skin wound is mainly caused by Gram Positive Cocci (GPC), but we take here only Gram Negative Bacilli (GNB) as ESBLs are mainly found in GNB. This is alarming to the patients as well as to the clinician. Proper burden of ESBL is needed to explore. Therefore, the present study was undertaken to see the status of ESBL producing bacteria isolated from skin wounds.

2. Methodology

This cross sectional study was conducted in the laboratory of the Department of Microbiology at Mymensingh Medical College, Mymensingh. This study was carried out from January 2011 to June 2011 for a period of 6 months. All the patients at any age with both sexes presented with skin wound infection who were attended at the OPD as well as the patients who were admitted in the IPD were taken as study population. Wound swab from all patients was taken by sterile swab stick. Thereafter, specimens were processed and bacteria were isolated and identified according to standard procedure [16]. Only GNB were taken in this study. All samples were routinely cultured on MacConkey's agar media and blood agar plates at 37˚C aerobically for 18 hours. Gram negative isolates were further identified by standard biochemical tests [16]. The susceptibility test was determined by Kirby Bauer method on Muller Hinton agar medium [17]. Two diagnostic tests were performed for phenotypic detection of ESBL producing bacteria which were disc diffusion test (DDT) [18] used as screening test for ESBL production and double disc diffusion test (DDDT) [18] as confirmatory test. MIC reduction methods [19] were also performed for detection of ESBL according to CLSI17 for further confirmation for ESBL. Screened for ESBL production by using disc diffusion test on Muller-Hinton agar where isolates showing inhibition zone size of ≥22 mm with ceftazidime (30 μg), ≥25 mm with ceftriaxone (30 μg), ≥27 mm with cefotaxime (30 μg), ≥ 27 mm with Aztreonam (30 μg) were suspected for ESBL production 18. ATCC 25,922 of *E. coli* was used as positive control strains. ATCC 25,922 of *E. coli* was used as a negative control. In double disk diffusion test (DDDT) a disc of ceftazidime (30 μg), cefotaxime (30 μg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10 μg) were used for each isolates. Both the discs were placed 25 mm apart, centre to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37˚C. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive 18. In MIC reduction test break point of ceftazidime was ≥8 μg/ml was taken ESBL positive 19. Statistical analysis was performed by SSPS 19.0. Qualitative variables were expressed by frequency and percentage.

3. Results

Among 84 specimens, wound swab were 45 (53.57%) and pus was 39 (46.42%). Majority bacterial isolates were ESBL positive which was 61 (72.6%) cases. The most common isolated bacteria was *Esch. coli* which was 39 (46.4%) followed by *Proteus* species, *Klebsiella* species, *Pseudomonas* species which were 23 (27.4%), 9 (10.71%) and 6 (7.1%). *Enterobacter* species and *Citrobacter* species were also detected in 7 (8.3%). ESBLs

production rate was higher among *Klebsiella* species (88.9%) followed by *Proteus* species (78.26%), *E. coli* (61.53%), *Pseudomonas* species (100%) and others (71.42%) (**Table 1**).

The antimicrobial susceptibility patterns of ESBL producers were measured. ESBL positive bacteria were 100.0% sensitive to imipenem and nitrofurantoin. On the other hand Aztreonam and Piperacillin were 100.0% resistant to ESBL producing bacteria. However, more than 80.0% resistance was found from Ampicillin (89.5%), Amoxiclav (89.3%), Ceftazidime (81.2%), Ceftriaxone (80.8%), Ciprofloxacin (84.4%) and Co-trimoxazole (85.1%). Quinolone and aminoglycosides were more resistant among ESBLs producers than non ESBL producers (**Table 2**).

4. Discussion

Skin wound infection is very common [4]. Multiple bacteria cause this infection. The irrational use of antibiotics to this infection cause partial elimination of susceptible of bacteria and favours the survival and multiplication of drug resistant bacteria in most of the occasions [15]. Proper use of antibiotics is very important for various reasons.

Table 1. Detection rate of different isolates in the study population.

Name of the organism	ESBL positive	ESBL negative	Total
E. coli	24 (61.5%)	15 (38.5%)	39 (100.0%)
Proteus species	18 (78.3%)	5 (21.7%)	23 (100.0%)
Klebsiella species	8 (88.9%)	1 (11.1%)	9 (100.0%)
Pseudomonas species	6 (100.0%)	0 (0.0%)	6 (100.0%)
Others	5 (71.4%)	2 (28.6%)	7 (100.0%)
Total	**61 (72.6%)**	**23 (27.4%)**	**84 (100.0%)**

**E. coli = Escherichia coli.*

Table 2. Antimicrobial resistance pattern of ESBLs producer and non ESBLs producer among skin wound isolates.

Antibiotics tested	Antimicrobials resistance to ESBL positive (n = 61)	Antimicrobials resistance to ESBL negatives (n = 23)
Ampicillin	89.5%	58.2%
Amoxiclave	89.3%	51.5%
Amikacin	5.6%	1.9%
Azithromicine	48.1%	21.0%
Aztreonam	100.0%	100.%
Ceftazidime	81.2%	50.8%
Ceftriaxone	80.8%	31.8%
Cefotaxime	77.3%	38.1%
Gentamicine	44.7%	38.0%
Ciprofloxacin	84.4%	74.4%
Nitrofurantoin	0.0%	0.0%
Piperacillin	100.0%	100.0%
Imipenem	0.0%	0.0%
Co-trimoxazole	85.1%	46.3%

In the present study, the isolated gram negative bacteria from skin wound were *E. coli* (46.4%), *Klebsiella* species (10.7%), *Proteus* species (27.4%), *Pseudomonas* species (7.1%) and others (8.3%) which correlate with the study done by Haque *et al*. [20] in the same hospital. *Klebsiella* species (88.9%) was the leading ESBL producers from skin wound followed by *Proteus* species (78.3%), *Enterobacter* species (71.4%), *E. coli* (61.5%) and *Pseudomonas* species (100.0%). In another study in Bangladesh Haque and Salam21 have reported that ESBL production for *Klebsiella* species was 57.9% followed by *Proteus* species (50.0%), *E. coli* (47.8%) and *Pseudomonas* species (31.3%) [21]. The high frequency of ESBLs in *Klebsiella* species is of great concern since infections caused by this bacterium were very common. In addition to that resistance of the organism may be due to the presence of some virulence factor like hyper viscosity, polysaccharide capsule and production of endotoxin, carbapenemase, which make it more resistant [7]. Furthermore, they also spread easily with pathogenic and efficient at acquiring and disseminating resistance plasmid [3] [13].

In this present study the occurrence of ESBLs observed among the *Pseudomonas* species may not reflect the actual picture because of very small sample size. Prevalence of ESBL in Bangladesh was 41.1% [14] and 41.7% [21] in 2004 and 2010 respectively. In few studies from Pakistan [22] it was found 40.0% and two other studies were 43% [23] and 58.7% [24] ESBLs producers. Several studies from India reported as ESBLs producers were 40.8% [10], 51.4% [25] and 53.8% [26] respectively. In Nigeria [13] ESBL production rate was 66.7%. The frequency of ESBL producer in the present study was 72.0% in general which was higher than the previous studies in Bangladesh [21], India [26] and other countries [22]. It may be due to steadily increasing the incidence of ESBL producing strains among the clinical isolates. Another two studies in Iran [27] and India [28] were reported 96.0% and 97.0% respectively. In the present study, some samples were taken which were sensitive to 3GCs and subsequently showed positive for ESBLs production by DDDT (40.0%) [29], as because failure to detect ESBL production by routine disc-diffusion tests has been well documented [30] [31]. However, the study that has been reported in Iran [27] and India [28] showed higher rates of ESBL producing bacteria than the present study and it may be due to the fact that they consider only 3GCs resistant organisms. Occurrence and distribution of ESBLs differs from country to country and from hospital to hospital [24].

Development of multidrug resistance in clinical isolates like *Pseudomonas* species and *Klebsiella* species has been reported in Bangladesh [32] ESBLs production coexisted with resistance to several other antibiotics because ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics [33]. It has been found such associated resistance with co-trimoxazole (85.1%), gentamicin (44.7%) and fluoroquinolones (84.4%). In this study aztreonam, ampicillin, amoxyclauve were found 95.0% - 100% resistant which is an agreement with other studies [10] [23]. In the present study ceftriaxone, ceftazidime and cefotaxime were found 80.8%, 81.2% and 77.3%, which correlates with the study done by Sasirekha *et al*. [10] and Singh and Goyal [34] which was found 84% resistance to cefotaxime and 75% and 85% resistant for ceftriaxone and ceftazidime respectively [10] [34]. Aminoglycosides have good activity against clinically important gram negative bacilli [35]. In the present study 82.1% isolates were susceptible to amikacin followed by gentamicin (41.8%). This is similar to Sasirekha *et al*. [10]. Several studies showed that amikacin was more sensitive than gentamicin; however, if it is used irrationally, then it may also become resistant. In another study it was reported that gentamicin was 59.0% resistant in India [10] and 55.5% in Bangladesh [21]. These variations may be due to increased use of gentamicin, caused by selection pressure of aminoglycosides in different region [36]. Carbapenem is the drug of choice for many infections caused by Gram positive and Gram negative bacteria [23]. In this study imipenem was 100% sensitive. These findings were similar to study done by Haque and Salam [21]; however, another study showed 3.1% resistant to imipenem in Bangladesh [21] [37]. Amikacin was the second most common sensitive drug after imipenem. Therefore, these drug resistant bacteria have limited therapeutic options and necessitated the increased use of carbapenem. Beta lactamases are found in *K. pneumoniae* as *K. pneumoniae* carbapenemase (KPC) which is resistant to imipenem and has been spread worldwide [38]. Therefore, there is a very limited option to treat imipenem resistant strains; in that situation, colistin may be the drug of choice [39], though it has many side effects. Since co-resistance to non β lactam antibiotics like ciprofloxacin, co-trimoxazole and gentamicin was observed, amikacin and nitrofurantoin were found to be alternatives for treating such patients at low cost.

5. Conclusion

In conclusion, skin wound is the most commonly infected by ESBL producing *E. coli* followed by *Proteus* spe-

cies with reduced sensitivity profiles among the GNB. An indiscriminate use of the higher antibiotics should be restricted as far as possible. The infection control programs should be monitored continuously in hospital. As a developing country adequate laboratory facilities should be provided to diagnose.

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