Detection of Extended-Spectrum β-lactamases' (ESBLs) Resistance among Wound Infection Pathogens in Khartoum State

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Abstract

The presence of extended-spectrum- β lactamases (ESBLs) in a clinical infection can result in treatment failure if one of the third-generation cephalosporins is used. ESBLs detection has both clinical as well as epidemiological relevance. The present study was conducted to detect the frequency of ESBL producing pathogens that causes wound infections. Hundred wound samples were collected from Khartoum state Hospitals were investigated for isolation and identification (colonial characteristics, Gram reaction, biochemical reactions, and antibiotic susceptibility). The frequency of isolates from all cases was 95 (95%) with 5 (5%) yielding no growth. The isolates were *E.coli*, *Klebsiella* species, *staph aureus*, *Proteus* species and *Pseudomonas aeruginosa* which isolated from 27(28.4%), 13(13.68%), 23(24.21%), 20(21.05%) and 12(12.63%) respectively. The isolates were subjected to show their susceptibility against the third generation cephalosporins (Cefotaxime, Ceftazidime and Ceftriaxone) using disk diffusion method. The results were observed that 60(63.1%) out of 95 isolates shown resistance to third generation cephalosporins, while35 isolates were sensitive to these antibiotics. The sixty resistant isolates were subjected to show their ability to produce ESBL using Kirby _Bauer Method (Calvulanic acid + Third generation cephalosporins). Among 18 *E.coli*, 4 *Klebsiella species*, 16 *Staph aureus*, 12 *Proteus* species, 10 *Pseudomonas aeruginosa*, isolates which were tested, 6(33.33%), 1(25%), 9(56.2%), 3(30%) and 3(30%) were found to be ESBL producer respectively. We conclude that the ESBL producers were found in large proportions which may refers to misusing of antibiotics or inadequate treatment.

Key words: Extended-Spectrum- β lactamases (ESBL), wound infection pathogens, Kirby _Bauer Method, Disk diffusion method, Cephalosporins, Sudan

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Introduction

Wound is a breach in the skin and exposure to subcutaneous tissue following loss of skin integrity providing a moist, warm and nutritive environment that is conductive for colonization and proliferation of opportunistic and pathogenic microorganism.
Wounds can be classified into two types (a) open wounds which include incisions, lacerations puncture wounds, gunshot wounds and abrasions. (b) Closed wounds which include contusions more commonly known as bruises, hematomas crush injury [(1).]
Beta lactamases are enzymes produced by bacteria that inactivate either penicillin or cephalosporins, but some can inactivate both classes of drugs. Most Gram positive bacteria secrete beta-lactamases so that beta-lactam drugs are inactivated extracellulry, i.e., in the surrounding medium. By contrast, the beta-lactamase of gram negative remains inside the cell and inactivate beta lactm drugs in periplasmic space, i.e., the space between the outer membrane and cytoplasmic membrane ([(2).] ESBLs are enzymes capable of hydrolyzing extended spectrum cephalosporins, penicillins and monobactam, but inactivated against cephamycin and imipenem. Wide spread use of third generation cephalosporins and azetronams is believed to be the major cause of the emergence of the ESBLs [(3).]

The term ESBLs is used to mean acquired class A β-lactamases that hydrolyze and confer resistance to oxyimino- '2nd - and 3rd -generation' cephalosporins, eg cefuroxime, cefotaxime, ceftazidime and ceftriaxone [(4).]

Criteria:

- Are capable of inactivating extended spectrum cephalosporins (first, second, third, fourth -generations and the monobactam aztreonam).
- Are inhibited by beta lactases inhibitors, such as clavulanic acid, cabapenem-sulbactum, tozabactam and to temocillin ([(5).]

These new enzymes were given the name ESBLs that reflect the fact that they were the older beta lactamases and had a new capability to hydrolyze a broader spectrum of beta lactam drugs [(6).]

ESBLs are not the only β-lactamases to confer resistance to 2nd and 3rd generation cephalosporins while sparing carbapenems, but are the most important. Moreover, as plasmid-mediated enzymes, they have great potential for spread. They occur mostly in Enterobacteriaceae (eg *E.coli Klebsiella* species and *Enterobacter* species) and rarely in non-fermenters (eg *P. aeruginosa*). They should be distinguished from other important modes of resistance to 2nd and 3rd generation cephalosporins [(7).]

The introduction of third-generation cephalosporins improved the effectiveness of therapy for the vast majority of infections caused by gram-negative bacteria; however, the use of these highly β-lactamase stable compounds has led to the emergence of resistant species[(8).]

In Dhaka medical college, Bangladesh a total of 320 samples were collected during July 2010 and June 2011 from the department of microbiology. Out of the 320 samples 169 (53%) gram negative bacteria were isolated, comprising 90(53%) *E*.coli, 25(15%) *K.pneumniae,* 15(9%) proteus spp and 39 (23%) pseudomonas spp. From the isolated gram negative

bacteria , 42 (25%) were ESBL producers detected by Double Disk Synergy test all of them were resistant to amoxicillin, azitroenam, cephradine, cefuroxime , cefotaxime , ceftraxione, and ceftazidime [9] .

Materials and Methods

This was Descriptive-cross-sectional study which had been conducted in Khartoum state during September to December 2014, 100 wound infected patients were enrolled. Ethical clearance was taken from research committee of AL Neelain University and verbal consent was taken from each individual with wound infection. Data were analyzed by Excel and Statistical Analysis System (SAS) soft wire.

Experimental Work

Specimen's collection:

Wound specimens were collected from 100 patients, under aseptic condition using sterile cotton swabs.

Culture and identification:

Wound specimens were inoculated onto blood agar and Macconkey media and incubated at 37°C over night. The isolates were identified by observing morphology and using conventional biochemical tests.

Antimicrobials susceptibility test

An overnight culture suspension of the test isolates which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller Hinton agar plate. A disc of 30-µg disc of Cefotaxime, Ceftazidime, and Ceftriaxone were placed 15 mm apart. After incubating overnight at 37°C, the presence of inhibition zones of discs was interpreted as positive for 3rd-generation cephalosporins if the zone read more than 22 mm (10).

Extended-spectrum β-lactamases' (ESBLs) resistance detection

Combination Disk Method

The combination-disk test using Cefotaxime, Ceftazidime, and Ceftriaxone alone and in combination with clavulanic acid was performed for the detection of ESBL according to the CLSI guidelines. In this test, an overnight culture suspension of the test isolates which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate. The Cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart on the agar, ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg / 10 µg) disks were placed 20 mm apart and Ceftriaxone(30 µg)and Ceftriaxone-clavulanic acid (30 µg / 10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, a ≥ 5-mm increase in the zone diameter for either

antimicrobial agent which will test in combination with clavulanic acid vs. its zone when test alone, was interpreted as positive for ESBL production(figure1) [5].

Results

A total of 100 wound specimens which were collected from hospitals of Khartoum State – Sudan during september to December 2014. The frequency of isolates from all cases was 95(95%) with 5(5%) showed no growth. Gram negative organisms accounted for 72(75.78%) of all isolates while gram positive accounted for 23(24.21%). Among gram negative organisms, *Escherichia coli* accounted for 27(28.4%), *Klebseilla spps* 13(13.68%), *Proteus spps* 20(21.05%) and *Pseudomonas aeruginosa 12(12.63%)*, while *Staphylococcus aureus* was only gram positive isolate with a frequency of 23(24.21%) (Table 1). From these 95 isolates 60(63.1%) microorganisms were showed resistance to third-generation cephalosporins and they were tested for the ESBL production and 35(36.8%) were sensitive to these antibiotics. These tested microorganisms exhibit resistance to the third-generation cephalosporins as *E.coli 18* (30.00%), *Klebseilla spps 4* (6.6%), *Staph aureus16* (26.6%), *Proteus spp* 12 (20%), and *Pseudomonas aeruginosa* 10 (16.6%). Among these isolates, 6 out of 18 *E.coli* (33.33%), 1 out of 4 *Klebseilla spps* (25.00%), 9 out of 16 staph aureus (56.2%), 3 out of 12 *Proteus spps* (25.00%), 3 out of 10 *Pseudomonas aeruginosa* (30%) were detected to be ESBL producers (table2). The overall of ESBL producers were 22 isolates (36.67%). Highest rate of ESBLS was observed in *Staph aureus 9* (56.2%) out of 16, followed by *E.coli 6* (33.33%) out of 18, *Pseudomonas aeruginosa* 3 (30%) out of 10, Proteus *spps 3*(25.00%) out of 12, *Klebseilla spps 1* (25.00%) out of 4.

Discussion

ESBL producing strains are gradually increasing, especially in nosocomial infections throughout the world. The occurrence of ESBL producers among clinical isolates vary greatly worldwide and are rapidly changing over time. Gram negative organisms accounted for four times the number of isolates than gram positive; this is in agreement to a similar study in Nigeria 2004 by Kehinde *et al* that reported a frequency of 72.0% for gram negative organisms and 28.0% for gram positive [11].

The isolated microorganisms are similar to the observation in some centers in Nigeria where *Pseudomonas spp, Klebsiella spp, E. coli* and Coliforms are the predominant pathogens responsible of wound and other nosocomial infections [12].The present study detect (21.6%) ESBL producing gram-negative bacteria which is closely simillar to Previous studies in Bangladesh which revealed 23% to 31% ESBL producers from gram-negative bacteria [13]. However, the proportion of ESBL producers was reported as 80% in another study in Bangladesh [14]. higher results of *staph aurues* (56.2%) which was the predominant ESBL

producers in this study is higher than the reported result from government hospital in south India(37.7%) [15] , also in this study it found 33.33% *E.coli* , 25% *Proteus spps and 25% Klebseilla spps* were ESBL producer which similar to result from Bangladesh *(*32% ,20% and 20%) respectively [9] , and 30% *Pseudomonas aeruginosa* which similar to result from Iran (28%) [16].

Table (1): The frequency of isolated organism from wound swabs specimens (n=95)

No	Bacterial isolates	Total No of isolates	Percintage %
1	*E.coli*	27	28.4
2	*staph aureus*	23	24.21
3	*Proteus spps*	20	21.05
4	*Klebseilla spps*	13	13.68
5	*Pseudomonas aeruginosa*	12	12.63
	total	95	100%

Table (2): Number and percentage of ESBL production among different wound infection pathogens

Organism	Positive ESBL (no %)	Negative ESBL (no %)	total
E.coli	6(33.33)	21(25)	27
staph aureus	9(56.2)	14(23.3)	23
Proteus spps	3(25)	17(28.33)	20
Klebseilla spps	1(25)	12(20)	13
Pseudomonas aeruginosa	3(30)	9(15)	12
total	22 %	73 %	100 %

Figure (1): ESBL production by *staph aureus* to Ceftriaxone-clavulanic acid (CTC), Ceftazidime-clavulanic acid (CZC), and Cefotaxime-clavulanic acid (CFC).

Conclusion

The routine susceptibility tests by clinical laboratories fail to detect ESBL producer strains**.** The result presented in this study confirms that the most commonly used antibiotics against wound infections become increasingly resistant and it is the right time to ensure the judicial use of antibiotics, and treatment will be done by using carbapinim group of drugs .National committee of clinical laboratory standers (NCCLS) recommended that when ESBL production is confirmed result should be reported as resistance to all pincillins, excluding cephamycins.

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