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Detection of Extended-Spectrum β-Lactamases producing Bacteria among Endoscopy Healthcare Workers in Khartoum State

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Abstract

Background: Extended-spectrum β-lactamases (ESBL) producing organisms pose unique challenges to clinical microbiology professionals and clinicians. The rapid and irreversible increase in antimicrobial resistance of pathogenic bacteria that had been observed over the last two decades is wildly accepted to be the major problems of human medicine today.

Objective: To detect ESBL producing bacteria among endoscopy healthcare workers in Khartoum State

Materials and methods: 150 samples were collected from the gloves of health care workers in these hospitals. Isolates were identified, tested for antimicrobial susceptibility, and screened for ESBL production as per standard methods. The double-disk diffusion method was used to confirm ESBL production using antimicrobial disks of ceftazidime (30 µg), ceftriaxone (30 µg), with or without clavulanic acid (10 µg). A zone difference of >5 mm between disks was considered indicative of ESBL production.

Results: ESBL producing isolates yielded high resistance rate than non-ESBL producing organisms. The resistance for trimethoprim-sulfamethoxazole was (42.4%) and for azithromycin was (65.5%). Hand hygiene was found to be the main procedure to decrease the nosocomial infection.

Conclusion: The world-wide prevalence of extended-beta-lactamase producing organisms is increasing rapidly. Infection due to ESBL producing organisms is associated with an increase in morbidity, mortality and health care cost. Hand hygiene is considered an important procedure to eliminate infections among healthcare workers.

Key words: ESBL producing bacteria, Endoscopy healthcare workers, Khartoum State

Introduction:

The main mechanism of bacterial resistance to the b-lactam class of antibiotics consists of the production of β-lactamases, which are hydrolytic enzymes with the ability to inactivate these antibiotics before they reach the penicillin-binding proteins located at the cytoplasmic

membrane. The extended-spectrum β-lactamases (ESBLs) are classified in the molecular (Ambler) class A and functional (Bushe Jacobye Medeiros) group 2be. They are characterized by the ability to hydrolyze an oxyimino-b-lactam at a rate of 10% of that for benzyl penicillin along with inhibition by clavulanic acid. The presence of ESBLs in various members of the *Enterobacteriacea*e family, particularly *Klebsiella pneumoniae* and *Escherichia coli*, is of great microbiological and clinical importance[1].

Since 20 years ago, the extended-spectrum β-lactamase (ESBL) producing organisms had become common organisms in medical institutions of many countries and of large geographic regions. Such infections are clinical problems created by nosocomial infection with very limited therapeutic options[2].

Various factors of different kinds facilitate their spread, including the diversity of parental B-lactamases in ESBL evolution, the worldwide prevalence of their specific types, the relative ease of emergence of ESBL, the motility of ESBL and finally the strong selective pressure of antibiotic use[3].

The insufficiently monitored movement of infected or colonized patients between different medical institutions often causes a very wide geographic spread of particular ESPL variant and their producer strains. All factors together illustrate that ESPL epidemiology today is very dynamic and the prediction of its consequences in the future is difficult[4].

In Sudan members *Enterobacteriaceae* specifically, *E. coli*, are the main causes of urinary tract infections (UTIs). *Staphylococcus saprophyticus (S. saprophyticus)* is the second most common cause, and lesser percentages are caused by others such as *Proteus* and *Klebsiella* or by *Enterococcus* or by *Pseudomonas* species[2].

ESBL producing *Entrobacteriaceae* were first reported in1980 in Europe and had since become a world problem. This had increased morbidity, mortality and costs in treating the infection they cause. ESBL are not the only extended-spectrum β-lactamases to form resistance to first, second, and third generation cephalosporin but are the most important. ESBL producing organisms cause infections such as urinary tract infection in hospital patients as well as those treated in community. *Entrobacteriaceae* are a well-known cause of healthcare association infection[5].

ESPL producing bacteria become resistant to almost all common anti-bacterial drugs. This resistance phenomenon had been blamed for the excessive use of antibiotics in the community and the hospitals. ESPL producing bacteria had spread across world. Health authorities must be aware of this problem to determine the enormity of the problem and to attempt to limit the spread of ESPL producing bacteria and enhance laboratory ESPL testing and establish surveillance programs in order to enormity of the problem as base for initiation of preventive measures.

The present study dealt with investigation of the types of ESPL producing bacteria carried over in hands of healthcare workers in endoscopy units in hospital. The study also looked in measures to prevent ESPL producing bacteria transmission in hospital. Detection of ESPL producing bacteria in the hands of healthcare workers may raise the awareness of healthcare workers as regard ESPL producing bacteria. Healthcare workers may also be aware of the role of hand-hygiene in infection control. Furthermore, the results of the study shall provide a base line for detection of ESPL producing bacteria.

Materials and methods

This study was a descriptive, cross-sectional, case finding, hospital and laboratory-based study. It had a qualitative approach aiming to screen healthcare workers in endoscopy units. Samples were collected from healthcare workers of endoscopy units in Khartoum State. The study was carried out during the period from June 2015 to February 2016, including the literature review, samples collection, laboratory work, data analysis, and paper writing. The study area was facility based and applied at hospitals of Omdurman, Al Ribat, and Ibn Sina Teaching Hospitals (Khartoum State). Inclusion criteria were all healthcare workers of endoscopy units. Exclusion criteria were all healthcare workers not working in endoscopy units. Sampling technique was a random, non- probability purposive sampling. The sample frame was healthcare workers of endoscopy units; and the sample strategy was a convenience type where healthcare workers were chosen on the basis of accessibility. The sample size was calculated according to the following standard, statistical equation:
$N = Z^2PQ / D^2 + 10\%$ (non-respondent rate) where: N is the sample size; P is the sample proportion; Q is equal to (1-P); Z is the appropriate cut-off point on the standard normal distribution (standard value of 1.96); D is the degree of precisions margin of error at 5% (standard value of 0.05). Considering the prevalence rate of this study as 8% then:
P = 0.8; Q =1-0; .8=0.2, Z =1.96; D =0.05. Therefore: N = 138 + 10% non-respondent rate.
i.e. 138 + 3.7 = 141.7. Approximately sample size was taken as 150 specimens.
The software used for the analysis of data was Statistical Package for Social Sciences (SPSS) program (version 14). Data was checked, validated and analyzed using Excel program. Frequencies, percentages, tables and graphs were used for presentation of the data. Complete information regarding risk factors was handed to all subjects under the study and no concealment what so ever. Confidentiality of information obtained from subjects under the study was maintained. Valid, verbal consent of the subjects under the study was taken. Permission to collect the samples was obtained from the different hospitals included in the study. The study findings may be shared with others by the following means: presentations in conferences, symposia, workshops, and scientific meetings; or by publishing results in scientific journals, memoranda, textbooks, and internet sites; or by lectures, seminars, and tutorials addressed to undergraduate and postgraduate students as well as members of the medical profession.
After explaining the procedure and aim of the study to the healthcare workers, 150 healthcare workers were investigated. Under aseptic conditions finger-prints were stamped on Mac Conkey agar culture medium plates. Then all plates were incubated at 37°C overnight under aerobic conditions. The next day, the resultant colonies were examined macroscopically and microscopically. Gram stain was performed to check the morphology of the colonies. The isolates were identified using different, standard biochemical reactions. Oxidase test, Kligler iron agar test, citrate utilization test, urease test, and indole test were used for identification of Gram negative bacilli. While catalase test, DNase test, coagulase test, and novobiocin sensitivity test were used for identification of Gram positive organisms.
Extended-spectrum cephalosporin antibiotics (ceftazidime and cefotaxime) were used for sensitivity testing. Antibiotic susceptibility tests were conducted using the agar diffusion technique. In this technique, 38g of Muller-Hinton agar were dissolved in one liter of distilled

water, sterilized by autoclaving and poured in Petri dishes to give a depth of 3-4 mm. The plates were left to solidify and dried until there were no droplets of moisture on the agar surface. Inocula were prepared by suspending one colony of the tested isolates in 3 ml of sterilized normal saline in test tubes. The plates containing Muller-Hinton agar were inoculated by immersing a swab in the inoculum and spreading it onto the entire surface of the medium. After drying, the third generation cephalosporins, i.e. ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg); were transferred with a forceps and pressed gently onto the surface of the medium to ensure even contact. The plates were then incubated overnight at 37°C. The diameters of inhibition zones were measured in mm, and read as per the guidelines of the Clinical Laboratory Standards Institute (CLSI). Inhibition zones were interpreted as susceptible, intermediate or resistant. If the organism was sensitive, it was considered as β-lactamase sensitive but not an ESBL producer. On the other hand, if the organism was resistant, it was considered an ESBL producer. All isolates showing resistant zones were confirmed by the double-disk synergy test using β-lactam and β-lactamase-inhibitor disks. This test is a convenient method of detecting extended-spectrum β-lactamase producing Gram-negative bacilli, according to the National Committee for Clinical Laboratory Standards (NCCLS). The test was performed by suspending an overnight culture of the test isolate in a McFarland broth (turbidity No. 0.5) and swabbed on a Mueller–Hinton agar plate. After drying, amoxicillin-clavulanic acid (amoxyclav) disc and the extended spectrum cephalosporin antibiotics (e.g. ceftazidime, cefotaxime, and ceftriaxone) were placed 20 mm apart. After overnight incubation, the presence of an enlarged inhibition zone indicating a positive synergy test was noted. This enlargement was due to inhibition of the ESBL by the amoxyclav disc, showing a key-hole phenomenon[15].

Fig. (1): Growth of ESBL by double disk synergy test on Muller Hinton agar showing the key-hole phenomenon

Results

150 gloves finger-print specimens were collected from 45 healthcare workers in endoscopy units at Omdurman, Al Ribat, and Ibn Sina Teaching Hospitals. The number of specimens that gave pathogenic growth was 34 (23%); while 116 specimens (77%) showed no bacterial growth. Of the 34 isolated organisms, 29 specimens (85.3%) were positive for ESBL. *E. coli* was the commonest pathogen (24/70.6%) among the positive isolates. Other organisms isolated were *Staphylococcus aureus*, *Proteus vulgaris*, and *Citrobacter freundii* (Table I).

Table (I): Frequency rate of isolated organisms

Isolated organisms	Frequency rate	ESBL Frequency rate
E. coli	24 (70.6%)	21 (72.5%)
Staphylococcus aureus	5 (14.7%)	3 (10.3%)
Proteus vulgaris	2 (5.9%)	2 (6.9%)
Citrobacter freundii	3 (8.8%)	3 (10.3%)
Total	34 (100%)	29 (100%)

The age incidence of healthcare workers investigated was 35-65 years (mean 50 years). The largest number of positive specimens (27/ 60%) was collected from healthcare workers aged 35-45 years. While the fewest number of positive specimens (5/11%) was collected from healthcare workers aged 56-65 years. The gender incidence of healthcare workers with positive were 16 (36.3%) males, and 29 (64%) were females (Table II)

Table (II): Frequency rate of positive isolates according to gender and age incidence

Age range (Years)	Frequency rate		Gender	Frequency rate
35-45	27 (60%)			
46-55	13 (29%)		Male	16 (36%)
56-65	5 (11%)		Female	29 (64%)
Total	45 (100%)		Total	45 (100%)

The distribution of positive isolates were 20 (44%) from Ibn Sina Teaching Hospital, 13 (29%) from Al Ribat Teaching Hospital, and 12 (27%) from Omdurman Teaching Hospital.
35 positive specimens (78%) were collected from postgraduate healthcare workers, and 10 specimens (22%) were collected from graduate healthcare workers.
Most positive isolates (35/78%) were isolated from healthcare workers used to wash their hands after patient contact. All positive isolates (45/100%) were found in specimens collected from healthcare workers washing their hands with soap and water only; and 44 isolates (98%) were found in specimens collected from healthcare workers using alcohol as a disinfectant. No isolates were found in specimens collected from healthcare workers using chlorhexidine as a disinfectant (Table III).

Table (III): Hand washing of healthcare workers

Hand Washing	Frequency rate		Disinfectants	Frequency rate
Prior to patient contact	10 (22%)		Alcohol	44 (98%)
After patient contact	35 (78%)		Soap & Water	45 (100%)
Total	45 (100%)		Chlorhexidine	0 (0%)

All healthcare workers used to wear gloves prior to patient contact; and they change gloves for every new patient. 34 positive isolates (76%) were detected in specimens collected from healthcare workers wear finger rings. No healthcare worker was using artificial finger nails.

Discussion:

Correct identification of ESBL-producing organisms is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms[16].
In this study 150 samples were collected from 45 healthcare workers in endoscopy units at Omdurman, Al Ribat, and Ibn Sina Teaching Hospitals. The outcome of the study was 34 isolates, 29 (85.3%) of them were ESBL producers organisms. In another study the prevalence of *E. coli*-ESBL producer was 30.2%[17].
Another study carried out in Nigeria reported that the frequency of ESBL production *E coli* and *Klebsiella* species was 53%[18]. Another study carried in Tanzania showed that ESBL production rate was 28.2%[19].
ESBL detection is not routinely carried out in many microbiology laboratories at hospitals in developing countries[20]. The emergence of ESBL producing strains created a need for laboratory testing methods to detect these enzymes among bacterial pathogens. Such methods are considered effective techniques for detection of ESBL producers[21].
In our study high resistance rates of ESBL-producing organisms were noticed against the first line therapeutic drugs such as trimethoprim and azithromycin. Similar findings had been

reported in other developing countries[22] as well as developed countries[23].
Also in Sudan, Ahmed and his co-workers[24] (2001) reported high resistance rates among ESBL-producing strains to the drugs: amoxicillin, trimethoprim-sulfamethoxazole, tetracycline, nalidixic acid, ciprofloxacin and amoxicillin-clavulanic acid.
High attention should be directed to maintain infection control practices in endoscopy units in order to prevent the spread of ESBL strains through the following means: ensuring health care professional practice hand hygiene, good processing to equipment, proper environment endoscopy, and prevention of bacterial colonization[25].
In Sudan, Eltayeb and his colleagues (2012) reported a frequency rate ESBL producing *E.coli* as 65.2%. This finding was lower than the frequency rate (85.3%) detected in our study[26].
Conclusion: The world-wide prevalence of extended-beta-lactamase producing organisms is increasing rapidly. Infection due to ESBL producing organisms is associated with an increase in morbidity, mortality and health care cost. Hand hygiene is considered an important procedure to eliminate infections among healthcare workers.
Recommendations:

1. Regarding the outcome of this study it may be recommended to apply hand hygiene as fundamental infection control program both prior and after gastrointestinal endoscopy.
2. Physicians should be aware of the ESB L producing bacteria, and should know how to treat infections caused by such organisms.
3. Laboratory staff should be well trained on methods used for detection of ESB L producing bacteria.
4. Measures should be adopted to ensure patient safety, optimal treatment, and control of the spread ESBL producing organisms.

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