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Prevalence of extended spectrum β-lactamases (ESBLs) among clinical *Enterobacteriaceae* isolates obtained from private diagnostic laboratory in Kano - Nigeria

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ABSTRACT

One hundred Gram negative bacterial isolates were collected from a private diagnostic center and identified by subjecting the isolates to biochemical tests using standard procedures. Confirmed Enterobacteriaceae isolates were further subjected to screening for extended spectrum β-lactamases (ESBLs) production using Clinical Laboratory Standards Institute (CLSI) breakpoint and Double Disc Synergy Test (DDST). Standard discs of Augmentin (AUG 30 µg) {Optudisc} and Ceftriaxone (CXM 30 µg) {Medireich} and Ceftazidime (CAZ 30 µg) {Glaxo-Smithkline} were used in the screening. The results of CLSI breakpoint test showed that 87% were ESBLs producers viz: *Citrobacter fruendii* (3), *Escherichia coli* (46), *Klebsiella pneumoniae* (13), *Morganella morganii* (1), *Proteus vulgaris* (23) and *Salmonella typhi* (1) while that of confirmed ESBLs producers using DDST was 49%. These included *Citrobacter fruendii* (2), *Escherichia coli* (25), *Klebsiella pneumoniae* (7), *Morganella morganii* (1), *Proteus vulgaris* (13) and *Salmonella typhi* (1). The implication of the results is discussed.

Keywords: Beta lactamases, Antibiotic resistance, Enzymes, Bacteria

INTRODUCTION

Betalactam antimicrobial agents constitute over 50% of global antibiotics used in the treatment of infections caused by bacteria (Kotra et al., 2002) and bacterial resistance to antibiotics has significantly increased in recent years due to spread of plasmid-mediated β-lactamases (Sanders, 1992).

Extended spectrum β-lactamases (ESBLs) are enzymes that confer variable level of resistance to oxyiminocephalosporins such as cephotaxime, ceftazidime and monobactams. They occur predorminantly in the family Enterobacteriaceae with *Klebsiella pneumoniae* being the most commonly reported worldwide and it is responsible for 5-20% of outbreaks of nosocomial infections in intensive care units, burn, oncology and neonatal units (Kotra et al., 2002). More than 200 different natural ESBLs variants are known at present which accounted for resistance in an increasing variety of Gram-negative species (Bradford, 2001) with their distribution being far from uniform (Marchandin et al., 1999). Patients at risk of infection with ESBLs-producing bacteria are seriously ill patients with prolonged hospital stays and those in whom invasive medical devices (such as catheters) are present for prolonged duration.

The length of hospital stays prior to isolation of ESBL producer range from 11-67 days (Lautenbach et al., 2001). Other risk factors for infection include presence of nasogastric tubes (Asensio et al., 2000), recent surgery and poor nutritional status (Paterson and Bonomo, 2005), haemodialysis (D'Agata et al., 1998) as well as selective pressure on the use and overuse of antibiotics (Cosgrove et al., 2002).

Four hundred Enterobacteriaceae isolates obtained from Muhammad Abdullahi Wase Specialist Hospital, Kano, Nigeria were screened for extended-spectrum β-lactamases (ESBLs) production using Double Disk Synergy Test (DDST) and the National Committee for Clinical Laboratory Standards (NCCLS) Test using standard disks of Augmentin (AMC 30 μg) (Oxoid, England), Cefotaxime (Ce 30 μg) (Hi-Media, India), Ceftazidime (Ca 30 μg) (Hi-Media, India) and Ceftazidime/Clavulanic acid (Cac 30μg) (Hi-Media, India). The results showed that 37 (9.25%) isolates were ESBL producers based on DDST while only 20 (5.00%) were positive using the NCCLS breakpoints (Yusha'u et al., 2007).

The collapse of primary healthcare system in Nigeria has resulted in most people purchasing antibiotics on counters and in some cases from hawkers, which expose them to the danger of acquiring ESBLs producing bacteria. The frequent prescription of β-lactam antibiotics coupled with the misuse of antibiotics in Kano (Nigeria) calls for the need to detect the occurrence or otherwise of ESBLs producing bacteria so as to assess the danger and address the problems associated with the use of β-lactam antimicrobials in treatment of infections. This work was aimed at detecting the occurrence and distribution of extended spectrum β-lactamases among clinical bacterial isolates belonging in the family Enterobacteriaceae.

MATERIALS AND METHODS

Sample collection site

The site for sample collection was a private Diagnostic Laboratory in Kano, a referral laboratory where people from different parts of the city and of different nutritional status patronize. This justified the choice of the laboratory as the study site as nutrition is among the predisposing factor for infection with resistant bacterial species (Paterson and Bonomo, 2005).

One hundred (100) Enterobacteriacae isolates used in the study were collected from a private diagnostic laboratory in Kano, Northern Nigeria. The identity of the isolates was confirmed using biochemical tests (citrate, indole, methyl red, urease and Triple Sugar Iron tests). Pure cultures of the identified isolates were preserved on nutrient agar slants and stored at 4 ^{o}C until required for use (Cheesbrough, 2004).

Standard antibiotics

The antibiotic discs used were Augmentin (AUG 30 μg, Optudisc), Cefriaxone (CXM 30 μg, Mederich) and Ceftazidime (CAZ 30 μg, Glaxo-Smithlkline).

Inoculum standardization

Loop full of each isolate were dispensed in sterile normal saline to match the 0.5 McFarland standard to obtain the standard bacterial cells concentration (3.3 x 10^6) for sensitivity tests as described by Clinical Laboratory Standards Institute (CLSI, 1999).

CLIS Breakpoint Test for ESBL screening

The standard inocula of the isolates from Brain Heart Infusion Agar plates were inoculated using sterile swab sticks onto the surface of Mueller Hinton agar and standard discs containing Ceftriaxone and Ceftazidime discs were placed independently. The plates were incubated at 35 ^{o}C for 18-24 hours after which the plates were read by measuring the zone of inhibition formed by the isolates in response to the cephalosporin discs (Paterson and Bonomo, 2005).

DDST for ESBL confirmation

The isolates were sub-cultured on Brain Heart Infusion Agar (BHI) {Biotech, England} using streak plate method and the plates incubated at 35 ^{o}C for 18-24 hours so as to obtain confluent growths. Improved procedure of Jarlier et al. (1998) was employed in the screening of isolates for ESBL production on Mueller Hinton Agar (Biotech, India) using standard inocula from BHI plates.

The isolates were inoculated using sterile swab sticks onto the surface of Mueller Hinton agar and discs containing Ceftriaxone and Ceftazidime were placed 20mm center to center from the Augmentin disc. The plates were incubated at 35 ^{o}C for 18-24 hours after which the plates were read by observing for the presence or otherwise of an increase in zone of inhibition of either or both cephalosporin discs towards the central Augmentin disc (synergy).

Statistical analysis

The results obtained for the different isolates were subjected to chi-square test (Mukhtar, 2007).

RESULTS AND DISCUSSION

Positive ESBL production in CLIS breakpoint test was indicated by inhibition zones of ≤25 mm for Ceftriaxone and ≤22 mm for Ceftazidime discs. Enhancement of inhibition zones of either or both cephalosporin discs towards the central Augmentin disc in DDST is positive confirmatory test as shown in Plate 1.

Prevalence of ESBLs among the enterobacteriaceae isolates was 46 representing 46% using CLSI breakpoint as shown in Table 1 while prevalence of confirmed ESBLs using DDST was 29 representing 29% as shown in Table 2.

Of the 100 enterobacteriaceae isolates obtained, 87 representing 87% were positive for ESBLs using CLSI breakpoint (Table 1). These include *Citrobacter fruendii* (3), *Escherichia coli* (46), *Klebsiella pneumoniae* (13), *Morganella morganii* (1), *Proteus vulgaris* (23) and *Salmonella typhi* (1). However, only 49 representing 49% were positive for ESBLs production using DDST (Table 2) as follows: *Citrobacter fruendii* (2), *Escherichia coli* (25), *Klebsiella pneumoniae* (7), *Morganella morganii* (1), *Proteus vulgaris* (13) and *Salmonella typhi* (1). There exist significant differences in ESBLs production among the isolates.

The variation in ESBLs positive results between the CLSI breakpoint and DDST procedures may be due to false positive results caused in bacteria with multiple β–lactamases that interfere with the test results, which can only be detected

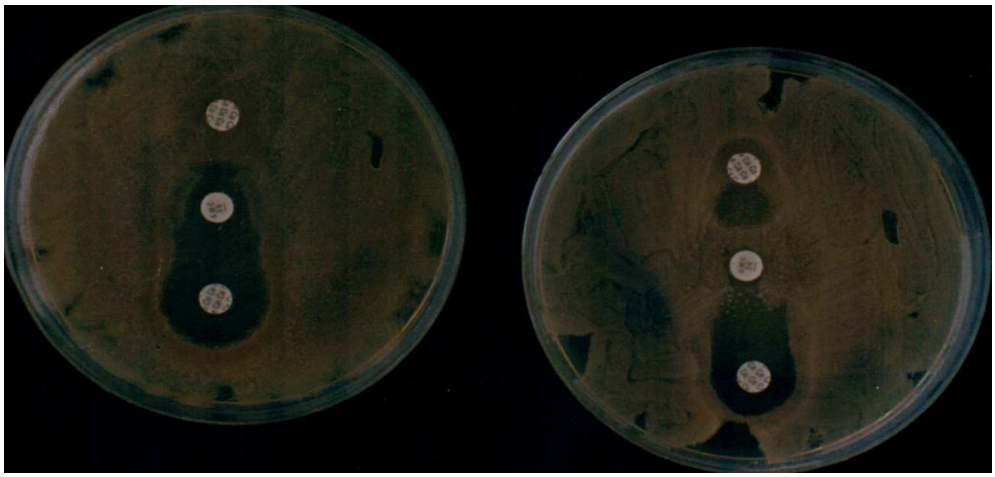

Plate 1: ESBL Double Disc Synergy Test (DDST).

Table 1: Prevalence of ESBLs among enterobacteriaceae isolates using CLSI breakpoint (Paterson and Bonomo, 2005).

Isolates	Number screened	Number positive	% occurrence
Citrobacter fruendii	4	3	3.45
Escherichia coli	51	46	52.87
Klebsiella pneumoniae	13	13	14.94
Morganella morganii	2	1	1.15
Proteus vulgaris	28	23	26.44
Salmonella typhi	2	1	1.15
Total	**100**	**87**	**87**

Table 2: Prevalence of ESBLs among enterobacteriaceae isolates using Double Disc Synergy Test (Jarlier et al., 1998).

Isolates	Number screened	Number positive	% occurrence
Citrobacter fruendii	3	2	4.08
Escherichia coli	46	25	51.02
Klebsiella pneumoniae	13	7	14.29
Morganella morganii	1	1	2.04
Proteus vulgaris	23	13	26.53
Salmonella typhi	1	1	2.04
Total	**87**	**49**	**56.32**

using iso-electric focusing and DNA sequencing (CLSI, 1999).

The prevalence observed was high considering poverty level of average Nigerians coupled with the collapse of primary healthcare delivery system, which indicates the possibility of treatment failure and/or outbreaks of infections caused by resistant bacteria (Ahmad et al., 1999).

Although the occurrence and distribution of ESBLs differs from country to country and from hospital to hospital (Bradford, 2001), the percentage prevalence of ESBLs among the isolates screened was higher in *Klebsiella pneumoniae* than other genera isolated in the present study, which conforms to the findings of Hanberger et al. (1999) and Moubareck et al. (2005). The high occurrence of ESBLs in *Klebsiella pneumoniae* observed in this research is of great concern since infections caused by this bacterium (particularly respiratory tract infections) are very common in this part of Nigeria due to the contagious nature and resistance of the organism to harsh conditions, which may be due to the presence of capsules that gives some level of protection to the cells (Paterson and Bonomo, 2005).

The occurrence of ESBLs among the different clinical isolates of enterobacteriaceae involved in this work calls for the need to improve control measures in healthcare settings so as to curtail the spread and possible outbreak of infections with resistant bacteria.

Recommendations

In view of the quick spread of ESBLs among bacterial pathogens and the problems that may be caused by treatment failure due to infections with ESBLs producing bacteria coupled with the results obtained in this work, it could be recommended that:

a- Government should strengthen awareness campaigns on improved hygienic practices so as to reduce the rate of infections and spread of ESBLs among both enterobacteria and other bacterial pathogens.

b- Healthcare settings should improve control measures such as proper handling and disinfection of equipment as well as detection of ESBLs producing bacteria among the patients and isolation of ESBLs colonized patients.

c- Clinicians should reduce the rate at which third and fourth generation cephalosporins are prescribed.

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