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Detection of Extended Spectrum β-Lactamase Producing Strains of (*Escherichia coli*) and (*Klebsiella* sp.) in a Tertiary Health Centre in Ogun State

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Abstract: ESBL producers have continued to draw increasing attention globally with their attendant problems of clinical failure to new generation antibiotics and nosocomial spread. In Nigeria, several reports have indicated the presence and severity of ESBL producers. In this study, researchers demonstrate the presence of ESBL strains of *E. coli* and *Klebsiella* sp. from the clinical samples in a tertiary medical institution in Abeokuta, Ogun State, Nigeria. About 160 clinical isolates samples were analyzed and cultured at the micro unit. Isolates of *E. coli* and *Klebsiella* were screened for ESBL producers by double disk synergy test on Mueller Hinton Agar. Susceptibility to some common antibiotics were also tested by disk diffusion method. About 80 isolates were recovered, 14 (17.5%), *Klebsiella pneumoniae*, 22 (27.5%), *Klebsillea oxytoca* and 44 (55%). *E. coli* ESBl producers was 75% *Klebsiella pneumoniae* 5%; *Escheirchia coli* 2.5%. Age group 1-10 gave highest percentage of isolate (66.7%) and age group 41-50 the lowest (15%). Sputum and wound swab were the only sample sites that yielded ESBL producers. The presence of ESBL producers has now been established in Abeokuta, concerted effort should be made toward initiating an early detection system for ESBL producing isolates in our hospitalized patients. In addition, appropriate control measures should be put in place to prevent nosocomial spread of infection.

Key words: ESBL, clinical failure, antibiotics, appropriate, infection, nosocomial spread

INTRODUCTION

Resistance to β-lactam antibiotics was reported in *Escherichia coli* even before the first β-lactam antibiotic, penicillin was released for use in medical practice (Ahamed and Kundu, 1999). A good number of enteric gram-negative bacteria have been shown to posses naturally occurring chromosomally mediated genes that confer resistance on them to β-lactam antibiotics (Brown *et al*., 2000).

Amongst the most prevalent bacteria pathogens capable of showing resistance to common antibiotics is *Escherichia coli* which is one of the most common causes of urinary tract infections and other opportunistic infections such as wound abscess which can have serious clinical implications (Iroha *et al*., 2009). *Escherichia coli* and *Klebsiella* sp. have also been reported to be common causes of hospital acquired infections which can also have severe clinical implications with corresponding multi-drug resistance (Aibinu *et al*., 2003). Extended spectrum β-lactamases are plasmid mediated enzymes that confer resistance to Penicillin, 3rd generation Cephalosporins and Aztreonam but are inhibited by Clavunalic acid. The first plasmid mediated β-lactamase was reported and named after a patient Temoniera (TEM) in Greece (Medeiros *et al*., 1985). Over the years TEM-1β-Lactamase has spread word wide and can now be found in many species of the family Enteriobacteriaceae (Okeshola and Makajuola, 2009). The first report of plasmid mediated Extended Spectrum β-Lactamases (ESBL) was published after a study done in 1983 at Germany on isolates of *Klebsiella pneumonia* (Urban *et al*., 1994). In Africa ESBL producing bacteria have been reported in Egypt, Morocco, Tunisia, Senegal and South Africa (Blomberg *et al*., 2005). In Nigeria, ESBL detection in the hospitals has been reported severally (Iroha *et al*., 2009; Okeshola and Makajuola, 2009). This is an indication of increasing degree of resistance to most available antibiotics. This development is very troubling in the environment as most of the 3rd generation cephalosporin is out of the reach of majority of the general population. Clinical outcome of patients with ESBL bacterial sepsis is also very poor (Blomberg *et al*., 2005). In this study researchers examined clinical isolates

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of *Escherichia coli* and *Klebsiella* sp. for ESBL production with general antibiotic susceptibility pattern to commonly used antibiotics in a tertiary health centers in Ogun State, Nigeria.

MATERIALS AND METHODS

Clinical isolates: About 160 clinical samples were cultured from various sample sites comprising 14 isolates of *Klebsiella pneumonia*e, 22 isolates of *Klebsiella oxytoca* and 44 *Escherichia coli* isolates. The isolates were identified by colonial appearance on Mac-Conkey agar, gram staining reaction and standard biochemical test (Farmer, 1999; Iroha *et al*., 2009).

Antibiotic susceptibility testing: Antibiotic sensitivity testing was done by disk diffusion method using commercially available multidisk (AB Biodisk) and Oxoid single disk consisting ampicillin (10 mg), tetaracyclin (10 mg), amoxil (30 mg), ceftazidime (30 mg), ceftriaxone (30 mg), azetronam (30 mg). The test was carried out on Molton Mueller Hinton agar. Innocular were standardized according to NCCLS standards, briefly 4-5 colonies of 24 h pure culture isolates were inoculated into 5 mL sterile normal saline and turbidity adjusted to match a McFarland 0.5 Barium Sulphate standard. The Molton Mueller Hinton agar plates were seeded with 0.1 mL of standardized suspension of each isolate allowed to solidify before placing the antibiotic disks and allowing it to pre-diffuse for 30 min before final incubation for 18-24 h at 37°C. The test was carried out in triplicate and a control plate containing no antibiotic was included for each isolate.

ESBL detection (Double disk synergy test): Prepared Mueller Hinton agar plates were inoculated with a 0.5 McFarland standard inoculum of *Escherichia coli* and *Pseudomonas aureginosa*. Control strains of *E. coli*, *E. coli* ATCC 35218 served as positive control while *E. coli* ATCC 25922 served as negative control. Control strain *Pseudomanas aureginosa* ATCC 27853 was used as control strain for *Pseudomonas aureginosa*. Double disk synergy test was performed by placing ceftrazidime (30 mg), ceftrazone (30 mg) and azetronam (30 mg) at a distance of 20 mm (center to center) from a disk containing Amoxicillin (20 mg) plus Clavulanate (10 mg), (Augmentin 30 mg). Positivity for ESBL production was interpreted if the zone of inhibition of the test antibiotic was increased toward the center disk (Augmentin).

RESULTS AND DISCUSSION

About 160 clinical samples were collected from various sites from patients at Federal Medical Center Abeokuta. Eighty isolates were recovered from the cultured samples consisting 14 (17.5%) *Klebsiella pneumonia*, 22 (27.5%), *Klebsiella oxytoca* and 44 (55%) *Echerichia coli*. The isolates were from wound swab 17 (21.25%), blood 11 (13.75%), urine 20 (25%), sputum 11 (13.75%), CSF 6 (3%), HVS 15 (18.75%) as shown in Table 1. The percentage distribution of isolated organisms was highest in age group 1-10 (66.7%) and the lowest in age group 41-50 (15%). All isolates showed resistance to ampicillin, amoxicillin and tetracycline. Isolates tested for ESBL production showed a distribution of 6 (7.5%) with *Klebsiella pneumonia* having 4 (5%) and *Esherichia coli* 2 (2.5%) as shown in Table 2. Table 3 shows the susceptibility pattern of non ESBL producers to ceftrazidime, caftrazone and azetronam. Only wound and sputum samples gave a positive ESBL producing isolates while other sample sites gave non ESBL producers.

In recent years, bacterial resistance to β-lactam antibiotics has risen dramatically (Medeiros, 1997) contributing to this increase has been the misuse of antibiotics and spread of Extended Spectrum Betalactamses (EBLSs) enzymes that hydrolyze the expanded-spectrum cephalosporin. A numner of studies have assessed the occurrence of EBSLs among members of the family Enterobactericeae. However *Klebsiella pneumonia* and *Escherichia coli* are on focused primarily.

In this study researchers study an overall prevalence rate of 7.5% of ESBL producers in all isolates tested. This is lower than an earlier report done at U.N.T.H Enugu with a rate of 11.4% ESBL producing strains of *Escherichia coli* (Iroha *et al*., 2009). This is also lower than that of a study done in Lagos with a rate of 20.3% ESBL producing strains of *Enterobacter* sp. (Aibinu *et al*., 2003).

Table 1: Distribution of isolates by sample site

Isolates	Wound swab	Blood	Sputum	CSF	HVS	Urine	Total
K. pneumoniae	3	4	5	2	0	0	14
K. oxytoca	4	3	0	0	7	8	22
E. coli	10	4	6	4	8	12	44
Total	17 (21.25%)	11 (13.75%)	11 (13.75%)	6 (3%)	15 (18.75%)	20 (25%)	80 (100%)

Table 2: Disribution ESBL producers by sample site

Clinical samples	Organisms			
	K. pneumoniae (%)	*K. oxytoca* (%)	*E.coli* (%)	Total (%)
Wound	0 (0)	0 (0)	2 (2.5)	2 (2.5)
CSF	0 (0)	0 (0)	0 (0.0)	0 (0.0)
Blood	0 (0)	0 (0)	0 (0.0)	0 (0.0)
Urine	0 (0)	0 (0)	0 (0.0)	0 (0.0)
Sputum	4 (5)	0 (0)	0 (0.0)	4 (5.0)

Table 3: Susceptibility pattern of non-ESBL producers

Antibiotics (30 µg)	Resistance (%)	Intermediate (%)	Sensitive (%)
Ceftazidime	34 (42.50)	18 (22.50)	28 (35.00)
Ceftrazone	23 (28.75)	3 (3.75)	54 (67.50)
Augmentine	50 (62.50)	6 (7.50)	24 (30.00)
Azetronam	15 (18.75)	7 (8.75)	61 (76.25)

In the study the rate is low in direct comparism to the earlier two reports discussed but a worrisome observation is the absolute resistance pattern to 3 of the commonest antibiotics in use in the environment, ampicillin, amoxil and tetracycline. This calls for an urgent action with regards to education of the pubic against the misuse of antibiotics and strict compliance to antibiotic regimen. Another worrisome development is the susceptibility pattern seen in the isolates tested against the 2nd and 3rd generation cephalosporins for instance only 35% of non ESBL producing isolates tested were sensitive to ceftazidime a rate of 18.75% resistance was observed to azetronam and only 30% of non ESBL producing isolates were sensitive to augumentin. Overall an increase in the number of isolates of *Escherichia coli* and *Klebsiella* sp. that have developed resistances to some of the most potent and widely used antibiotics has now been reported for the first time in a tertiary health institution in Abeokuta, Ogun state.

CONCLUSION

From the findings, there is a need to further study the general ESBL prevalence rates in other health facilities in Abeokuta to give a brighter picture to the level risk of exposure patients particularly those going in for admission are faced with. A surveillance system is also recommended in the tertiary hospitals in other to detect early patients with these ESBL producers and manage them appropriately to prevent nosocomial as well as community spread of this infections. Controlled use of antibiotics is also advised and physicians should try and correspond with the laboratory before any of the cephalosporins are prescribed.

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