**INCIDENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING Klebsiella pneumoniae AMONG PATIENTS WITH URINARY TRACT INFECTIONS IN KANO METROPOLIS NIGERIA**

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ABSTRACT

Extended Spectrum Beta-Lactamase (ESBLs) production is one of the ways by which bacteria become resistant to antibiotics and pathogens of UTIs such as Klebsiella pneumoniae have been incriminated at global scale. This study was conducted to investigate the incidence of Extended Spectrum Beta lactamase producing Klebsiella pneumoniae from Urinary Tract Infections in Kano metropolis. The work involved One hundred and forty seven K. Pneumoniae isolates obtained from patients with suspected urinary tract infections were studied from January to July 2017. The identity of the isolates was confirmed using Microgen\textsuperscript{TM} Gna + B-ID System. Antibiotic susceptibility testing was carried out using the Kirby-Bauer Disc Diffusion Technique. Screening for ESBLs production was done using Clinical Laboratory Standards Institute breakpoint. Suspected ESBLs producers were subjected to confirmation using Double Disc Synergy Test. Standard Discs of Augmentin (AMC 30µG Oxoid England), Ceftazidime (CAZ 30µG, Oxoid England) and Cefotaxime (CTX 30µG, Oxoid England) were used for the screening and confirmation. Accordingly, Multidrug Resistant K. pneumoniae were found to be 63.3% and all were ESBLs producers. The Double Disc Synergy Test however confirmed 6.8% ESBLs producing K. pneumoniae. Antimicrobial sensitivity of the ESBLs producing organisms showed 100% resistance to Augmentin, ceftriaxone, ceftazidime, cefotaxime while resistance to gentamicin was 91.5%, chloramphenicol 23.4%, Nitrofurantoin 61.7%, Ciprofloxacin 93.6% and cotrimoxazole 95.7%. However, Imipenem was the most pharmacologically active drug. ESBL producing K. pneumoniae are incident in Kano and are resistant to commonly prescribed antibiotics. We, therefore, suggest screening and confirmation for ESBL in any attempt to treat UTIs due to such pathogens

Keywords

Extended Spectrum Beta Lactamases, K. pneumoniae, Urinary Tract Infection, Incidence, Kano

INTRODUCTION

Urinary Tract Infections are the most commonly reported hospital and community acquired infections, affecting more than half of the population at least once in their lifetime and has been associated with a high mortality rate. Majority of the causative agents in UTIs are Gram negative pathogens, primarily Enterobacteriaceae with Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis being the main culprits (Tenney et al., 2018). K. pneumoniae has been named as one of the top three pathogens of international concern documented in the 2017 World Health Organization’s (WHO) Global Priority List of Antibiotic Resistant Bacteria to Guide Research, Discovery and Development of new antibiotics (WHO, 2017).

An increasing frequency in multi drug resistant K. pneumoniae that possess Extended Spectrum Beta Lactamases has been reported (Paterson and Bonomo, 2005). Multiple drug resistance has significantly increased among bacteria causing nosocomial infections and there is a growing concern for multi drug resistant Gram-negative bacteria which produce Extended Spectrum Beta Lactamases (ESBLs) (Coque et al., 2008).
ESBLs are class A beta lactamases that hydrolyse penicillin, oxymimo-cephalosporins and monobactams. They are plasmid mediated enzymes with the most common types being TEM, SHV and CTX-M types. ESBLs are primarily produced by Enterobacteriaceae E. coli, Klebsiella pneumoniae and Klebsiella oxytoca (Paterson and Bonomo, 2005). An increase in antibiotic resistance among uropathogenic organisms to most common cephalosporins used in hospitals has been reported (Al janaby et al., 2017; Khalid et al., 2017)

The prevalence of ESBLs is increasingly being reported worldwide with it varying from one geographical location to another and being directly linked to the misuse and abuse of antibiotics (Jarlier et al., 1988). In many parts of the world, 10% -40% of strains of E. coli and K. pneumoniae express ESBLs (Rupp and Fey, 2003). In Nigeria, prevalence rates range from 5% to 44.3% as shown by (Olomotola et al., 2007; Olowe and Aboderin, 2010; Yushau et al., 2010; Akujobi and Ewuru, 2010; Ogerefe et al., 2015; Mohammed et al., 2016; Giwa et al., 2018).

Several outbreaks of infection due to ESBL producing organisms have been described on every continent thereby posing as a challenge to infection control issues. Some initial outbreaks have been supplanted by endemicity leading to increase in patient morbidity and mortality (Paterson and Bonomo, 2005).

The aim of this study is to phenotypically investigate the incidence of Extended Spectrum Beta Lactamases (ESBLs) producing Klebsiella pneumoniae isolated from patients with UTIs in Kano Metropolis.

MATERIALS AND METHODS

A total of 1500 urine samples were collected from Aminu Kano Teaching Hospital, Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Specialist Hospital from In and Outpatients with suspected UTIs between January to July 2017 following approval from the Aminu Kano Teaching Hospital Ethical Committee and Kano State Ministry of Health.

Urine microscopy was carried out using a drop of uncentrifuged urine to determine significant pyuria. The samples were inoculated on Cysteine Lactose Electrolyte Deficient Agar and incubated at 37°C for 18-24 hours. Discrete colonies were picked, and Gram staining was carried out. Further identification was done using Microgen™GnA+B 1D biochemical identification system according to manufacturer’s instructions. Klebsiella pneumoniae isolates that were obtained as a pure and predominant growth from the clinical specimen were only considered for the present study.

Extended Spectrum Beta Lactamase Screening Test

Screening for ESBL production by disc diffusion test

Resistance to cefotaxime ceftriaxone and ceftazidime was detected by disc diffusion test as recommended by (CLSI, 2017). From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5 x 10⁶CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria was spread on Mueller Hinton agar. After allowing the plate to dry, the antibiotic discs mentioned above were placed on the surface and the plates incubated at 37°C for 24-18 hours. Following growth, the diameter of the zone of inhibition around the disks were measured and recorded. The disc potency and zone diameters for inferring resistance were as follows; cefotaxime (30µg) ≤27mm, ceftriaxone (30 µg) ≤25 mm, ceftazidime (30 µg). Resistance to at least one of the antibiotics will be considered as positive in the screening test for possible ESBL production (CLSI, 2017)

Confirmation of ESBL production by Double Disc Synergy Test (DDST)

Double Disc Synergy Test was carried out using 3 antibiotics, namely Amoxycillin-Clavulanic acid (20/10µg), Cefotaxime (30µg) and Ceftazidime (30µg). The discs were placed 25mm (centre to centre of the discs) from Amoxycillin-Clavulanic acid on Mueller Hinton Agar. Enhancement of the zone of inhibition towards the clavulanate disc after 24hours incubation at 37°C was considered indicative of a potential ESBL producer (Jarlier et al., 1988)

Antibiotic Sensitivity Testing of ESBL producing Klebsiella pneumoniae

This was carried out using Kirby-Bauer-CLSI modified Disc Agar Diffusion technique (DAD) (Hudzicki, 2009). One milliliter (1.0 ml) of standardized overnight culture of each isolate (containing 10⁶ CFU/ml) was used to flood the surface of Mueller Hinton Agar (MHA) plates and excess drained off and dried while the Petri dish lid was in place. The standard antibiotic discs (Gentamicin, Ceftazidime, Chloramphenical, Cefotaxime, Nitrofurantoin, Ciprofloxacin, Amoxicillin/Clavulanic acid, Imipenem, Ceftriaxone and Cotrimoxazole) were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h.
The plates (prepared in duplicates for each isolate) were then incubated at 37°C for 18 hours. The diameter of the zones of inhibition produced by each antibiotic disc was measured and recorded.

**RESULTS AND DISCUSSION**

A total of 147 *K. pneumoniae* isolates were obtained from the total samples examined. Table 1 shows the distribution of the organism across the different sampling sites and ESBL production with AKTH having the highest number of isolates followed by MMSH and MAWSH. This is similar to the study carried out by Kumurya and Sule (2016) at AKTH and Sule and Kumurya (2016) at MMSH where the number of *K. pneumoniae* isolates were 26.7% and 7% respectively. Several studies on uropathogens has shown *Klebsiella pneumoniae* to be the second most predominant pathogen after *Escherichia coli* (Ehinmidu 2003; El Mahmood 2009; Pondei et al., 2013; Azekhueme et al., 2015; Mohammed et al., 2016; Onanuga et al., 2019).

In this study, ESBLs producing *K. pneumoniae* was 10.6%. A higher prevalence was reported by Yushau et al. (2010) at 25.5%, in a study in India, nearly 40% of urinary isolates of *K. pneumoniae* were ESBL positive (Babypadmini and Appalaraju, 2004). ESBL producing *K. pneumoniae* were 54.4% in a study in Latin America (Aminazadeh et al., 2008). Mekki et al. (2010) reported ESBL producing *K. pneumoniae* from the patients suffering from Urinary Tract Infections. Ejaz et al. (2011) reported ESBL producing *K. pneumoniae* to be 71.7% in Pakistan. About 26.5% ESBLs producing *K. pneumoniae* has been reported in Zaria (Giwa et al., 2018) and Port Harcourt (Onanuga et al., 2019) respectively. These observed differences could be due to regional and attitudinal behaviour towards prescription and consumption of antibiotics especially the cephalosporins in both hospital and community settings.

Distribution of ESBLs across the hospitals showed that AKTH had the highest occurrence of ESBLs producing *K. pneumoniae* being 70%. The spread of an ESBL variant can be facilitated by referral system where the presence of a single ESBL variant in a different centre may be imported by a patient on referral to another centre (Nordmann et al., 2009). This situation could hold for AKTH because it is a tertiary referral centre receiving patients from different parts of north western Nigeria.

Table 1: Distribution of isolate based on sampling site and ESBL production

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sampling site</th>
<th>ESBL Production</th>
<th>Distribution of ESBL production across the sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AKTH</td>
<td>MMSH</td>
<td>MAWSH</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>68</td>
<td>53</td>
<td>26</td>
</tr>
<tr>
<td>N = 147</td>
<td>(46.3)%</td>
<td>(36.1)%</td>
<td>(17.7)%</td>
</tr>
</tbody>
</table>

Table 2 shows the Antibacterial Susceptibility pattern of ESBLs producing *K. pneumoniae*. A high level of resistance was observed among the isolates to Amoxicillin/Clavulanic acid and Cephalosporins has been widely reported in Nigeria and other parts of the world as expected because ESBLs production in Gram Negative bacteria is the key factor that confers resistance to beta lactam antibiotics except cephemycins and carbapenems (El Bouamri et al., 2015; Garbati et al., 2016; Pang et al., 2018; Onanuga et al., 2019). A high resistance of ESBLs producing *K. pneumoniae* to Gentamicin, Ciprofloxacin, Chloramphenical and Cotrimoxazole (70-100%) has also been reported by Onanuga et al. (2019) which poses a significant problem to the treatment of urinary tract infections with this commonly used antibiotics thereby narrowing the choice of antimicrobial agents effective against ESBLs producing organisms (Paterson et al., 2001). Multi drug resistance among ESBLs producing *K. pneumoniae* in this study is similar to the findings of Esthe et al., 2015 in Ethiopia who reported 87.4% MDR *K. pneumoniae* and Onanuga et al. (2019) also reported 100% while Giwa et al. (2018) reported a lesser value of 40%.

Imipenem was the most effective antibiotic with 100% sensitivity. A similar result has been reported by Ejikeugwu et al. (2012) and Igbinoba and Osazuwa (2012) in Nigeria.
However, carbapenem resistance has been reported widely as an increasing public health problem (Garbati et al., 2016; Pang et al., 2018). Effectiveness of Imipenem could be due to its late arrival in the Nigerian market therefore to ensure its continued relevance a coordinated rationale usage must be implemented.

Table 2: Antibacterial Susceptibility pattern of ESBLs producing *K. pneumoniae* (N=10)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive (%)</th>
<th>Resistant (%)</th>
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<tbody>
<tr>
<td>Gentamicin (10µg)</td>
<td>1(10)</td>
<td>9(90)</td>
</tr>
<tr>
<td>Ceftazidime (30µg)</td>
<td>0(0)</td>
<td>10(100)</td>
</tr>
<tr>
<td>Chloramphenical (30µg)</td>
<td>3(30)</td>
<td>7(70)</td>
</tr>
<tr>
<td>Cefotaxime (30µg)</td>
<td>0(0)</td>
<td>10(100)</td>
</tr>
<tr>
<td>Nitrofurantoin(300µg)</td>
<td>6(60)</td>
<td>4(40)</td>
</tr>
<tr>
<td>Ciprofloxacin(5µg)</td>
<td>2(20)</td>
<td>8(80)</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic acid(30µg)</td>
<td>0(0)</td>
<td>10(100)</td>
</tr>
<tr>
<td>Imipenem(10µg)</td>
<td>10(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Ceftriaxone (30µg)</td>
<td>0(0)</td>
<td>10(100)</td>
</tr>
<tr>
<td>Cotrimoxazole (1.25/23.75µg)</td>
<td>0(0)</td>
<td>10(100)</td>
</tr>
</tbody>
</table>

CONCLUSION

The incidence of phenotypically expressed ESBLs producing *K. pneumoniae* is high and they are generally multi drug resistant. Carbapenems remain the most useful therapy for infections caused by this organism. A functional antibiotic prescription policy that involves the rationale use of carbapenems needs to be implemented to prevent failure. It is recommended that a continued surveillance using well-equipped laboratories for prompt detection and reporting of ESBLs producing *K. pneumoniae* should be implemented as well as making it a routine procedure in hospitals.

REFERENCES


