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Detection of extended spectrum Beta – Lactamase in Enterobacteriace isolated from a general hospital in Lagos

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Abstract

Extended spectrum beta-lactamases (ESBLs), mainly produced by Gram-negative bacteria, hydrolyze quinolones leading to multi-drug resistance challenges encountered in clinical settings. Detection of ESBLs will help in treatment option. This study, therefore, investigated the presence of ESBL encoding gene by polymerase chain reaction (PCR) in Enterobacteriaceae isolated from clinical samples. A total of 100 clinical samples submitted for routine microbiological analysis were obtained and examined for Enterobacteriaceae using standard microbiological methods. Bacterial identity confirmation was performed by API 20E kit and PCR using ropB specific primers. Sensitivity test was determined using agar diffusion method while ESBLs were confirmed by PCR. Thirty (30) Enterobacteriaceae isolates consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica* and *Serratia* were obtained from 100 clinical samples. The rpoB gene was confirmed in all the 30 isolates. All isolates were resistant to the all tested antibiotics (ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, amoxicillin/clavulanate, nitrofurantion, and ciprofloxacin). The PCR-based ESBLs confirmation revealed that 21 (70%) of the 30 isolates possessed gene for SHV type enzyme while TEM and CTM-X types were not amplified.

Conclusions

The study indicates a high level of multi-drug resistant ESBL-producing Enterobacteriaceae in clinical setting in Lagos. Hence, a proactive antibiotic surveillance system is urgently needed to curtail spread of resistant bacteria in the community.

Keywords: Enterobacteriaceae, Extended spectrum beta-lactamase, Multidrug resistance.

1.0 Introduction

Plasmid-mediated enzymes that inactivate β-lactam antibiotics mainly of oxyimino-cephalosporins and oxyimino-monobactam are known as extended spectrum β-lactamases (ESBLs; Pitout *et al*., 2005). are located within large plasmids that code for genes of resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol (Deepti and Deepti, 2010).

Extended spectrum β-lactamase-producing organisms are a problem in hospitals worldwide (Bradford, 2001). To date, more than 200 different types of ESBLs have been identified. There is increase in the prevalence of ESBLs among clinical isolates of Enterobacteriaceae in Europe and Asia (Bonnet, 2004). ESBLs are mainly acquired by Gram-negative bacteria; thus, infections caused by ESBLs-producing bacteria are on the increase. Infections can involve the respiratory tract (pneumonia), urinary tract and bladder, skin and soft tissue, blood, gastrointestinal tract, reproductive organs, and the central nervous system. Generally speaking, individuals with infections caused by bacteria carrying ESBLs have a higher mortality rate and require longer stays in hospitals, thus increasing the costs of patient care. Plasmids coding for ESBL enzymes may carry co-resistance genes for other non-β-lactam antibiotics; this limits the number of useful drugs against these organisms (Schwaber *et al*., 2005; Morosini *et al.*, 2006; Agrawal *et al.,* 2008).

In general, organisms with ability to produce ESBL have reduced susceptibility to oxyimino-aminothiazolylcephalosporins (Livermore *et al*., 2001). ESBLs are the most evolving mechanism of antibiotic resistance among the family Enterobacteriaceae. This is due to the selective pressure imposed by inappropriate use of third generation cephalosporins most often encountered in the intensive care unit (Bradford, 2001). Reliable detection of ESBL production by clinical microbiology laboratory is therefore essential to guide the clinicians in providing appropriate therapy. In Nigeria, only few studies used molecular methods for detection of ESBLs (Mohammed *et al.,* 2016; Olufemi *et al*., 2016; Onanuga *et al*., 2019), thus, most laboratories depend on low sensitive phenotypic detection of ESBLs for routine clinical investigation. Hence, this study was designed to access the prevalence of ESBLs among members of the family Enterobacteriaceae isolated from clinical samples of patients in a General Hospital in Lagos, Nigeria, using molecular method and to determine the antibiotic susceptibility of the isolates to different antibiotic classes.

These enzymes pose a major therapeutic challenge in the treatment of hospitalized and community-based patients globally (Paterson *et al.,* 2005; Deepti and Deepti, 2010). The genes responsible for the expression of ESBL

Materials and methods

Ethical considerations, isolation and identification of Enterobacteriaceae

Ethical clearance to conduct the study was obtained from Babcock University Health Research Ethics Committee under the approval number (BUHRC543/19). Clinical samples (midstream urine, high vaginal swabs, and sputum) from patients at a General Hospital in Lagos, Nigeria, which were submitted for microbiological analysis at the hospital, were collected during June to July 2019. Samples were processed microbiologically within 48 h of collection by surface-plating on prepared plates of MacConkey agar plates (Biomark Laboratories, India) and incubated at 37°C for 24 h. Thereafter, morphologically distinct colonies were sub-cultured and purified on fresh MacConkey plates. Cultures were stored in vials containing nutrient broth supplemented with 40% glycerol at -20 ºC (Oladipo *et al* 2012; Moghaddam *et al* 2015).

For the identification of Enterobacteriaceae, a two-stage identification was performed. First, all isolates were identified using the Analytical Profile Index (API) kit 20E (BioMerieux, France) according to the manufacturer's instructions. Thereafter, a PCR-based identification using the rpoB gene was performed as detailed below.

Extraction of DNA by Boiling Method

An overnight suspensions of the organisms were boiled to extract its DNA and then centrifuged at 15,000 x g for 15 min. The supernatant was then dispensed, and the pellet was re-suspended in sterilized distilled water prior to centrifugation at 15,000 x g for 10 sec. The supernatant from this step was then transferred into a fresh Eppendorf tube prior to storage at -20°C. Aliquots of 2µl of template DNA were used for PCR (Pe'rez-roth *et al*., 2001).

Detection of rpoB and ESBL Genes by PCR

A 2.0µl aliquot of DNA suspension was added to 25µl of PCR mixture consisting of 5.0µl reaction buffer (New England BioLabs) following previous protocol (Ezeamagu *et al*., 2018). Polymerase Chain Reaction assay was employed with a negative control containing all of the reagents devoid of DNA template for the detection of the coding genes (Table 1). Amplification was performed on 9700 thermocycler (Applied Biosystems) under the following protocol: initial denaturation at 95°C for 3 min, denaturation 94°C for 30 sec, annealing (Table 1) for 30 sec, extension 72°C for 30 sec and final extension 72°C for 5 min which was programmed for 30 cycles. After PCR amplification, 10µl of PCR product was resolved by agarose gel electrophoresis.

Antimicrobial Susceptibility Test

Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2015) was applied to test the response of the isolates to antibiotics. Briefly, a single colony of pure isolate was inoculated into a test tube containing 1 ml of nutrient broth (Oxoid, UK) and incubated overnight at 37 °C. The overnight broth was then standardized to match 0.5 McFarland standard. A sterile swab stick was dipped in the standardized suspension and streaked over the surface of freshly prepared Mueller Hilton agar plates (Oxoid LTD, Basingstoke, Hamshire, England). The antibiotic disc; gentamicin (10 µg), ceftazidime (30 µg), cefturoxime (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), amoxicillin/clavulanate (30 µg), ofloxacin (5 µg) and cefixime (5 µg) were placed on the agar surface maintaining a distance of 30mm edge to edge. The plates were incubated at 37 °C for 24 h and clear zones of inhibition were measured with a ruler to the nearest diameter. Results were interpreted in accordance with Clinical and Laboratory Standards Institute breaking point for Enterobacteriaceae (CLSI 2015).

Results and discussion

Frequency of Enterobacteriaceae isolation

The API kit analysis and PCR-based rpoB gene amplification identified all the encountered Enterobacteriaceae. Consequently, 30 Enterobacteriaceae consisting of *E. coli* (50%), *Klebsiella pneumoniae* (16.7%), *Salmonella enterica* (10%) and *Serratia* (23.3%) species were identified (Figure 1).

The rpoB primers have been equivocally used for the identification of Enterobacteriaceae in this study as with previous reports and the primers consistently showed sensitivity of 100% (Arabestani *et al*., 2012). The PCR results showed that Enterobacteriaceae were distributed in the clinical samples and this agrees with previous information that this group of bacteria are found in both clinical and environmental settings (Woerther *et al*., 2010).

Resistance pattern and ESBL status

All examined isolates (n=30) were resistant to the selected antibiotics (ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, amoxicillin/clavulanate, Nitrofurantion and Ciprofloxacin). Of all the 30 isolates, 26 (70%) indicated the SHV gene. Figure 2 shows the electrophoregram of the SHV genes in selected isolates. None of the isolates showed the presence of TEM and CTX-M genes.

The observed resistance of all isolates to the tested antibiotics contrasts a study in Ado Ekiti, Nigeria,that applied the same set of antibiotics but reported varying resistant isolate patterns of 96%, 83%, 58%, 49%, 39%, 5% and 5% to amoxicillin/clavulanate, cefuroxime, ceftazidime, gentamicin, cefixime, ofloxacin, and ciprofloxacin, respectively (Olowe *et al*., 2015). It is important to note that the study in Ado Ekiti focused on Enterobacteriaceae obtained from animal sources (faecal) and this could have accounted for this variation. The antibiotic pressure in clinical setting is higher than that of non-clinical setting, accounting for such wide variation. The consequences of increasing multi-drug resistant pathogens have been the subject of discussion globally (Bradford, 2001).

Table 1: Primers used in this study with their expected molecular weight

Gene	Primer name Primer sequence, 5′–3′	Gene target	Amplicon length	Tm (°C)	Reference
rpoB 1	CAGGTCGTCACGGTAACAAG	β-subunit of RNA polymerase	512	55	Arabestani *et al*., 2014
rpoB 2	GTGGTTCAGTTTCAGCATGTAC				
TEM 1	ATAAAATTCTTGAAGACGAAA	ESBL	1097	40	Moosavian & Deiham, 2012
TEM 2	GACAGTTACCAATGCTTAATCA				
SHV 1	TCGGGCCGCGTAGGCATGAT	ESBL	660	55	Moosavian & Deiham, 2012
SHV 2	AGCAGGGCGACAATCCCGCG				
CTX-M 1	TTAATGATGACTCAGAGCATTC	ESBL	901	46	Kim *et al*., 2005
CTX-M 2	GATACCTCGCTCCATTTATTG				

Key: Tm: Annealing temperature

ESBL-producing organisms are problematic in hospitalized patients worldwide (Bradford, 2001; Perez *et al*., 2007; Zeina *et al*., 2017). Infections can involve the respiratory tract (pneumonia), urinary tract and bladder, skin and soft tissue, blood, gastrointestinal tract, reproductive organs, and central nervous system (Sharon *et al*., 2018). This phenomenon of resistance to antibiotics makes infections more difficult to treat. CTX-M, TEM and SHV enzymes are the dominating ESBLs globally, but the dominating enzyme differs somewhat between countries (Perez *et al*., 2007). Reliable detection of ESBL production by clinical microbiology laboratory is essential to guide the clinicians to provide appropriate therapy (Rudresh, & Nagarathnamma, 2011). Hence, the importance of molecular detection method as have been shown in this study. The high rate of SHV-enzymes detected in this study could be due to the high level of penicillin used in medical settings over time. Another study on the characterization of ESBL-producing *E. coli* obtained from animal fecal samples revealed the presence of TEM (42.1%) and CTX-M (44.7%) whereas SHV gene was absent (Olowe *et al*., 2015). Thus, it is likely that ESBL type depends on the source of isolates. However, in order to ascertain the assumption, several studies are required from various sources to determine the prevalence and distribution of ESLBs in this region. This will provide basis and resourceful epidemiological data to guide the government and policy makers in mapping out strategies necessary to curtail the rising trend in ESBLs and antibiotic resistance in this region.

Conclusion

The study revealed a high level of multi-drug resistant ESBL-producing Enterobacteriaceae in clinical setting in Lagos. For this reason, treatment failure may be enormous and inevitable. Hence, a proactive antibiotic surveillance system and antibiotic stewardship are urgently needed to curtail spread of resistant bacteria our community.

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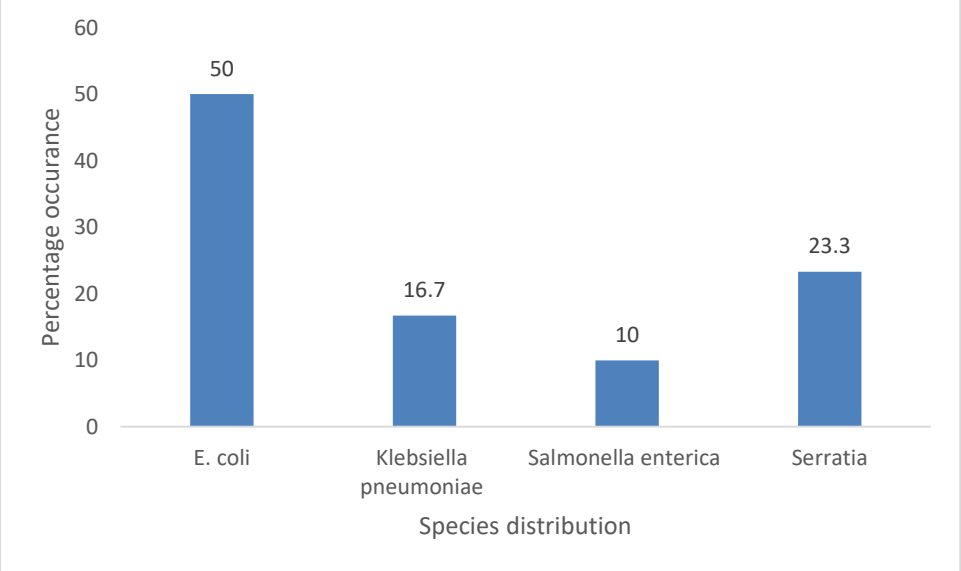

Figure 1: Members of Enterobacteriaceae identified in clinical samples

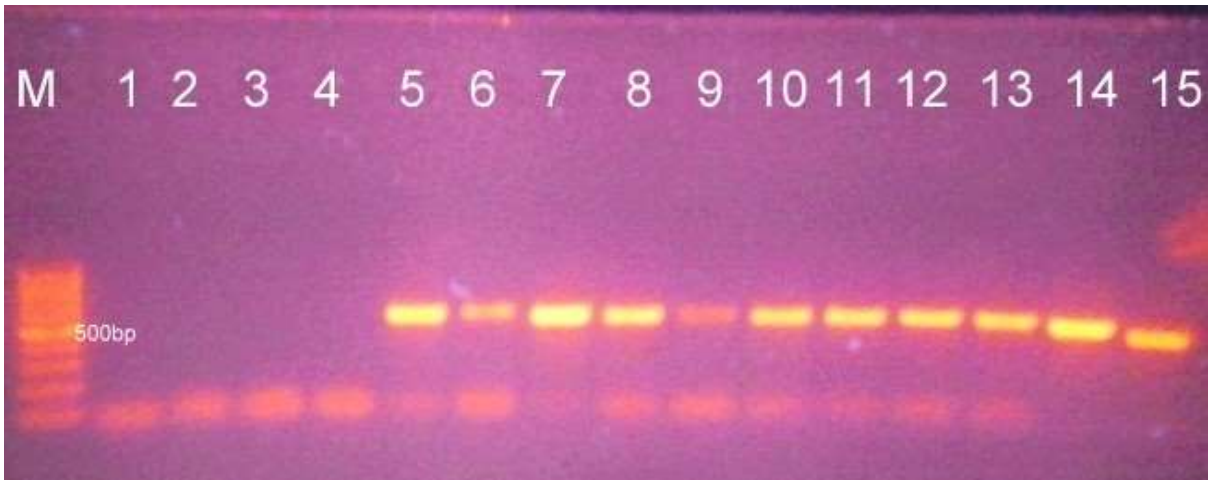

Figure 3: Electrophoregram of SHV genes amplified in PCR.

M: Molecular ladder (100bp), Lane 1: Negative control, Lane 5-15: SHV positive.

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