DETECTION OFBETA-LACTAMASE GENES IN UROPATHOGENS OF DIABETIC AND NON-DIAB TIC SUBJECTS IN ELEME GENERAL HOSPITAL, RIVERS STATE, NIGERIA.

ABSTRACT

One of the leading antimicrobial resistance mechanisms for many uropathogens among diabetic subjects is Extended–Spectrum Beta-Lactamase (ESBL) enzyme production which has become a public health concern. The aim of the study is to investigate the presence of Beta-Lactamase genes(TEM gene, CTX-M gene and SHV gene) in uropathogens of diabetic and non-diabetic subjects in Eleme, Rivers State. The 16s rRNA classification of bacteria was used and isolates were identified as *Staphylococcus aureus, Serratia marcescens, Serratia ficana, Kluyvera sp., Escherichia coli, Klebsiella aerogenes, Pragia fontium, Kocuria palustris, Pantoea dispersa, Enterobacter hormaechie, Enterobacter mori,* and *Morganella morganii*. Isolates were screened for resistant genes of $_{\text{blaTEM}}$(Temoniera), $_{\text{blaCTX-M}}$(Cefotaxime Munich) and $_{\text{blaSHV}}$(Sulfydryl Variable). These genes were identified using the 27F : 5- AGAGTTTGATCMTGGCTCAG-3 and 1492R: 5-CGGTTACCTTGTTACGACTT-3 primers on a AB19700 Applied Biosystems thermal cycle at a final volume of 40 microlitres for 35 cycles (Polymerase Chain Reaction).Twenty (20) multi drug resistant isolates(antimicrobial resistance shown by a species of micro-organism to at least one antimicrobial drug in three or more antimicrobial category) were investigated for the presence of ESBL resistant genes and 17 were found to possess ESBL genes representing 85% of the isolates investigated. The study revealed that CTX-M had an overall percentage prevalence of 41.2%, TEM had 35.3% and SHV had 23.5% respectively. A statistical significance of p=0.0195 was observed in the SHV gene in the distribution pattern of ESBL genes. The TEM and CTX-M genes had p=0.9999 and p=0.8282 respectively which showed no statistical significance.

1. INTRODUTION

The most widely used group of antimicrobial agents are the β-lactam drugs. The members of this drug group all share a specific core structure which consists of a four-sided β-lactam ring. Resistance to the β-lactam drugs occurs through three general mechanisms: (1) preventing the interaction between the target PBP and the drug, usually by modifying the ability of the drug to bind to the PBP (this is mediated by alterations to existing PBPs or acquisition of other PBPs; (2) the presence of efflux pumps that can extrude β-lactam drugs; (3) hydrolysis of the drug by β-lactamase enzymes (Bush & Bradford, 2016).

The β-lactamases (originally called penicillinases and cephalosporinases) inactivate β-lactam drugs by hydrolyzing a specific site in the β-lactam ring structure, causing the ring to open. The open-ring drugs are not able to bind to their target PBP proteins. The known β-lactamases are wide-spread, and the group contains enzymes that are able to inactivate any of the current β-lactam drugs. The production of β-lactamases is the most common resistance mechanism used by gram negative bacteria against β-lactam drugs, and the most important resistance mechanism against penicillin and cephalosporin drugs (Kumar & Mukherjee, 2013).

The β-lactamase enzymes are classified based on their molecular structure and/or functional characteristics. Structurally they are placed into four main categories (A, B, C, or D). There are three functional groupings based on the substrate specificity: the cephalosporinases, the serine β-lactamases, and the metallo (zinc-dependent) β-lactamases. These enzymes may also be commonly known by their enzyme family; for example: the TEM (named after the first patient) family, the SHV (sulphydryl variable) family, and the CTX (preferentially hydrolyze cefotaxime) family. Gram negative bacteria may produce β-lactamases from all four structural groups. The β-lactamases found in gram positive bacteria are mainly from group A, with some from group B (Reygaert, 2013).

These enzymes may be innately found on the bacterial chromosome or may be acquired via a plasmid. Many members of the *Enterobacteriaceae* family of gram-negative bacteria possess chromosomal β-lactamase genes. Other gram-negative bacteria that possess these include *Aeromonas* spp., *Acinetobacter* spp., and *Pseudomonas* spp. Plasmid-carried β-lactamase genes are most commonly found in the *Enterobacteriaceae*, but may also be found in some species of gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium* (Schltsz & Geelings, 2012).

Globally, diabetes as a chronic metabolic disorder of multiple aetiologies is assuming epidemic proportions with an estimated 415 million adults affected in the world and 14.2 million adults aged 20-79 years have diabetes in the African region (International Diabetes Federation (IDF), 2015).Investigating the presence of Beta-Lactamase genes in uropathogens of diabetic and non-diabetic subjects in General Hospital Ogale, Nchia-Eleme, Rivers State was the focus of this work.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was General Hospital, Ogale, Nchia- Eleme, Rivers State. Eleme is a local government area in Rivers State, Nigeria. It is a part of the Port Harcourt metropolitan city, covering an area of 138 km2. The Eleme people are Eleme's main indigenous ethnic group. Ogale in Eleme is located at Latitude 4.78711^{o} N and Longitute 7.12684^{o} E. Eleme has two of Nigeria's four petroleum refineries and one of Nigeria;s busiest seaport located at Onne a famous town with numerous industries (en.m.wikipedia.org).

Study Design

The case control study design was used to collect urine samples from diabetic and non-diabetic subjects (male and female) totaling three hundred (300) attending General Hospital, Ogale, Nchia-Eleme. The non-diabetic subjects were used as controls.

Ethical Consideration

Ethical approval was obtained from the Rivers State Hospitals Management Board. Consent forms were also given to participants for their consent.

ESBL Genes	**Total Occurrence**	**Diabetics (%)**	**Non-Diabetics (%)**	**Bacterial Isolates**

Sample collection

The clean catch procedure as described by Herrero *et a*l., (2015) was used to collect mid-stream early morning urine samples from three hundred (300) subjects(Two Hundred and forty (240) diabetic and Sixty (60) non-diabetic) that participated in the study.

Cultivation and Identification of Bacterial Isolates

Using the streak plate method, a sterile standard wire loop was used to inoculate urine samples aseptically onto McConkey, Mannitol salt and CHROMagar orientation plates and incubated aerobically at 37^{o}C for 24-48hrs. Bacterial isolates were subcultured onto nutrient agar plates to obtain pure colonies and preserved in 10% glycerol(Gilbert *et al.,*) for molecular identification. The following bacteria isolates were identified using 16s rRNA classification of bacteria:*Staphylococcus aureus, Serratia marcescens, Serratia ficana, Kluyvera sp., Escherichia coli, Klebsiella aerogenes, Pragia fontium, Kocuria palustris, Pantoea dispersa, Enterobacter hormaechie, Enterobacter mori*, and *Morganella morganii*.Isolates were screened for resistant genes of ${}_{\text{blaTEM}}$(Temoniera), ${}_{\text{blaCTX-M}}$(Cefotaxime Munich) and ${}_{\text{blaSHV}}$(Sulfydryl Variable) molecularly.

Statistical Analysis

Data obtained were analysed using chisquare and percntages where necessary.

RESULTS

Out of the Twenty (20) bacterial isolates that were investigated for multi drug resistance (when a micro-organism is resistant to more than three antibiotic agents), seventeen (17) representing 85% were found to possess ESBL resistant genes. The study revealed that CTX-M had an overall percentage prevalence of 41.2%, TEM had 35.3% and SHV had 23.5% respectively. The SHV gene in the distribution pattern of ESBL genes was found to be statistically significant, p=0.0195with diabetic subjects having higher percentage prevalence than their non-diabetic counterparts (Fig 1.) while TEM and CTX-M genes were statistically insignificant having p=0.9999 and p=0.8282 respectively.In the TEM gene, diabetic subjects had higher percentage prevalence than their non-diabetic counterparts which was also applicable in the CTX-M gene. In this piece of work, it was also discovered that *Pragia fontium* and *Kocuria palustris* were resistant to Vancomycin among diabetics isolates but were susceptible among the non-diabetics using the Kirby –Bauer disc diffusion procedure.

TEM	6(35.3)	4 (66.7)	2 (33.3)	*Serratia fonticola, Pragia fontium,S. aureus, Serratia liquefaciens, E coli, Proteus mirabilis, Rauotella planticola*
SHV	4(23.5)	3 (75)	1(25)	*S.aureus, E. coli, Enterobacter hormaechie, Proteus mirabilis,Rauotella planticola*
CTX-M	7(41.2)	5(71.4)	2(28.6)	*S.aureus, E.coli, Enterobacter hormaechie, Proteus mirabils, Rauotella planticola, Photorhabdus asymbiotica, Enterobacter cloacea, Enterobacter cancergenes, Serratia liquefaciens*

Table 1: Percentage Prevalence of ESBL Genes

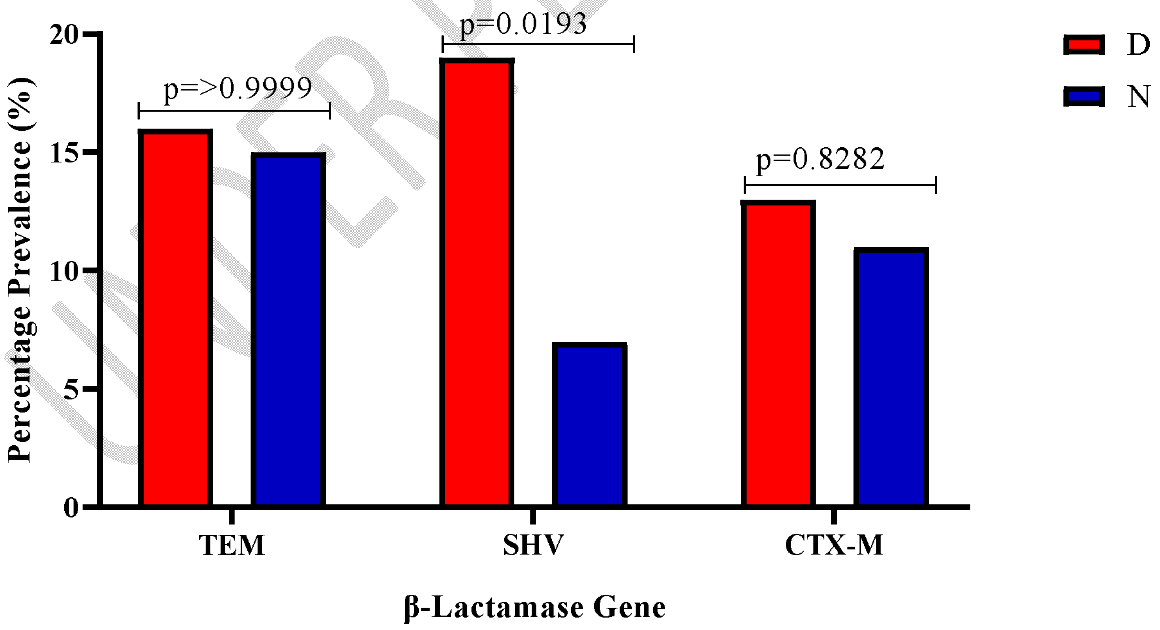

Figure 1: Distribution Pattern of Beta-lactamase Genes.

KEY:TEM- Temoniera, SHV- Sulfydryl Variable, CTX-M- Cefotaxime Munich

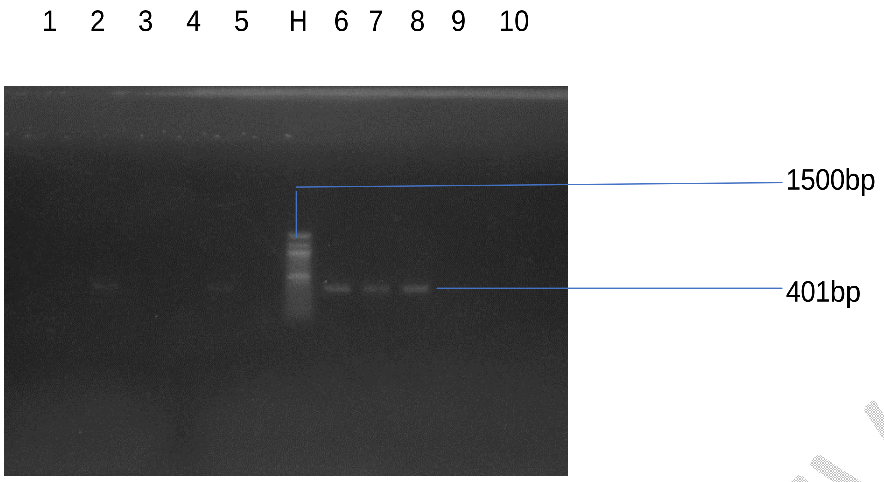

Plate 1: Amplified Tem gene on Agarose Gel after Electrophoresis. Lane H represents the 100bp Molecular ladder indicated at 401bp

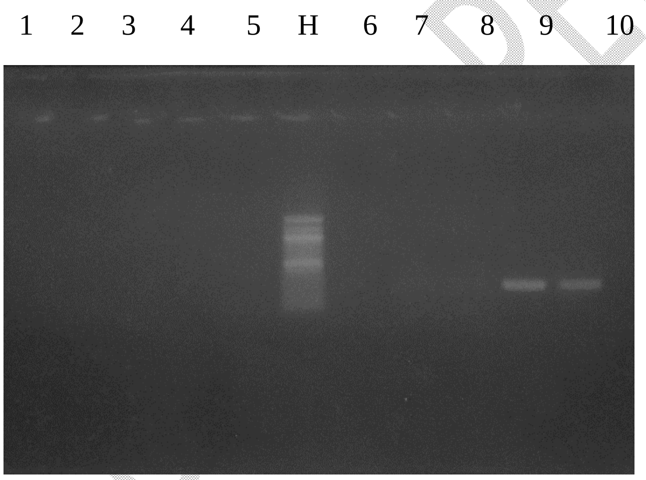

Plate 2. Agarose gel electrophoresis of SHV gene of bacterial isolates. Lane 9 and 10 represents the SHV gene bands (293bp). Lane H represents the 100bp Molecular ladder.

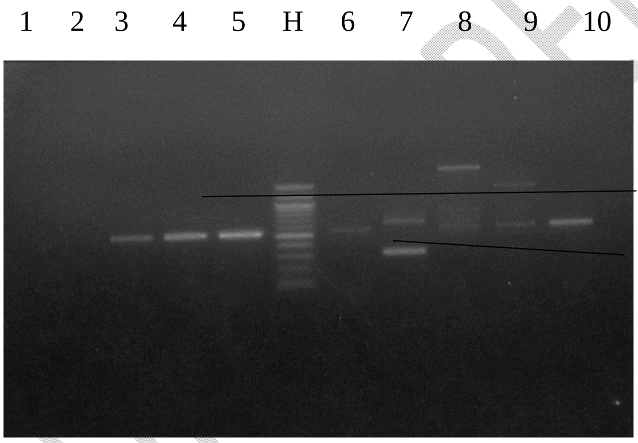

Plate 3. Agarose Gel Electrophoresis of Bacterial Isolates. Lane 3, 4, 5, 6, 7, 8, 9 And 10 Represent CTX-M Gene Bands (550bp). Lane H represents the 100bp Molecular ladder.

Total Number of Isolates	Total Number of ESBL	ESBL Isolates	ESBL Isolates

Table 2. Showing Percentage Occurrence of ESBL Isolates

Based on Bacteriuria			for Molecular			in Study Population (%)	Among Gram-Negative bacteria (%)
	Gram-Negative (%)	Gram-Positive (%)		ESBL- (%)	ESBL +(%)		
95	71	24	20	3(15)	17(85)	17.9	23.9

DISCUSSION

One of the leading antimicrobial resistance mechanisms for many UTI causing gram negative bacteria is Extended - Spectrum Beta-Lactamase (ESBL) enzyme production that hydrolyses the beta-lactamase ring of antimicrobials which confer bacteria resistance to commonly prescribed antibiotics including penicillins and first, second and third-generation cephalosporins (Belete, 2020). When a microorganism is resistant to more than 3 antibiotic agents, it is said to be multidrug resistant. The significant high level of drug resistance especially among the diabetic subjects found in this study could be attributed to indiscriminate use of antibiotics and the presence of resistant genes in the bacterial isolates. Rupp and Fey, (2003) showed that bacterial resistance can be acquired by the production of beta-lactamase enzymes that cause multidrug resistance to beta-lactamase antibiotics such as pencillin and broad spectrum cephalosporins. It also revealed that Extended Spectrum Beta Lactamases (ESBL) are generally derived from common beta-lactamases that have undergone amino-acid substitution near the active site of the enzyme. In clinical isolates worldwide, there is an increase in ESBL- producing organisms (Rupp and Fey,2003). In gram-negative bacteria, TEM-1 and SHV-1, two- broad spectrum Beta-lactamases have greatly increased in frequency as a result of the introduction of the first and second generation cephalosporins. This necessitated the development and introduction of new classes of beta- lactamase resistant to hydrolysis by these enzymes and these are present and encoded for enzymes hydrolysing expanded- spectrum cephalosporins such as Ceftazidime and Cefotaxin (Castanheira *et al.,* 2021).

Isolates with ESBL genes were identified in this study and these genes include, TEM (Temoniera), SHV (Sulfydryl Variable) and CTX-M (Cefotaxime Munich) among diabetic and non-diabetic subjects. This is in agreement with the findings of previous studies where *E. coli* and *Enterobacter sp.* were found to have TEM, SHV and CTX-M (Obasi *et al.*, 2017).

Seventeen (17) multi drug resistant isolates were found to possess ESBL genes representing 85% (Table 2) out of the Twenty isolates investigated. This is slightly lower than the report of the study conducted by Legese *et al.,* (2017) that recorded 78.6% in ESBL producing *Enterobacteriaceae*.

In this study, 23.9% of the Gram-negative isolates were found to be ESBL producers (Table 2). This again is higher than that reported by Belete, (2020) (15.8%), but almost similar to the report of Onwuezobe and Orok, (2015) (20.0%). The rise in the prevalence of ESBL producing pathogens discovered in this present study might be attributed to the habit of empirical treatment practice without drug susceptibility testing and poor compliance of subjects with prescribed drugs. The findings of this study is however in contrast and lower than the reports of Krishnakumar *et al*., (2012) (44.4%). This could be due to differences in the study population and health care trends. Isolates found as ESBL producers in this study among diabetic and non-diabetic subjects include: *Serratia marcescens, Pragia fontium, E coli, Staphylococcus aureus, Enterobacter hormaechie, Enterobacter cloacea, Enterobacter bugandensis, Pantoea dispersa, Seriatia surfactantfaciens, Proteus vulgaris and Kocuria palustris* (all Gram-Negatives, Table 1). These findings collectively showed that the sufficiently rising occurrence of ESBL producing isolates showing resistance to commonly used antibiotics in the subjects under study (diabetics and non-diabetics) calls for much concern and attention. Discoveries of the prevalence of ESBL producing micro-organisms in clinical isolates in different geographical locations have been carried out. Studies in United Arab Emirates revealed about 38-39% of *E. coli* isolates as having ESBL (Machado *et al.,* 2007). In another study that was conducted over a period of 2 years in Portugal 39% of isolates of the *Enterobacteriaceae* family were confirmed to possess ESBL (Al-zaroumi *et al.,* 2008). In Nigeria, ESBL has also been reported in clinical isolates of *Enterobacteriaceae* (Iroha *et al*., 2009, Yusha'u *et al*., 2010).

It was discovered from this study that CTX-M has an overall percentage prevalence of 41.2% (Table 1). This is contrary to the report of Lavigne *et al.*, 2007 (68%) and Mendonca *et al.,* 2007 (66%). A study revealed that CTX-M gene can be gotten by the horizontal gene transfer from other bacteria using genetic apparatuses such as conjugative plasmid or transposon; they preferentially hydrolyze Cefotaxin and are mainly in members of the *Enterobacteriaceace* family (Gazouli *et al*., 1998). Percentage prevalences of 35.3% (TEM) and 23.5% (SHV) were also discovered in this study (Table 1). Findings from this work also revealed that TEM has a prevalence of 66.7% among diabetics and 33.3% among non-diabetics, SHV has 75% prevalence in diabetics and 25% in Non-diabetics while CTX-M has 71.4% in Diabetics and 28.6% in Non-Diabetics (Table 1). Distribution patterns of ESBL genes among diabetic and non-diabetic subjects as revealed by this study is shown in Fig 1. A statistical significance was observed in the SHV gene with diabetic subjects having higher percentage prevalence than their non-diabetic counterparts with p=0.0193. In the TEM gene, diabetic subjects had higher percentage prevalence than the non-diabetic subjects. This was also applicable in the CTX-M gene with P=0.9999 and P=0.8282 respectively which showed no statistical significance. The higher percentage prevalences of these ESBL genes among the diabetic subjects could be responsible for the high resistance to antibiotics by these subjects as discovered in a previous study (Hanson *et a*l., 2023).

CONCLUSION

The bacterial isolates of diabetic and non-diabetic subjects demonstrated possession of SHV, TEM and CTX-M Extended Spectrum Beta-Lactamase (ESBL) genes as revealed from this study which conferred resistance to antibiotics on spme of the isolates. Presence of Beta-Lactamase genes caused multi-drug resistance to beta-lactamase antibiotics thereby making treatment options difficult. There is therefore need for the development and introduction of new classes of beta-lactamases resistant to hydrolysis by these enzymes.

CONSENT

Written informed consent was obtained from all patients as declared by all the authors.

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