Molecular Epidemiology of Extended Spectrum Beta-lactamases Producing *Escherichia coli* and *Klebsiella* Species in Catheterized Patients

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Abstract — Irrational antibiotics use has added to the escalation of antibiotics resistance, especially among hospitalized patients on prolonged urethral catheterization, a significant risk factor for urinary tract infection and urosepsis. Extended-spectrum β -lactamases are transferable plasmid-mediated resistance mechanism orchestrated majorly by Enterobacteriaceae, which confer resistance to β -lactam antibiotics and other classes of antibiotics. This work was aimed at determining the molecular characteristics of uropathogenic *Escherichia coli* and *Klebsiella* spp involved in urinary tract infections among patients on prolonged urethral catheterization in two major tertiary hospitals in Lagos. One hundred and one samples were collected from participants in Lagos University Teaching Hospital and 68 Army Reference Hospital Yaba, between November 2015 and May 2016. The mean age of the participants was 49.04 ± 8.8 years.

Single, non-repeat aseptically aspirated urine specimens from the catheter ports were obtained from consenting participants and processed immediately. Bacterial species were isolated and characterized by conventional methods. Antibiotics susceptibility testing was done using a modified Kirby Bauer method. Further analysis was done by Polymerase Chain Reaction amplification aimed to detect bla SHV, bla TEM, and bla CTX-M resistance genes. Isolates were considered significant if there were up to 10^4 CFU/ml in symptomatic participants and $\geq 10^5$ CFU/ml in asymptomatic participants with analyzed. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 (Inc., Chicago 111). Forty (39.6 %) males and 61 (60.4 %) female participants' catheter urines were sampled, with male to female ratio of 1:1.5.

Fifty-nine (58.4%) out of 101 samples had significant growth, while 32 (54.2%) of these were lactose fermenters. Of the 32 lactose fermenters, 26 were identified as *E. coli* and *Klebsiella* spp, while 23 (88.5%) of these 26 (identified as *E. coli* and *Klebsiella* spp) were ESBL producers carrying ESBL gene(s) and revealed various degrees of antibiotics resistance. We conclude by discussing the epidemiological importance of improving the infection control practices and antibiotics stewardship program as central dogma to controlling antibiotics resistance in hospitals.

Index Terms — CAUTI, ESBL, Significant bacteriuria, Antibiotics resistance, gene.

I. INTRODUCTION

Enterobacteriaceae, notably *Escherichia coli* and *Klebsiella* spp, are among the most significant etiologic agents of serious hospital-associated and community-onset infections in humans [1], [2].

Beta-lactam antibiotics are significant groups of antibiotics class commonly employed in the treatment of most infections caused by Enterobacteriaceae. Therefore, resistance to these drugs will pose a severe challenge to clinical therapeutic choices [3]. Extended-spectrum beta-lactamase (ESBL) production is associated predominantly with Enterobacteriaceae particularly *E. coli* and *Klebsiella pneumoniae* [4].

This group of organisms (ESBL producers) represents an emerging clinical threat not only for their rapid and widespread dissemination but also because they often exhibit multidrug-resistant phenotypes leaving only limited therapeutic options. A significant impact on morbidity, mortality, and healthcare-associated cost has been demonstrated previously for infections caused by ESBL producing organisms [5].

Urinary tract infections (UTI) represent one of the most common (34%) types of nosocomial infections closely following pneumonia [6] and indwelling urethral catheterization has been found as a predominant risk factor in the majority (about 80%) of UTI among hospitalized patients and people in long term care facilities (LTCF) whereas, others may be due to genitourinary procedures [7-9] also, catheter-associated UTI (CAUTI) is the most common cause of bacteremia in LTCF [10].

In spite of the closed drainage system, about 48% of hospitalized patients with the indwelling urethral catheter still develop CAUTI. The duration of catheterization, female gender, inadequate catheter care, and lack of appropriate antibiotics use are other risk factors leading to the development of CAUTI [8].

It has been previously reported that infection caused by bacteria in the group of ESBL producing Enterobacteriaceae is highly associated with a delay in the initiation of appropriate antibiotics therapy, increased patients' morbidity, mortality, prolonged hospital stays and increased hospital cost when compared to infections caused by susceptible strains [11], [12]. The prevalence of ESBL producing Enterobacteriaceae has been on a steady global increase in the past decades [13], with mounting evidence suggesting that non-therapeutic use of antibiotics in foods, animals and consequent contamination of food with ESBL

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producers has played an importantly significant role in the spread and dissemination [14]. It was reported recently that among the enterobacteriaceae, the TEM and SHV derived types are the most predominant enzyme types among those ESBL phenotypes [15], [14] and that other ESBL genes are derived from these classic types SHV and TEM via mutation and plasmid or amino acids substitution around the active sites. These organisms are resistant to all penicillins, first, second and third-generation cephalosporins (except cephamycins and carbapenems) and Aztreonam; there is also associated resistance to aminoglycosides, trimethoprim-sulphamethoxazole and high co-existence of fluoroquinolone resistance [16]-[19].

Reports from many regions of the world showed different prevalence rates of ESBL producing Enterobacteriaceae causing urinary tract infections, however, *E. coli* and *K. pneumoniae* are the most prevalent ESBL positive spp, but all Enterobacteriaceae can harbour plasmid mediated ESBL genes [20], [21]. In 2004 Colodner and co-workers postulated that the risk factors associated with UTI caused by ESBL producing bacteria were infections due to *Klebsiella spp*, previous hospitalization, previous antibiotics treatment, and male gender, age over 65 years and diabetes mellitus. The spread of ESBL within the community of people without prior contact with either health facility or antibiotic consumption has been demonstrated [20]-[22]. Also, these uropathogens have shown slow but steady resistance to several agents over the last decades [23].

Carbapenems remain the drug of choice for serious infections caused by ESBL producing organisms through their indiscriminate use should be avoided. Fourth-generation cephalosporins can be a therapeutic alternative for mild to moderate infections, provided their pharmacokinetics and pharmacodynamic targets can be easily achieved. However, in the uncomplicated lower urinary tract infections, Fosfomycin and Nitrofurantoin are the best treatment alternatives [24], [25].

Therefore, diligent hand hygiene by healthcare workers and avoidance of indiscriminate (inappropriate) antibiotics use are keys in preventing infection and further development and spread of this antimicrobial resistance [26]. Establishment of an institutional antibiotics stewardship program/ committee, among other methods, has contributed in no small measure to the prevention and control of bacterial resistance especially in hospital settings [27].

Based on those above, this study will attempt to determine the molecular characteristics and epidemiology of uropathogenic *E. coli* and *Klebsiella spp* genes involved in antibiotics resistant UTI among participants on prolonged catheterization in Lagos, Nigeria.

II. MATERIALS AND METHODS

A. Study Population

One hundred and one (101) catheter urine samples were collected from consenting participants admitted in all the wards in Lagos University Teaching Hospital (LUTH) and 68 Nigerian Army Reference Hospital Yaba (68 ARHY). To assist to further establish some risk factors for CAUTI and possible carriage of ESBL uropathogenic *E. coli* and

Klebsiella spp, simple structured questionnaire was developed to determine patients demographic data, the duration of patient's admission, the indication for admission, the primary diagnosis, the indication for urethral catheterization, the duration of catheterization, history of dysuria/ suprapubic pain or tenderness, possible history of UTI, antibiotics history, history of previous hospital admission, etc

B. Ethical Consideration

Ethical review was obtained from the Health Research Ethics committee of Lagos University Teaching Hospital and 68 Army Reference Hospital Yaba (Approval number: ADM/DCST/HREC/APP/2168 and 68NARHY/G3/ERC10/10). Informed consent was obtained from participants after explanation of the study while the privacy and confidentiality of data obtained from the study participants were kept.

C. Sample Collection and Processing

Catheter urine samples were collected from all consenting participants on an indwelling catheter for more than three days whether symptomatic or asymptomatic for urinary tract infection (UTI). Detailed specimen collection procedures and transportation were based on WHO 2014 guidelines on sample collection.

Standard/ calibrated wire loop (that delivers 0.001ml of urine was used) for semi-quantitative urine culture. Samples were cultured on 5% sheep blood agar (SBA) and Cysteine lactose electrolyte deficient (CLED) agar (OXOID, UK) incubated aerobically for 18-24hours at 35-37°C. After 24hours, those with significant growth (those with colony count up to $\geq 10^5$ CFU/ml) were identified by standard bacteriological methods using colonial morphology, Gram stain, motility and the use of MICROBACT 12A (OXOID, UK).

Antibiotics susceptibility testing following the modified Kirby Bauer method [www.clsi.org] was employed. The *E. coli* and *Klebsiella spp* isolates confirmed to be ESBL producers using the double-disc synergy test (DDST) were further processed for molecular characteristics and the primer sequences as shown in table 1 was employed in the molecular analysis.

TABLE 1: PRIMERS USED FOR DETECTION OF RESISTANT GENES

Primers	Oligonucleotide Sequence (5' to 3')	References	Expected amplicon size (bp)
TEM-Forward	ATGAGTATTCAACATTTCCG	28	517
TEM-Reverse	CTGACAGTTACCAATGCTTA		
SHV-Forward	GGTTATGCGTTATATTCGCC	28	620
SHV-Reverse	TTAGCGTTGCCAGTGCTC		
CTX-M ₁ -Forward	ATGTGCAGYACCAGTAARGT	28	585
CTX-M ₂ -Reverse	TGGGTRAARTARGTSACCAGA		

QUALITY CONTROL: *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used.

III. RESULTS

Out of 101 participants recruited for this study, 39.6% (40/101) were males, and 60.4% (51/101) were females. The mean age of the participants was 49.04 ± 8.7 years while 12.9% were < 30years, 37.6%, 30-49years, 34.7%, 50-69 years and 14.9%, > 70 years (Table 2)

TABLE 2: GENDER AND AGE DISTRIBUTION OF STUDY PARTICIPANTS

Age range	Sex		Total
	Males (%)	Females (%)	
<30	6 (5.9)	6 (5.9)	12 (11.8)
30-49	13 (12.9)	25 (24.8)	38 (37.7)
50-69	16 (15.8)	20 (19.8)	36 (35.6)
≥70	5 (5.0)	10 (10.0)	15 (15.0)
Total	40 (39.6)	61 (60.4)	101 (100)

Fifty-nine 59 (58.4%) of the samples had significant bacteriuria, while 42 (41.6 %) had no growth, mixed growth, or non-significant growth (Table 3). The following organisms were also isolated from the 59 samples with significant bacteriuria: *Enterococcus spp* (4%), *Proteus spp* (2%), *Staphylococcus spp* (4%), *Acinetobacter baumannii* (2%) and *Candida spp* (12%) and were predominantly non-lactose fermenters while 32 (54. 2%), were lactose fermenters and made up of- *E. coli* (9.9%) *Klebsiella spp* (15.8%), *Enterobacter [Pantoea] agglomerans* (6.9%), *Serratia marcescens* (1.7%), employing further biochemical identification using Microbact 12A (Oxoid, USA).

TABLE 3: BACTERIAL PROFILE OF CULTURED URINE SAMPLES FROM STUDY PARTICIPANTS

Organisms	Significant Bacteriuria (%)	Total (%)
<i>Klebsiella spp</i>	16 (15.8)	101 (100.0)
<i>Candida spp</i>	13 (12.9)	101 (100.0)
<i>E. coli</i>	10 (9.9)	101(100.0)
<i>Enterobacter agglomerans</i>	7 (6.9)	101 (100.0)
<i>Staph. Spp</i>	4 (4.0)	101 (100.0)
<i>Enterococcus spp</i>	4 (4.0)	101 (100.0)
<i>Acinetobacter</i>	2 (2.0)	101 (100.0)
<i>Proteus spp</i>	2 (2.0)	101 (100.0)
<i>Serratia rubidae</i>	1 (1.7)	101 (100.0)
Total	59 (58.4)	101(100.0)

Out of the 26 (81.1%) *E coli* and *Klebsiella spp*, 23(88.5%) were ESBL producing while 3(11.5%) were ESBL producing uro-pathogens (Table 4). Twenty-three (23) of the isolated organisms were phenotypically confirmed (with DDST) to be ESBL producing and were analyzed using Polymerase chain reaction (PCR). Among the isolates analyzed were, 9 (39.1%) *E. coli*, 14 (60.9%) *Klebsiella spp* (8 were *Klebsiella oxytoca*, 5 *K. pneumoniae*, and 1 *Klebsiella. ozanae*). Three (*E. coli* and 2 *Klebsiella spp*) of the study isolates were phenotypically confirmed to be non-ESBL producing (Table 4).

Participants with urogenital pathologies were noticed to have higher carriage rate of ESBL organisms [7/23 (30.4%)], followed by participants with central nervous system pathologies [6/23 (26.09%)], those with obstetrics conditions had [3/23 (13.04%)], while those with cardiovascular-related diseases had 2/23 (8.7%). Participants

with endocrine disorders had the least carriage rate of ESBL organisms 1/23 (4.3%), while those with orthopedics and other conditions (burns, chronic myeloid leukemia, paracolic abscess etc.), had 4/23 (17.4%) (Fig. 1).

TABLE 4: ESBL/NON-ESBL PRODUCING ENTEROBACTERIACEAE ISOLATES FROM CAUTI OF STUDY PARTICIPANTS

ORGANISMS	ESBL		TOTAL (%)
	POSITIVE (%)	NEGATIVE (%)	
<i>E.coli</i>	9 (15.3)	1 (1.7)	10 (17.0)
<i>Klebsiella spp</i>	14 (23.7)	2 (3.4)	16 (27.1)
<i>Enterobacter agglomerans</i>	4 (6.8)	3 (5.1)	7 (11.9)
<i>Serratia rubidae</i>	1 (1.7)	0 (0.0)	1 (1.7)
TOTAL	28 (47.5)	6 (10.2)	34 (57.7)

Fig. 1. Proportions of ESBL positive carriers in relation to their pathology.

Out of the 14 isolates that expressed the *bla* genes, 1 (7.1%) expressed all (*bla CTX-M*, *bla TEM* and *bla SHV*) the three genes under study, 1 (7.1%) expressed *bla CTX-M* and *bla TEM* 4(28.6%) expressed *bla SHV* and *bla TEM*, 1 (7.1%) expressed *bla SHV* and *bla CTX-M*, 2 (14.3%) expressed only *bla CTX-M* genes, 4 (28.0%) expressed *bla SHV* only and 1(7.1%) expressed *bla TEM* only (Fig. 2).

Fig. 2: Carriage rate of the different bla genes the resistant isolates.

All (100%) isolates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in this study possess *bla* genes whereas, 60% of *Klebsiella oxytoca* and 55.5% of *Escherichia coli* respectively possess the *bla* genes (Fig. 3). PCR Amplification of *bla SHV* group and *bla CTX-M* group 1are shown in Fig. 4 and 5.

Fig. 3. Proportion of different isolates with bla genes.

Fig. 4: PCR Amplification of bla_{SHV} group 1. Lane M: Hyperladder IV; 13 Bands from 250-1000bp, lane 4, 10, 11 and 13 are positive for bla_{SHV} genes. Lanes 1-3, 5-9 and 12 are negative for bla_{SHV} genes. Product size is 620 bp.

Fig. 5. PCR amplification of bla_{CTX-M} group 1. Lane M: Hyper ladder IV; 9 Bands from 250-1000bp, lane 1, 2, and 9 are positive for bla_{CTX-M} genes. The product size is 585 bp for bla_{TEM} genes.

IV. DISCUSSION

The magnitude of bacterial resistance to antibiotics has continued to rise, and unfortunately, infections caused by resistant organisms results in massive morbidity and mortality worldwide. Also, the growth of global trade and travel allows resistant microorganisms to spread rapidly to distant countries and continents through humans, food and medical tourism [29], [30].

The Infectious Diseases Society of America (IDSA) recognizes antimicrobial resistance as “one of the greatest threats to human health worldwide” [31]. To highlight the

importance of this subject, antimicrobial resistance was on the front burner at the World Health Organization (WHO), 2011 World Health Day. The impact of multidrug resistance (MDR) extends into all aspects of medicine, transcending the field of human medicine to the field of Agriculture and threatens the significant progress which has been made in transplantation, oncology, and surgery to mention but a few [25]. Antibiotics resistance is currently a global pandemic driven by increased irrational antibiotic consumption resulting in antibiotic pressure and selection of resistant strains especially among hospitalized individuals or those in long term care facilities [7]-[8]. The selection pressure is made possible as a result of poor/irrational antibiotics prescription and lack of antibiotics stewardship program in our health institutions.

In this study, 59 (58.4%) catheterized urine samples had significant bacteriuria, which is far higher than the prevalence obtained in work done by Onyegbule et al. in 2015 [32] (11.4%) at Nnewi South Eastern Nigeria. The disparity may not be unconnected with the fact that their study participants were not on prolonged catheterization as the participants in this study. However, our work was in agreement with that of other workers [33], [34] but much lower than the findings of Taiwo and Aderounmu in 2006 [35], all of which were within the same geographic region. It is also interesting to note that in this study, *Klebsiella spp* (16.8%) was the most prevalent etiologic agent of CAUTI which is contrary to most of the earlier studies in which literature where *E. coli* appears to be the most prevalent agent [33], [36], [37] but it is consistent with the work of Panders et al. in 2003 [38].

Globally, the prevalence of ESBL production and antibiotics resistance has increased remarkably among uropathogenic isolates over time, as observed by Datta and others in 2012 [39], raising concern over the therapeutic management of the infections due to these agents. The remarkable increase in ESBL production among *E. coli* and *Klebsiella spp* isolates may not be unconnected to the fact that they are both gastrointestinal flora predisposed to a high level of quorum sensing and resistance genes (plasmids) transfer within this biome [40]. Poor infection control practices; poor catheter care etc., may have fueled this increase. Fourteen (60%) of the studied isolates possessed various ESBL genes, about 7.1% carried all the genes (CTX-M, TEM and SHV), 42.8% possessed two of the genes each while about 50% has only one of the genes. This correlates with the work done by Manoharan et al. in 2011 [41]. All the *Klebsiella pneumoniae* in this study had an SHV gene, agreeing with the study carried out by Tolentino and co-workers in 2011 [42]. More than half of the *Klebsiella spp* with SHV genes were from internal Medicine wards, whereas the remaining were from obstetrics and gynecology wards, revealing the possibility of cross-contamination of patients from hands of HCWs and shared instruments/wares. This may also be due to the dissemination of plasmids among genetically related isolates or clusters of *Klebsiella spp* as plasmid encoding ESBLs are efficiently transferred among *Klebsiella spp* [42]. There was an interrelationship found between ESBL carriage and pathologic conditions of participants: those with urogenital conditions (e.g. Benign Prostatic Hyperplasia) and central

nervous system (Cerebrovascular accident) had higher ESBL carriage than others. This may be because of the prolonged duration of catheterization in this group of patients and hospital stay.

V. CONCLUSION

From this study, we suggest that periodic monitoring and surveillance for ESBL producing organisms should be encouraged in our hospitals to track the current trend of antimicrobial resistance and possible predisposing factors to enable appropriate implementation of remedies. More so, education and infection control practices should be re-enforced in the hospitals to curb cross infections/transmission of MDR, and as well urethral catheterization should be reserved only for patients that require it, and the procedure should be aseptically carried out. Also, regular screening for CAUTI and catheter care/change should be encouraged, together with the institution of antibiotics stewardship committee/programs in all hospitals. Conclusively, research into the development of novel antibiotics are urgently needed to combat the superbugs ravaging our reserved antibiotics agents and rendering them ineffective.

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