

RESEARCH ARTICLE

## High level detection of extended spectrum beta-lactamase gene encoding Enterobacteriaceae in public toilets in Abeokuta, Nigeria

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**Abstract:** Increasing reports of the pandemic nature of extended spectrum  $\beta$ -lactamase (ESBL) producing bacteria in clinical infections called for more surveillance to identify possible sources of infection. This study examined the dissemination of ESBL producing Enterobacteriaceae in hospital and public toilet facilities in Abeokuta. Swab samples were collected from toilet seats, door handles, toilet bowls and flush handles. Detection of ESBL producing isolates was done using double disc test. Presence of ESBL gene was done using multiplex PCR. Overall, ESBL-producing bacteria was detected in 44 (27.5%) out of 160 samples. ESBL-producing bacteria were detected at significantly higher rate ( $p < 0.001$ ) in hospital toilets than in community toilets. Isolates were identified as *Klebsiella pneumoniae* (n=25), *Klebsiella oxytoca* (n=2), *Citrobacter freundii* (n=6), *Citrobacter youngae* (n=3), *Escherichia coli* (n=5), *Enterobacter cloacae* (n=2) and *Enterobacter kobei* (n=1). Analysis for the presence of ESBL gene showed that all the isolates harboured the  $bla_{CTX-M}$  gene while  $bla_{TEM}$  and  $bla_{SHV}$  were detected in six and two isolates respectively. All the ESBL-producing isolates demonstrated resistance to at least three different classes of antimicrobials. The study showed that public toilets could serve as sources for nosocomial and community transmission of ESBL-producing bacteria in the environment.

**Keywords:** ESBLs, enterobacteriaceae,  $bla_{CTX-M-15}$ , public toilets, nosocomial transmission, multidrug resistance.

### INTRODUCTION

The extended spectrum  $\beta$ -lactamases (ESBLs) are enzymes capable of hydrolyzing and inactivating beta lactams antimicrobials especially penicillins, monobactams as well as first to fourth generation cephalosporins (Bradford, 2001). Thus, bacteria that produce ESBLs are resistant to many clinically important old and new generation penicillins and cephalosporins (Bradford, 2001). The  $\beta$ -lactam antimicrobials are among the most commonly used antimicrobials in human medicine because of their relatively high efficacy, low toxicity and low cost (Bush and Bradford, 2016). Diseases caused by ESBL-producing bacteria could be very difficult to treat because of non-responsiveness to antimicrobial therapy and limited choice

of antimicrobials (Lautenbach *et al.*, 2001; Hu *et al.*, 2019). Most ESBL strains are not only resistant to  $\beta$ -lactam antimicrobials but display multidrug resistance to other classes of antimicrobials due to co-location and co-selection of resistance genes to other antimicrobial agents alongside genes that mediate synthesis of ESBLs (Ruppé, 2015). The production of ESBLs is particularly high among members of the family Enterobacteriaceae with easy intra- and inter-species sharing of ESBL genes via mobile genetic elements (Ruppé, 2015). The Enterobacteriaceae are widely distributed in nature and are the most commonly isolated bacteria strains in clinical samples. They are associated with hospital- and community-acquired extra-intestinal infections including septicaemia, meningitis and urogenital tract diseases (Vading *et al.*, 2018).

Public toilets could serve as important source of indirect human-to-human transmission of pathogens, which may lead to widespread dissemination of disease during an outbreak (Suen *et al.*, 2019). People spend most of their time outside their homes for many reasons relating to works, social gatherings, travelling and other outdoor activities. Therefore, they need public toilets to relieve themselves. Ready access to clean toilets is essential to maintain hygienic environments and promote public health (Greed, 2006). Public places such as schools, transport stations, hospitals, religious centres and business organizations have toilets facilities, which members of the community may use. In most developing countries, inadequate access to public toilets contribute to environmental pollution and food/water contamination leading to diseases outbreaks (Peprah *et al.*, 2015). High pressure on few available public toilets, poor maintenance and unsanitary conditions make the toilets hot spots for contracting and quick dissemination of pathogens.

Earlier studies have reported the detection of ESBL-producing bacteria from clinical samples in hospitals in Abeokuta (Olowe and Aboderin, 2010). However, there is a dearth of information on the role of public toilets in the dissemination of ESBL-producing Enterobacteriaceae among human population in Abeokuta. Hence, the present study investigated the presence and antimicrobial resistance

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of ESBL-producing Enterobacteriaceae in selected public toilets in Abeokuta, Nigeria.

## MATERIALS AND METHODS

### Collection of samples

One hundred and sixty swab samples were collected from 40 public toilets in Abeokuta. In each toilet, four swab samples were taken from surfaces as follows: toilet seats, toilet bowls, toilet flush handle and door handle. Samples were collected within a period of 10 weeks from April to June 2017. Toilets located within hospitals (n=20) and community (n=20) settings were investigated. Samples were collected from toilets in three public hospitals (hospital setting) as well as from a religious worship centre, a bus station and a tertiary institution (community setting). Samples were properly labelled and preserved in icepack for transportation to the laboratory. Microbiological analysis commenced within 12 hours of sample collection.

### Isolation of ESBL-producing isolates from swab samples

Each swab sample was inoculated into nine millilitres of buffer peptone water (BPW) and incubated at 37°C overnight for pre-enrichment. A loopful of the pre-enrichment broth was streaked on MacConkey agar supplemented with ampicillin (100mg/L). This was incubated at 37 °C for 18 hours. Discrete colonies on MacConkey-ampicillin agar were separately transferred onto MacConkey agar supplemented with cefotaxime (1mg/L) for selective isolation of cefotaxime resistant strains. All cefotaxime resistant isolates were tested for phenotypic detection of ESBL producer using cefpodoxime/cefpodoxime-clavulanic acid combination disk test kit (Oxoid DD0029) according to the manufacturer's instruction and in line with recommendations of Clinical and Laboratory Standard Institute (CLSI, 2016). Briefly, test isolate was thoroughly emulsified in normal saline. The suspension was adjusted to an optical density corresponding to 0.5 McFarland standard. This was uniformly spread over Mueller Hinton agar (MHA) plate. Cefpodoxime and cefpodoxime-clavulanic acid disks were firmly placed on the surface of the inoculated MHA. The plate was incubated at 35 ± 2 °C for 18 hours. The difference in the diameter of zones of inhibition between cefpodoxime and cefpodoxime-clavulanic acid disks was determined. Isolates with a difference of five millimeter or above were confirmed as phenotypic ESBL producers.

### Bacterial Identification

Preliminary bacterial identification was based on cultural characteristics on MacConkey agar. Further identification of phenotypic ESBL-producers into genera and species was done based on biochemical characterization using commercial biochemical kit for the identification of Gram negative bacteria (Microbact GNB 24E, Oxoid<sup>®</sup> Basingstoke, UK) and by the MALDI-TOF MS technique.

### Phenotypic antimicrobial susceptibility testing of ESBL producing isolates

All identified ESBL-producing isolates were tested for susceptibility to selected antimicrobial agents using the Kirby-Bauer disk diffusion method. Isolates were tested for susceptibility to the following antimicrobials: amikacin (30 µg), amoxicillin/clavulanic acid (20/10 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), chloramphenicol (10 µg), ciprofloxacin (5 µg), ertapenem (10 µg), gentamycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), streptomycin (10 µg), sulphamethoxazole/trimethoprim (23.75/1.25 µg), tetracycline (30 µg) and trimethoprim (5 µg). Antimicrobial susceptibility test was performed on Muller Hinton agar (MHA) with bacterial suspension of approximately 1 x 10<sup>6</sup> colony forming units/ml of bacterial cells (0.5 McFarland standard) in normal saline. Test procedures and result interpretation were done according to the guideline of Clinical and Laboratory Standard Institute (CLSI, 2016). *Escherichia coli* (ATCC 25922) was included in the test for quality control.

### Detection of ESBL genes

Genomic DNA was extracted from all phenotypic ESBL-producing isolates by thermolysis as previously describe (Ojo *et al.*, 2016). Amount of DNA was quantified by nanospectrophotometry. The DNA concentration was adjusted to a final working concentration of 100ng/µl. ESBL-determinant genes (*bla*<sub>CTX-M-group1</sub>, *bla*<sub>CTX-M-group9</sub>, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub>) were detected by multiplex (*bla*<sub>CTX-M-group1</sub>, *bla*<sub>CTX-M-group9</sub> and *bla*<sub>SHV</sub>) or simplex (*bla*<sub>TEM</sub>) polymerase chain reaction assay using previously described specific primers and amplification conditions (Olesen *et al.*, 2004; Grobner *et al.*, 2009; Cullik *et al.*, 2010). A second round of PCR assay followed by nucleotide sequencing and sequence analysis were used for further determination of *bla*<sub>CTX-M-group1</sub> gene variants (Ojo *et al.*, 2016; Okpara *et al.*, 2018). *Escherichia coli* isolates were assigned to phylogenetic groups (A, B1, B2, C, D, E, and F) using a PCR-based classification method (Clermont *et al.*, 2013).

### Statistical analysis

The prevalence of ESBL-producing bacteria in toilet facilities in Abeokuta was compared using Chi-squared test and P values of <0.05 were considered statistically significant. All data were analysed using Statistical package for the Social Sciences software (SPSS version 16).

## RESULTS

One hundred and thirteen (70.6%) of all 160 swab samples from public toilets yielded ampicillin resistant bacteria (Table 1). Sixty-one (76.3%) of the ampicillin-resistant isolates were from samples collected from hospital toilets while 52 (65.0%) were from community toilets samples. Sixty-five (40.6%) of all 160 samples were positive for cefotaxime-resistant isolates. The cefotaxime resistant isolates were predominantly from hospital toilets (48/80; 60.0%). Only 17 (21.3%) cefotaxime-resistant isolates originated from community toilets.

Among the 65 cefotaxime-resistant isolates, 44 were confirmed as phenotypic ESBL-producers. The positive samples represented 27.5% of the total 160 swab samples examined (Table 1). The rate of detection of ESBL-producing bacteria was significantly higher ( $p > 0.05$ ) in hospital toilets (41.3%) than in community toilets (13.8%). All (100.0%) the hospital toilets tested positive for ESBL-producing bacteria as at least one of the four swab samples collected from each of the 20 hospital toilets yielded an ESBL-producer. In contrast, only five (25.0%) of the 20 community toilets tested positive for ESBL-producing bacteria. The five positive community toilets were located within religious worship centre and tertiary institution. None of the samples from toilets located within the bus station tested positive for ESBL-producing bacteria.

The ESBL-producing bacteria were identified as *Klebsiella pneumoniae* (25/160; 15.6%), *Klebsiella oxytoca* (2/160; 1.3%), *Citrobacter freundii* 6/160; 3.8%), *Citrobacter youngae* (3/160; 1.9%), *Escherichia coli* (5/160; 3.1%), *Enterobacter cloacae* (2/160; 1.3%) and *Enterobacter kobei* (1/169; 0.6%) (Table 2). The rate of detection of ESBL-producing *K. pneumoniae* was significantly higher ( $p > 0.001$ ) than other bacterial species.

All the ESBL-producing isolates tested positive for the

*bla*<sub>CTX-M-group1</sub> gene. Nucleotide sequence analysis showed that all the isolates harboured the ESBL gene variant *bla*<sub>CTX-M-15</sub>. In addition, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> were detected in two and six isolates respectively. The *bla*<sub>TEM</sub> was present only in *E. coli* (n=2) while *bla*<sub>SHV</sub> gene was found only in *K. pneumoniae* (n=6). The *E. coli* isolates belonged to phylogenetic groups A (n=1), D (n=1) and F (n=3). All the *E. coli* isolates originated from hospital toilets.

The 44 ESBL-producing isolates were all susceptible to ertapenem but showed 100% resistance to ampicillin, cefotaxime, ceftazidime, sulphomethoxazole/trimethoprim, tetracycline and trimethoprim. In addition, there was 95.5% resistance to ciprofloxacin, 88.6% to nalidixic acid, 86.4% to kanamycin, 84.1% to streptomycin, 81.8% to gentamicin, 75.0% to amoxicillin/clavulanic acid, 56.8% to chloramphenicol, 18.2% to amikacin and 15.9% to cefoxitin (Table 3). All the isolates demonstrated multiple drug resistance trait with resistance to at least an antimicrobial each from more than three different classes of antimicrobials.

**Table 1:** Phenotypic detection of extended spectrum  $\beta$ -lactamase producing bacteria in public toilets in Abeokuta, Nigeria.

Toilet/sample category (sample size)	Number (%) of ampicillin-resistant isolate	Number (%) of cefotaxime-resistant isolate	Number (%) of phenotypic ESBL-producers
<b>Hospital toilets</b>			
Toilet seats and bowls (40)	35 (87.5)	29 (72.5)	19 (47.5)
Flush and door handles (40)	26 (65.0)	19 (47.5)	14 (35.0)
Sub-total (80)	61 (76.3)	48 (60.0)	33 (41.3)
<b>Community toilets</b>			
Toilet seats and bowls (40)	27 (67.5)	8 (20.0)	5 (12.5)
Flush and door handles (40)	25 (62.5)	9 (22.5)	6 (15.0)
Sub-total (80)	52 (65.0)	17 (21.3)	11 (13.8)
Total (160)	113 (70.6)	65 (40.6)	44 (27.5)

**Table 2:** Distribution pattern of ESBL-producing *Enterobacteriaceae* in public toilets in Abeokuta, Nigeria.

Bacteria isolates	Hospital toilets		Community toilets		Total (N=160)
	Toilet seats and bowls (N=40)	Flush and door handles (N=40)	Toilet seats and bowls (N=40)	Flush and door handles (N=40)	
<i>Klebsiella pneumoniae</i>	12 (30.0%)	6 (15.0%)	4 (10.0%)	3 (7.5%)	25 (15.6%)
<i>Klebsiella oxytoca</i>	1 (2.5%)	1 (2.5%)	-	-	2 (1.3%)
<i>Escherichia coli</i>	2 (5.0%)	3 (7.5%)	-	-	5 (3.1%)
<i>Citrobacter freundii</i>	2 (5.0%)	2 (5.0%)	-	2 (5.0%)	6 (3.8%)
<i>Citrobacter youngae</i>	1 (2.5%)	1 (2.5%)	-	1 (2.5%)	3 (1.9%)
<i>Enterobacter cloacae</i>	-	1 (2.5%)	1 (2.5%)	-	2 (1.3%)
<i>Enterobacter kobei</i>	1 (2.5%)	-	-	-	1 (0.6%)
Total	19 (47.5%)	14 (35.0%)	5 (12.5%)	6 (15.0%)	44 (27.5%)

**Table 3:** Antimicrobial rates of ESBL-producing *Enterobacteriaceae* from public toilets in Abeokuta, Nigeria.

Antimicrobial agents	Number (%) of resistant isolates				
	<i>Klebsiella</i> spp (N=27)	<i>Citrobacter</i> spp (N=9)	<i>Escherichia coli</i> (N=5)	<i>Enterobacter</i> spp (N=3)	Total (N=44)
Amikacin	5 (18.5)	3 (33.3)	0 (0.0)	0 (0.0)	8 (18.2)
Amoxicillin/clavulanic acid	18 (66.7)	7 (77.8)	5 (100.0)	3 (100.0)	33 (75.0)
Ampicillin	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)
Cefotaxime	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)
Cefoxitin	2 (7.4)	4 (44.4)	0 (0.0)	1 (33.3)	7 (15.9)
Ceftazidime	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)
Chloramphenicol	13 (48.1)	6 (66.7)	3 (60.0)	3 (100.0)	25 (56.8)
Ciprofloxacin	25 (92.6)	9 (100.0)	5 (100.0)	3 (100.0)	42 (95.5)
Ertapenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gentamicin	22 (81.5)	9 (100.0)	4 (80.0)	1 (33.3)	36 (81.8)
Kanamycin	22 (81.5)	9 (100.0)	4 (80.0)	3 (100.0)	38 (86.4)
Nalidixic Acid	24 (88.9)	7 (77.8)	5 (100.0)	3 (100.0)	39 (88.6)
Streptomycin	23 (85.2)	7 (77.8)	5 (100.0)	2 (66.7)	37 (84.1)
Sulphamethoxazole/trimethoprim	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)
Tetracycline	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)
Trimethoprim	27 (100.0)	9 (100.0)	5 (100.0)	3 (100.0)	44 (100.0)

## DISCUSSION

ESBL-producing bacteria constitute an important group of antimicrobial resistant bacteria with significant challenge to infection management in the healthcare sector. In the present study, ESBL-producing bacteria were detected in high frequency in public toilets within hospital and community settings. All the investigated public toilets within hospitals yielded ESBL-producing bacteria as against 25% of the public toilets within the community. The high level detection of ESBL-producing bacteria in hospital toilets signifies the level of contamination and ease of transmission of these bacteria within hospital settings. These bacteria could be transmitted to in-patients, healthcare workers and visitors to the hospitals. Using public toilets within the hospital is thus more likely to predispose to contracting ESBL-producing bacteria than using toilets within the community. The higher detection of ESBL-producing bacteria in hospital toilets than in community toilets also suggests infectious sources within hospitals as the likely origins of toilets contamination. Extended spectrum  $\beta$ -lactamase producing bacteria has been reported in sewage (Mahato *et al.*, 2019) and air (Solomon *et al.*, 2017) samples from hospitals. Moreover, the aggregation of patients who are carriers of ESBL-producing bacteria in hospital wards could enhance easy dissemination of these bacteria within hospital settings. Earlier authors have reported high isolation rates of ESBL-producing bacteria from clinical specimens from hospitalized patients (Rubio-Perez *et al.*, 2012). It could also be that the use of antimicrobial agents in the hospitals facilitate the selection and proliferation of ESBL-producing bacteria in patients receiving antimicrobial therapy, which in turn serve to contaminate toilets and help the dissemination of the

bacteria. Initial observations of resistance to cefotaxime and other new generation cephalosporins in clinical isolates from hospitals was attributed to extensive use of the cephalosporins (Sirot *et al.*, 1987).

The present study showed that the possible transmission of ESBL-producing bacteria through toilets is not limited to toilets within hospitals. Public toilets in the community could also be sources of dissemination of ESBL-producing bacteria within human population in the study area. Contamination of community toilets could be from apparently healthy carriers, hospital outpatients or clinically infected individuals who are not seeking medical attention from hospitals. A previous study identified ESBL-producing *Enterobacteriaceae* in apparently healthy people in the community with higher prevalence of *Klebsiella* spp than *E. coli* (Maharjan *et al.*, 2018). Wherever might be the primary source of contamination, public toilets are likely foci for contracting ESBL-producing bacteria similar to other pathogens. Earlier studies have identified public toilets as sources of possible spread of infections within the community especially during disease outbreaks (Johnson *et al.*, 2013; Suen *et al.*, 2019). The use of public toilets within the community could be a risk factor for insidious transmission of ESBL-producing bacteria to unsuspecting people (Dayan, 2004).

Findings from the present study showed that ESBL-producing trait is present among different species of *Enterobacteriaceae* being detected in *Klebsiella* spp, *Citrobacter* spp, *Escherichia coli* and *Enterobacter* spp. Diverse species from the family *Enterobacteriaceae* including those identified in the present study are known to produce ESBLs (Mesa *et al.*, 2006; Guet-Revillet *et al.*, 2012; Dziri *et al.*, 2016). The production of ESBLs

is common among members of the *Enterobacteriaceae* bacteria due to intra- and inter-species sharing of ESBL traits via transferrable mobile genetic elements (Vaidya, 2011). In this study, *Klebsiella* spp was the most commonly encountered ESBL-producing species. This finding is similar to earlier reports by other authors who reported higher occurrence of *Klebsiella* spp among ESBL-producers than other species (Sturenburg and Mack, 2003; Gröbner *et al.*, 2009). *Klebsiella* spp are dominant ESBL producer especially from clinical samples in hospitals (Gröbner *et al.*, 2009). The CTX-M enzyme, which is the most widely spread ESBL type was first reported in *Klebsiella* spp from hospital setting (Sirost *et al.*, 1987). All the bacterial species identified in this study are known to produce clinical infection in humans either as major or opportunistic pathogens (Ledeboer *et al.*, 2015). These pathogens have also been implicated in cases of community-acquired and nosocomial infections in hospitalized patients (Ling *et al.*, 2005; Weinstein *et al.*, 2005; Mehrad *et al.*, 2015). Previous studies have identified ESBL-producing *Enterobacteriaceae* in clinical samples in hospitals within Abeokuta (Olowe and Aboderin, 2010) but none has investigated the possible roles of public toilets in the transmission of these pathogens. The detection of ESBL-producing bacteria in public toilets as observed in this study is of significant public health implication. The involvement of ESBL-producing bacteria in clinical infections has enormous socioeconomic implications. The prolonged hospitalization arising from non-response to antimicrobial therapy could increase the cost of medical care, enhance the spread of infectious agents, precipitate complications and increase the level of anxiety and frustration among family members and caregivers.

In the present study, all the ESBL-producing bacterial isolates harboured *bla*<sub>CTX-M-15</sub> ESBL gene variant. Few of the isolates also possessed *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>. The *bla*<sub>CTX-M</sub> gene is globally disseminated with wide bacterial and plasmid host range (Novais *et al.*, 2007; Woerther *et al.*, 2013). All variants of the *bla*<sub>CTX-M</sub> gene mediate production of ESBLs. Only fractions of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> variants mediate production of ESBLs while the rest mediate the production of narrow spectrum  $\beta$ -lactamase. Thus, the ESBL statuses of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes detected in this study were not determined because isolates that had *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> also had *bla*<sub>CTX-M-15</sub>. The *bla*<sub>CTX-M-15</sub> is a mediator of a very potent ESBL enzyme with pandemic distribution (Woerther *et al.*, 2013). It is the most commonly identified ESBL-gene variant in clinical bacterial isolates (Šeputienė, 2010). In Nigeria, the *bla*<sub>CTX-M-15</sub> gene has been reported in bacteria from clinical samples from humans (Soge *et al.*, 2006) as well as from commensal bacteria from environmental and animal sources (Okpara *et al.*, 2018).

The ecological niche, lifestyle and pathogenicity of *E. coli* strains vary with phylogenetic origins (Clermont *et al.* 2015). Studies have shown that *E. coli* causing extra-intestinal infections are more likely to be members of phylogenetic group B2 and to a lesser extent group D (Clermont *et al.*, 2000; Daga *et al.*, 2019). Three *E. coli* isolates from the present study belonged to phylogroup F and one isolate each belonged to groups D and A. The

phylogroup F is very closely related to the pathogenic phylogenetic group B2 (Clermont *et al.*, 2013). Previous studies have shown that some pathogenic strains of *E. coli* isolates were members of the phylogenetic group A (Daga *et al.* 2019). All the *E. coli* isolates detected in this study were from samples collected within hospital toilets. Thus, they could be pathogenic considering that they might have originated from infectious sources within the hospital.

In addition to being resistant to the  $\beta$ -lactam antimicrobials, the ESBL-producing isolates in this study showed high degrees of resistance to the fluoro/quinolones (ciprofloxacin and nalidixic acid), aminoglycosides (gentamicin, kanamycin, streptomycin), folate inhibitors (sulphamethoxazole, trimethoprim), tetracycline, and phenicol (chloramphenicol). All the isolates demonstrated multidrug resistance trait. Resistance to multiple antimicrobials is a common phenomenon among ESBL-producing bacteria (Ojo *et al.* 2016). This has been attributed to co-location of resistance genes to different classes of antimicrobials on the same mobile genetic element harbouring ESBL genes (Ojo *et al.* 2016). Thus, the use of antimicrobials other than the  $\beta$ -lactams could lead to co-selection of ESBL traits. This could probably explain the preponderance of ESBL-producing bacteria in hospital setting where antimicrobial usage is common. Despite the high level of resistance to many of the tested antimicrobials, the isolates in this study were all susceptible to ertapenem and showed considerable susceptibility to cefoxitin and amikacin. Thus, these three antimicrobial agents could be considered in cases of failed therapy to the other antimicrobials. However, to maximize the benefits of antimicrobials and preserve their efficacy, antimicrobials should be used with caution after appropriate microbiological investigations with antimicrobial susceptibility testing.

The present study highlighted the possible role of public toilets in the dissemination and transmission of ESBL-producing bacteria in hospital premises and within the community. To minimize the risk of spreading ESBL-producing bacteria through public toilets, more attention should be given to the sanitary conditions of these toilets. In particular, there should be adequate supply of water as well as provision of cleaning materials including detergents and disinfectants. Individual user of public toilets should be aware of the health risks associated with unsanitary toilets and thus must adhere to strict practice of personal hygiene at all times in the overall interest of the general public. Periodic routine microbiological assessment of public toilets for monitoring purposes should be incorporated into the sanitary inspection role of the government. This could be made part of the policies guiding the operations of toilets in public places. This is especially important in hospital settings where toilets could be foci for nosocomial transmission of resistant pathogens to healthy individuals such as hospital workers and visitors or serve to complicate ongoing illnesses in hospitalized patients.

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## STATEMENT OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

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