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# High burden of extended spectrum β–lactamase (ESBL)–encoding genes in third–generation cephalosporin–resistant *Escherichia coli* recovered from frequently contacted surfaces and wastewater of selected healthcare institutions in Nigeria

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## ABSTRACT

**Objective:** This study aimed to determine the carriage of extended spectrum β-lactamase (ESBL) genes in third-generation cephalosporin-resistant (3GCR) *Escherichia* (*E.*) *coli* from frequently contacted surfaces, wastewater and disinfectant-cleaning solutions of selected healthcare institutions in South-western Nigeria.

**Methods:** Samples were collected over three months for the isolation of 3GCR *E. coli* on MacConkey agar containing 6 µg/mL of cefotaxime. 3GCR *E. coli* isolates were identified by detection of *uidA* gene and susceptibility to selected antibiotics was performed using disc-diffusion method. Detection of ESBL genes was done using primer-specific PCR.

**Results:** A total of 22 ESBL-producing *E. coli* (11 each from the frequently contacted surfaces and wastewater) were obtained from the pool of 3GCR isolates in this study. No isolate was recovered from the disinfectant-cleaning solution. All the ESBL-producing *E. coli* obtained from the frequently contacted surfaces and wastewater were multidrug resistant, with complete resistance observed to ampicillin, cefotaxime, cefpodoxime, tetracycline and ertapenem. The ESBL genotyping showed that 54.5% carried $bla_{\text{CTX-M}}$, 63.6% carried $bla_{\text{TEM}}$ and 9.1% carried $bla_{\text{SHV}}$ in isolates from the frequently contacted surfaces, while 63.6%, 9.1% and 18.2% carried $bla_{\text{CTX-M}}$, $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$, respectively, in the isolates obtained from the wastewater.

**Conclusions:** This study showed a high burden of multidrug resistance *E. coli* on frequently contacted surfaces and wastewater of the studied healthcare institutions, indicating the need for good hygiene and proper mitigation measures to prevent potential public health and environmental challenges.

**KEYWORDS:** Multi-drug resistance; Wastewater; ESBL-producing *E. coli*; $bla_{\text{SHV}}$; $bla_{\text{TEM}}$; $bla_{\text{CTX-M}}$

## 1. Introduction

The physical environment of healthcare facilities serves as a major reservoir of microorganisms. The presence of multiple drug resistant bacteria in the environment is an important contributing factor to healthcare-associated infections. Studies on hospital environments played an important role to characterize the dissemination of resistance determinants, which in some cases extended far beyond patients primary colonized areas[1,2].

The reality of hospital surfaces' contribution to extended spectrum β-lactamase (ESBL) spread was not fully appreciated

**Significance**

The occurrence of ESBL-producing bacteria in the clinical settings has been on the rise globally. The present study shows that frequently contacted surfaces in hospitals and hospital wastewater are hotspots of ESBL-producing *E. coli* in Nigeria. Our study highlights a need to enforce the treatment of hospital wastewater before discharge and effective disinfection of surfaces in hospitals to prevent potential public health challenges.

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until the emergence of molecular evidence-based outcomes linking environmental isolates with isolates from hospital surfaces and those from the patients themselves[3]. The last decade especially, have witnessed mounting reports on the contributions of hospital surfaces to the spread of resistant bacteria carrying resistant genes.

Studies have reported the reservoir nature of hospital environmental surfaces and their propensity to breed and disseminate several hospital-acquired pathogens, of which the enteric bacteria are notable candidates[4,5]. Bacteria expressing extended spectrum β-lactamases are important group of pathogens linked to nosocomial outbreaks in the hospital settings[2,6]. Most bacteria residing on inanimate hospital surfaces owe the ability to survive for long outside their primary niche from few days to months, due to the innate resilience they possess[7]. Most hospitals use traditional disinfection as standard recommended practice to control these surface pathogens[7]. It has been reported that in many cases, especially in developing countries, gaps in disinfection procedure and processes have led to sub-optimal cleaning, which has contributed significantly to the colonization and spread of multidrug resistant bacteria carrying resistance genes in hospitals[7,8]. In addition to the contribution of hospital surfaces to the expansion of ESBLs, untreated hospital wastewater is another important contributor to antibiotic resistance in the environment[9,10]. What makes it more daunting is that many healthcare facilities are sited in close proximity to residential buildings in Nigeria. Previous studies reported the practice of discharging untreated wastewater directly into municipal drain or directly into the ground which extended the impact farther beyond the hospital to the community and the wider environment[11–13]. There are continuing concerns over ESBL-producing bacteria due to the rapid increase of variants and transferability between and within bacterial species as a result of the plasmid-borne nature of the ESBL genes. In multiple studies, the incidence and spread of *Escherichia* (*E.*) *coli* strains producing different variants of the ESBL genes have been associated with the hospital environment[14,15].

Although some studies have been done on the spread of ESBL-producing bacteria and their carriage of ESBL genes in Nigeria gaps still exist owing to the rapid increase and evolution of ESBLs[9,10,12,13]. The few existing literatures available on hospital surfaces are mostly limited to the assessment of microbial loads and phenotypic screening for antibiotic resistance[16,17]. In Nigeria, inadequate research on hospital surfaces has led to their under-appreciation as potential sources for the spread of ESBL-producing bacteria. This study assessed the occurrence of ESBL-producing bacteria on selected high-touch hospital surfaces and provided more enlightenment on the contribution of these surfaces to the growing reservoirs of ESBL-producing bacteria in the hospital environment, as well as the carriage of ESBL determinants by *E. coli* isolated from the wastewater of the selected healthcare institutions, with a view to contributing to existing reports on the subject.

## 2. Materials and Methods

### 2.1. Study design

The study was carried out in two Local Government areas in Ogun state (Ijebu North and Ijebu-Ode) located in Southwest Nigeria. Five healthcare institutions comprising three privately-owned hospitals coded as MPH, VSH and BPH, one state government-owned hospital (SGH) and a clinic located in an institution of higher education (OCC), were used for the collection of samples.

### 2.2. Collection of samples

Over the sampling course of three-month (April-June, 2021), samples (12 each) were taken from the hospital surfaces (bedside rails, bedside chairs, kidney dishes, ward sinks, overbed tables) using sterile swab sticks, which had been moistened in sterilized saline solution. A defined portion of the surfaces (3 cm × 3 cm) were sampled for consistency. Personnel (cleaners) were encouraged to follow the usual cleaning and disinfecting practice to erase any form of bias and the samples were taken 45-60 minutes after disinfection. Samples (12 each) of the disinfectant-cleaning solution and wastewater were collected into sterile sample bottles from the final wastewater disposal outlet of each institution. Samples were transported on ice to the microbiology laboratory for analysis within two hours of collection.

### 2.3. Enrichment of samples for the selective isolation of third–generation cephalosporin–resistant E. coli

Swabs from the frequently contacted surfaces, aliquots of the disinfecting solution and wastewater were each inoculated into Luria Bertani broth containing 6 μg/mL cefotaxime and incubated overnight at 35±2 ℃. This was done using the cefotaxime breakpoint of ⩾ 4 μg/mL according to Clinical and Laboratory Standards Institute[18]. The streak plate method was used for the isolation of *E. coli* from the incubated broth cultures on MacConkey agar (Oxoid, UK). Morphologically distinct presumptive isolates were selected and further subcultured and stored. Isolates were initially characterised using conventional methods, and also by targeting the housekeeping *uidA* gene[19]. The details of the *uidA* primer are shown in Table 1.

**Table 1.** Primers used in this study.

| Target Gene | Primer sequences (5'-3') | Amplicon size (bp) | Reference |
|---|---|---|---|
| *uid*A | AAAACGGCAAGAAAAAGCAG<br>ACGCGTGGTTACAGTCTTGCG | 147 | [18] |
| $bla_{CTX-M}$ | TTTGCGATGTGCAGTACCAGTAA<br>CGATATCGTTGGTGGTGCCATA | 543 | [20] |
| $bla_{TEM}$ | GAGTATTCAACATTTTCGT<br>ACCAATGCTTAATCAGTGA | 857 | [21] |
| $bla_{SHV}$ | TCGCCTGTGTATTATCTCCC<br>CGCAGATAAATCACCACAATG | 768 | [21] |

### 2.4. Antibiotic susceptibility testing and phenotypic detection of ESBL production

The disc diffusion method was employed in determining the antibiogram of the isolates to nine selected antibiotics (ampicillin, ciprofloxacin, gentamicin, cefotaxime, cefpodoxime, amoxicillin-clavulanate, sulfamethoxazole-trimethoprim, tetracycline and ertapenem), while the detection of ESBL production in the isolates was done using the double disc synergy test[18]. Isolates were designated as sensitive, intermediate or resistant using the zone of inhibition interpretation[18]. The multiple antibiotic resistance index (MARI) of each isolate was calculated as the number of antibiotics resisted by each isolate divided by the total number of antibiotics tested against the isolate.

### 2.5. Detection of $bla_{TEM}$, $bla_{SHV}$ and $bla_{CTX-M}$ in the ESBL–producing E. coli

Duplex PCR was used for the detection of $bla_{TEM}$ and $bla_{SHV}$ following the procedures of Maynard *et al*.[20], while detection of $bla_{CTX-M}$ was done using a monoplex PCR following the methods of Mendonça *et al*[21]. The primer sequences of the genes are shown in Table 1.

## 3. Results

### 3.1. Frequency and Distribution of ESBL–producing E. coli obtained from the samples

A total of 22 confirmed ESBL-producing *E. coli* isolates were detected from the samples, accounting for 62.85% of 35 strains of third-generation cephalosporin-resistant *E. coli* obtained. Of the 22 isolates, eleven were from the frequently contacted surfaces and the remaining eleven were from the wastewater samples. No ESBL-producing *E. coli* was obtained from the disinfecting solutions being used at the healthcare institutions.

Figure 1 shows the distribution of ESBL-producing *E. coli* obtained based on the healthcare institutions sampled. VSH and MPH had four isolates each from the frequently contacted surfaces, while SGH had two isolates and one from BPH. No ESBL producer was recovered from OCC. From the wastewater, three isolates were obtained respectively from VSH, SGH and OCC, two isolates from MPH and none from BPH.

### 3.2. Resistance of the ESBL–producing E. coli to selected antibiotics

The resistance of the ESBL-producing *E. coli* obtained from the frequently contacted surfaces and wastewater to the panel of antibiotics is shown in Figure 2. All the isolates obtained from the surfaces and wastewater showed complete resistance to ampicillin, cefotaxime, cefpodoxime, tetracycline and ertapenem. The gentamicin resistance among the isolates from frequently contacted surfaces and wastewater was 27.3% and 45.5% respectively, which was the lowest in this study. The resistance to ciprofloxacin was 72.7% for isolates from the frequently contacted surfaces and 63.6% for isolates obtained from wastewater. The resistance to amoxicillin-

**Table 2.** Resistance phenotypes and ESBL gene profile of the ESBL-producing *E. coli* isolated from frequently contacted surfaces.

| S/N | Isolate code | Sample source | Hospital source | Resistance phenotypes | MARI* | $bla_{CTX-M}$ | $bla_{TEM}$ | $bla_{SHV}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | *E. coli* S02 | Bedside Chair | BPH | amp, cip, gen, ctx, cpd, amc, sxt, tet, ert | 1.00 | - | - | - |
| 2 | *E. coli* S12 | Ward sink | SGH | amp, cip, gen, ctx, cpd, amc, sxt, tet, ert | 1.00 | + | + | - |
| 3 | *E. coli* S31 | Ward sink | SGH | amp, cip, gen, ctx, cpd, amc, sxt, tet, ert | 1.00 | - | + | - |
| 4 | *E. coli* S60 | Overbed table | VSH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | + | - |
| 5 | *E. coli* S2E | Bedside chair | VSH | amp, gen, ctx, cpd, amc, sxt, tet, ert | 0.88 | - | + | - |
| 6 | *E. coli* S62 | Bedside rail | VSH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | - | + |
| 7 | *E. coli* S04 | Bedside rail | VSH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | - | - |
| 8 | *E. coli* S05 | Bedside rail | MPH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | + | - |
| 9 | *E. coli* S58 | Ward sink | MPH | amp, ctx, cpd, tet, ert | 0.55 | - | + | - |
| 10 | *E. coli* S01 | Overbed table | MPH | amp, ctx, cpd, tet, ert | 0.55 | + | - | - |
| 11 | *E. coli* S63 | Kidney dish | MPH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | - | + | - |

amp: ampicillin; cip: ciprofloxacin; gen: gentamicin; ctx: cefotaxime; cpd: cefpodoxime; amc: amoxicillin-clavulanate; sxt: sulfamethoxazole-trimethoprim; tet: tetracycline; ert: ertapenem. *Multiple antibiotic resistance index.

**Table 3.** Resistance phenotypes and ESBL gene profile of the ESBL-producing *E. coli* isolated from hospital wastewater

| S/N | Isolate code | Sample source | Hospital source | Resistance phenotypes | MARI* | $bla_{CTX-M}$ | $bla_{TEM}$ | $bla_{SHV}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | *E. coli* WW14 | Wastewater | SGH | amp, ctx, cpd, amc, sxt, tet, ert | 0.77 | + | - | - |
| 2 | *E. coli* WW34 | Wastewater | SGH | amp, gen, ctx, cpd, amc, sxt, tet, ert | 0.88 | - | - | - |
| 3 | *E. coli* WW15 | Wastewater | SGH | amp, gen, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | - | + |
| 4 | *E. coli* WW76 | Wastewater | OCC | amp, cip, ctx, cpd, sxt, tet, ert | 0.77 | - | - | - |
| 5 | *E. coli* WW2 | Wastewater | OCC | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | + | - |
| 6 | *E. coli* WW32 | Wastewater | OCC | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | - | - |
| 7 | *E. coli* WW44 | Wastewater | VSH | amp, gen, ctx, cpd, amc, sxt, tet, ert | 0.88 | - | - | - |
| 8 | *E. coli* WW35 | Wastewater | VSH | amp, cip, gen, ctx, cpd, amc, sxt, tet, ert | 1.00 | + | - | - |
| 9 | *E. coli* WW10 | Wastewater | VSH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | + | - | - |
| 10 | *E. coli* WW36 | Wastewater | MPH | amp, cip, ctx, cpd, amc, sxt, tet, ert | 0.88 | - | - | - |
| 11 | *E. coli* WW11 | Wastewater | MPH | amp, cip, gen, ctx, cpd, amc, sxt, tet, ert | 1.00 | + | - | + |

amp: ampicillin; cip: ciprofloxacin; gen: gentamicin; ctx: cefotaxime; cpd: cefpodoxime; amc: amoxicillin-clavulanate; sxt: sulfamethoxazole-trimethoprim; tet: tetracycline; ert: ertapenem. *Multiple antibiotic resistance index.

clavulanate for isolates from wastewater was higher (90.9%) than that from the frequently contacted surfaces (81.8%), while there was complete resistance to sulfamethoxazole-trimethoprim by the isolates from wastewater as against 81.8% resistance observed in the isolates from the frequently contacted surfaces to the same antibiotic.

### 3.3. Resistance phenotypes and ESBL gene profile of the ESBL–producing E. coli

The resistance phenotypes and gene profile of isolates obtained from the frequently contacted surfaces and wastewater is shown in Table 2 and Table 3. The MARI of the isolates to the antibiotics ranged from 0.55 to 1.00 in isolates from the frequently contacted surfaces and 0.77 to 1.00 for isolates recovered from wastewater. The data suggested a high risk of antibiotic resistance from the samples studied. There were three isolates from the frequently contacted surfaces showing complete resistance to the panel of nine antibiotics, while two isolates from the wastewater showed 100% resistance to all the antibiotics tested.

### 3.4. Frequency of ESBL genes obtained in the ESBL–producing E. coli from frequently contacted surfaces and wastewater

The frequency of occurrence of ESBL genes in the isolates obtained from the frequently contacted surfaces and wastewater is shown in Table 4. The most predominant ESBL gene in the *E. coli* isolated from the frequently contacted surfaces was $bla_{TEM}$ (63.6%) whereas $bla_{CTX-M}$ (63.6%) was the most predominant in the wastewater. Six isolates (54.5%) from the frequently contacted surfaces carried $bla_{CTX-M}$, while only one isolate carried $bla_{SHV}$. Two isolates (18.2%) and one isolate (9.1%) respectively carried $bla_{SHV}$ and $bla_{TEM}$ from the wastewater generated by the healthcare institutions. Overall, the percentage occurrence of ESBL genes in isolates from both frequently contacted surfaces and wastewater was 59.1% ($bla_{CTX-M}$), 36.4% ($bla_{TEM}$) and 13.6% ($bla_{SHV}$).

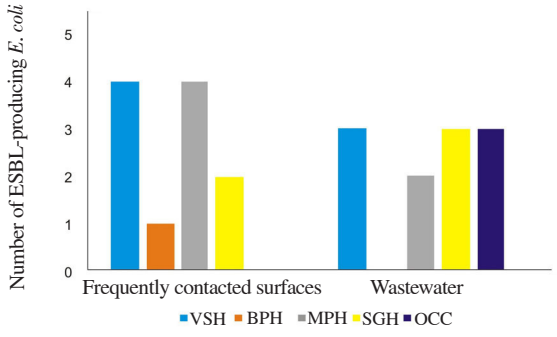


**Figure 1.** Distribution of the ESBL-producing *E. coli* in the healthcare institutions.

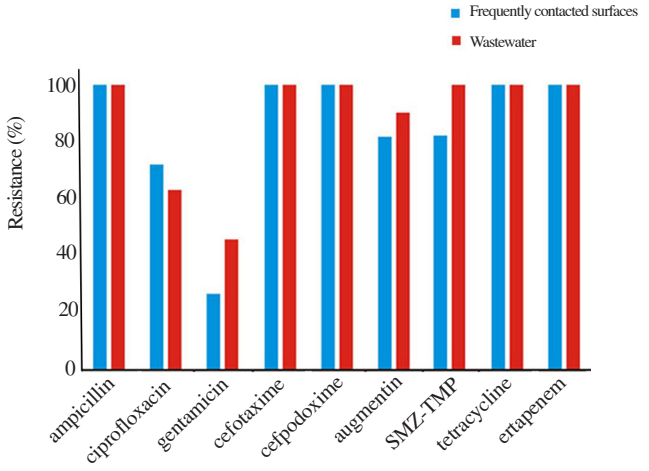


**Figure 2.** Resistance of the ESBL-producing *E. coli* from frequently contacted surfaces and wastewater to selected antibiotics. augmentin: amoxicillin-clavulanate; SMZ-TMP: sulfamethoxazole-trimethoprim.

**Table 4.** Occurrence (%) of ESBL genes in *E. coli* from frequently contacted surfaces and wastewater.

| ESBL gene | Frequently contacted surfaces ($n$=11) | Wastewater ($n$=11) | Overall occurrence ($n$=22) |
|---|---|---|---|
| $bla_{CTX-M}$ | 54.5 (6/11) | 63.6 (7/11) | 59.1 (13/22) |
| $bla_{TEM}$ | 63.6 (7/11) | 9.1 (1/11) | 36.4 (8/22) |
| $bla_{SHV}$ | 9.1 (1/11) | 18.2 (2/11) | 13.6 (3/22) |

## 4. Discussion

Hospitals were considered as a high-risk environment carrying several resistance genes in numerous studies. Wastewater generated from the operations of healthcare institutions contain antibiotic residues which could trigger the development of resistance in resident bacteria, as a result of adaptation and other mechanisms[22]. Grundmann *et al*.[23] and Baquero *et al*.[24] in their respective studies opined that high concentration of antibiotic residues in wastewater from healthcare institutions could select for bacteria showing resistance to different classes of antibiotics, thus further confirming the role of wastewater from healthcare institution as an important contributor to the increasing incidence of antibiotic resistance in bacteria.

In this study, eleven ESBL-producing *E. coli* isolates were obtained from wastewater generated by five healthcare institutions in two local government areas of Ogun State, South-west Nigeria. All the isolates obtained were resistant to more than three antibiotics (multidrug resistant), with MARI ranging from 0.77-1.00. Several other studies have reported the isolation of multidrug resistant *E. coli* in hospital wastewater and clinical origins. In a study by Ogbolu *et al*.[25], high resistance to antibiotics was reported in some clinical Gram-negative bacteria, same as Adekanmbi *et al*.[13,26], with a reported case of multidrug resistance in *E. coli* recovered from wastewater of a University sickbay and a tertiary hospital in Ibadan, Nigeria. Gundogdu *et al*.[27] reported the same trend in *E. coli* from the hospital environment.

The frequency of ESBL ($bla_{CTX-M}$, $bla_{TEM}$ and $bla_{SHV}$) genes were detected in the isolates obtained from the wastewater of the healthcare institutions. The carriage of these genes by *E. coli* obtained from hospital wastewater has been reported in several studies conducted in different parts of the world. In this study, 63.6% of the total ESBL-producing *E. coli* obtained from the hospital wastewater carried $bla_{CTX-M}$. This could be linked to the diversity and known significant clinical impact of the CTX-M, and the occurrence of many phylogenetic groups of the gene, in comparism with other ESBL genes[28]. Adekanmbi *et al*.[13] reported the predominance of $bla_{CTX-M}$ in their study, which corroborates the observation in this study. The same authors in another closely-related study also reported $bla_{CTX-M}$ as the most prominent ESBL gene in *E. coli* associated with wastewater and sludge of a healthcare institution in South-west Nigeria[26]. Other notable studies have reported a high frequency of $bla_{CTX-M}$ in bacteria from hospital wastewater and other samples of clinical origin[29–32].

The ability to resist first-generation cephalosporins, ampicillin and penicillin in ESBL-producing bacteria is encoded by $bla_{TEM}$. The relative carriage of $bla_{TEM}$ gene (9.1%) in the isolates obtained from wastewater in this study was very low, as it was detected in only one isolate. The low frequency of $bla_{TEM}$ is contrary to the results obtained in some other studies on ESBL-producing bacteria from hospital wastewater and the clinical settings generally. Lien *et al*.[33] reported the predominance of $bla_{TEM}$ in *E. coli* from hospital wastewater in Vietnam, while the high carriage of the same gene was reported by Varela *et al*.[34]. The $bla_{SHV}$ gene was initially associated with infection-causing members of the Enterobacterales and has normally been domiciled in the hospital settings. Some studies have reported a worrisome scenario on the spread of the gene into the environment[35–37]. In comparison with other ESBL genes, the frequency of $bla_{SHV}$ is generally very low[11,38].

The carriage of bacteria especially the multidrug resistant ones on inanimate objects in hospitals may have been on the rise partly due to some unhygienic practices in our healthcare facilities. Several studies have reported the contamination of inanimate surfaces by multidrug resistant bacteria notably *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. Getachew *et al*.[39] reported the occurrence of bacterial contaminants on inanimate surfaces at a referral hospital in Ethiopia, while Olowokere *et al*.[17], Dziri *et al*.[40], and Matthew et al.[41] all reported the occurrence of different genera of bacteria on inanimate surfaces within the hospital environment. In this study, a total of eleven ESBL-producing *E. coli* were isolated from inanimate surfaces and equipment of the five selected healthcare institutions. The unabating incidence of ESBL-producing *E. coli* in hospital environment could be largely due to several factors including the cross transmission of strains from the hospital environment to patients, patients to patients and the transfer of resistance through mobile genetic elements[14,42–44].

All the isolates obtained from the surfaces were multidrug resistant which showing a high level of resistance to eight of the tested nine antibiotics, with the only exception being gentamicin. The occurrence of bacteria showing elevated resistance to most commonly used antibiotics has been reported in several studies and the hospital environment has been identified by several studies as a 'hotspot' for the spread of multidrug resistant bacteria, especially ESBL-producing enteric bacteria[45].

Of the eleven ESBL producers obtained from frequently contacted surfaces in this study, $bla_{SHV}$ was detected in one isolate *(E. coli* S62), six isolates (54.5%) carried $bla_{CTX-M}$, while seven of the isolates (63.6%) carried $bla_{TEM}$, making it the predominant ESBL gene. In contrast however to the report of Dziri *et al*.[40], the percentage carriage of the three target ESBL genes in this study was higher, although the increased number of healthcare institutions sampled in this study could be a major factor contributing to this. This observation has further confirmed the assertion in many

other studies of the contribution of frequently contacted surfaces in hospitals and other healthcare facilities to the ever-increasing incidence of antibiotic resistance globally.

## 5. Conclusions

ESBL-producing *E. coli* with multiple resistance to antibiotics and harboring genes encoding ESBL production were obtained from the frequently contacted surfaces and wastewater in this study. This is very alarming as people especially hospital patients and other personnel have constant interaction with these surfaces, which could eventually lead to the spread and transmission of potentially infectious agents. Another thing of note is the discharge of wastewater from the healthcare institutions to the environment without any treatment, thus exposing receiving aquatic bodies to antibiotic resistant organisms. This study calls for concerted efforts by relevant agencies to enforce the treatment of wastewater from healthcare settings before discharge and the effective disinfection of surfaces in hospitals to prevent a potential public health challenge.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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## Authors' contributions

Banjo OA and Adekanmbi AO developed the original idea and the protocols. All authors performed the experiments, and were involved in the collection of data. Banjo OA and Adekanmbi AO wrote the preliminary draft and analyzed the data. All authors read, revised and approved the manuscript for publication.

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