Asian Journal of Biology

7(1): 1-9, 2018; Article no.AJOB.46352
ISSN: 2456-7124

Occurrence and Resistance Profile of Extended Spectrum Beta- Lactamase *Escherichia coli* from Inanimate Surfaces of Student Toilets

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Authors' contributions

This work was carried out in collaboration between both authors. Author ACC managed the literature searches, carried out the research work and performed statistical analysis. Author OEA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2018/46352
Editor(s):
(1) Dr. P. Dhasarathan, Department of Biotechnology, Prathyusha Engineering College, Anna University, India.
Reviewers:
(1) Daoudi Abdellatif, Cadi Ayyad University, Mohammed VI University Medical Hospital of Marrakesh, Morocco.
(2) Fatunla Oluwatoba Ayodele, Federal University of Technology, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/46352

Original Research Article

Received 30 October 2018
Accepted 05 January 2019
Published 29 January 2019

ABSTRACT

Aim: The study was carried out to determine the occurrence and resistance profile of extended spectrum beta-lactamase *E. coli* from inanimate surfaces in public and private toilets in student lodge within the University of Port Harcourt Nigeria.
Study Design: The employs experimental design and data collection.
Place and Duration of Study: The study was carried out for a period of 9 months from December 2017 to August 2018 in the Medical Microbiology laboratory of the department of Microbiology, University of Port Harcourt Nigeria.
Methodology: A total of 105 swabs were swabbed from floors, seats and door handles of the 6 toilets, the isolates were identified using standard microbiological methods. The positive cultures of *E. coli* were subjected to antimicrobial susceptibility testing using Gram negative disk by Kirby Bauer disk diffusion method. ESBL-producing *E. coli* were detected using several combinations of cephalosporin disks with clavulanic acid disks.

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Results: Out of the bacteria identified from the swabbed area 33% (35 isolates) were identified as *E. coli.* Antibiotic susceptibility testing showed high resistance of the isolates to Cefuroxime, Gentamicin, Cefixime, Augmentin, Ceftazidime, Ciprofloxacin, Ofloxacin. but were more susceptible to Nitrofurantoin. Ninety-five (95) % of isolated *E. coli* was resistant to at least resistant 3-5 antibiotics. The ESBL production of the isolated *E. coli* was noted from seats of both public and private toilets with 67% respectively than the floors with 20% and 33%. This study reveals ESBL producing *E. coli* can occur in large numbers on surfaces which users of toilets readily contact. **Conclusion:** Efforts should be made in monitoring the excessive use of antibiotics as these contributes to the resistance ability of the organism and also, daily cleaning and disinfection in conjunction with a regular hygiene service are recommended to prevent the spread multidrug resistant strains and ESBL producing *E. coli*.

Keywords: Escherichia coli; extended spectrum beta-lactamase; inanimate surfaces; resistance; toilets.

1. INTRODUCTION

E. coli are responsible for urinary tract infection and other intestinal infections that can be expelled into the environment via fecal matter [1]. When implicated in infection they slow down effective treatment-based resistance to some broad-spectrum antibiotics. Antibiotic resistant strains of *E. coli* can spread through human population increasing diseases in the community and hospital [2]. ESBLs are Gram-negative bacteria that produce the enzyme beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillin and cephalosporins and render them ineffective for treatment. ESBLs can be transmitted through unwashed hands by health care workers and individuals. They were initially associated with hospital- acquired outbreaks, currently ESBLs are now associated with fecal carriage in the community [3,4]. The increase of drug resistant organism has become a major challenge, because of this there is an increase of untreatable diseases in the world today, especially in developing countries. The fact that it takes years for new antibiotics to be produced makes the situation alarming. Antibiotics inhibit the growth of susceptible bacteria while the once that are resistant tend to adapt to environmental conditions and can be the channel through which drug resistant strains and genes be transferred from environmental isolates to human [5,6]. Toilets are sanitation facilities at the user interface that permits the safe and convenient urination and defecation, toilets can be with or without flushing water. In private homes, the toilet, sink, bath, or shower may be in the same room. Another option is to have one room for body washing (bathroom) and a separate room for the toilet and hand washing sink (toilet room). Public toilets consist of one or more toilets which are available for use by the general public [7]. Aerosols toilets from door handles, tap head, sink, towels and floor are possible routes through which diseases can be transmitted [8]. This is possible because aerosols can persist for a minimum of 12 minutes after the toilet is flushed [9]. The toilets when flushed produce micro droplets which contain viable bacteria. These bacteria can rest on inanimate surfaces in the toilet or restroom as the case maybe. The bacteria can persist on inanimate surface for several days. Self-contamination can occur from touching such surfaces without washing hands after wards [6,10,11]. Contamination of the toilet environment has a key role to play in the transmission of infectious diseases. The study was carried out to determine the occurrence and resistance profile of *E. coli* from student lodge toilets.

2. MATERIALS AND METHODS

2.1 Description of Study Area and Sample Collection

A total of 105 swabs were swabbed from door handles, seats, and floors of 6 toilet facilities in Moses villa lodge Alakahia, Obi city lodge, Choba (private facilities), Nelson Mandela block A, C, D toilets and Ofrima girls' toilets (public facilities) in the University of Port Harcourt Rivers State Nigeria [12]. The swabs were soaked in sterile tubes containing Brain Heart Infusion Broth (BHI) and transported immediately to the Medical Microbiology laboratory of University Port Harcourt and incubated for 24 h at 37°C.

2.1.1 Duration of study

The study was carried out for a period of 9 months from December 2017 to August 2018 in

the department of Microbiology, University of Port Harcourt Nigeria.

2.1.2 Identification of *Escherichia coli* characterization and identification of *Escherichia coli*

The bacteria isolates were grown on Eosin-Methylene Blue Agar (LAB M), they were sub cultured onto Nutrient Agar (LAB M). *E. coli* was characterized and identified based on their motility, microscopic morphology, colonial morphology and biochemical characterization as described in medical laboratory manual for tropic countries and taxonomic scheme of Bergey's Manual of Determinative Bacteriology [13,14].

2.2 Antimicrobial Susceptibility Testing

All isolates were tested for the antimicrobial susceptibility through the Kirby Bauer diffusion according to the Clinical and Laboratory Standard guidelines [15]. The antimicrobial agents used in the susceptibility testing included Augmentin (30µg), Nitrofurantoin(30µg), Ofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg), aminoglycosides (Gentamicin (10µg) and a fluoroquinolone (Ciprofloxacin (5µg) (TOKU-E, USA). Inoculums were adjusted 0.5 McFarland turbidity standards on nutrient broth and swabbed across the entire surface of Muller Hinton agar plate. The plates were incubated within 15 minutes of the disk's application at 37˚C for 18 to 24 hours. Isolates were considered as multidrug resistance (MDR), when it shows resistance to ≥ 3 antimicrobial agents [9,15].

2.3 ESBL Screening and Detection

Detection of ESBL production was carried out on isolates that exhibited intermediate susceptibility/resistance to any one of the third generation cephalosporins ceftazidime / cefotaxime [15,16]. An inhibition zone of < 18mm for cefotaxime and <14m for ceftazidime indicated that the isolated strain is an ESBL producer.

2.3.1 Double disc diffusion testing

An overnight culture of identified isolates was inoculated onto Mueller-Hinton agar plates. Disks containing the standard 30 µg of ceftazidime, and ceftriaxone were placed 15 mm apart (edge to edge) and from an amoxicillin-clavulanic acid disk containing 10 µg was placed in the center of the plate. Following incubation for 16-20 hours at 35°C, zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor indicated the presence of an ESBL.

2.3.2 Phenotypic detection of ESBLs by modified CLSI ESBL confirmatory method

The modification of the CLSI ESBL confirmatory test was performed employing disks of Ceftazidime with or without Clavulanic acid, on which both boronic acid and EDTA were dispensed. Boronic acid (400µg) was dispensed onto commercially available antibiotic disks containing ceftazidime (30µg) with or without clavulanic acid (10µg)[17]. Additionally, 10µl of 0.1 M EDTA (containing 292µg of EDTA) was dispensed onto the same antibiotic disks on MHA plates inoculated with identified isolates. The agar plates were incubated at 37 ˚C for 18 hours. An augmentation of ≥ 5mm in the growth inhibitory zone diameter of either ceftazidime-clavulanic in combination with boronic acid and EDTA, compared with the zone diameter of ceftazidime disk containing boronic acid and EDTA, was considered a positive result for ESBL production [17].

3. RESULTS

Results obtained from the study showed that out of the 105 swabs collected from both public and private toilet restrooms within the University of Port Harcourt 35 (33%) isolates were positive for *E. coli*. The isolates were identified by Standard Microbiological Technique and Biochemical tests. Sixty – seven (67) percent of the isolates showed growth of other organisms. Percentage occurrence of *E. coli* from the swabbed surfaces is presented in Fig. 1, results obtained showed that the lowest occurrence of *E. coli* was in the door handles followed by the seats and the highest was the floor. The private student hostels toilets had lower occurrence of *E. coli* compared to those of the public toilets as shown in Fig. 1.

Confirmed *E. coli* isolates from specific areas in toilets showed resistance pattern to various antibiotics, they were resistant to Cefuroxime, Gentamicin, Cefixime, Augmentin Ceftazidime, Ofloxacin, Ciprofloxacin and Nitrofurantoin in various levels. The isolates from the seats had the highest level of resistance which was followed by the door handle isolates and the least resistance was among isolates from the floor. were next in resistance and the floor isolates were least resistant to antibiotics disks, the highest level of resistance was observed in

seat isolates followed by the door handles and the least resistance was found in the isolates from the toilet floors of the private toilet. The floor isolates differed only in the Nitrofurantoin.

The resistance profile of *E. coli* from public and private toilets are presented in Table 1, results obtained showed that *E. coli* isolated from public and private toilets were resistant to CAZ and AMP. *E. coli* isolates from public toilets had higher percentage of resistance to more than 3 antibiotics, 22 of the isolates were multidrug resistant. Nineteen of the isolates were resistant to 7 antibiotics. This confirms the high level of resistance observed in *E. coli* from the public toilets. From the private toilets 13 of *E. coli* were resistant to more than 3 antibiotics as shown in Table 1.

3.1 Detection of Extended Spectrum Beta-Lactamase Producing *E. coli* Isolates

ESBL detection was carried out on confirmed *E. coli* isolates, results obtained showed that 38% of the floor isolates and 25% of the seat isolates from the public toilets showed zone size of inhibition ≥22mm around either Ceftazidime disc or combined disc of Ceftazidime and Clavulanic acid were considered as ESBL producer according to the Clinical Laboratory Standard Institute as shown in Table 1. ESBL was detected in all the sample sites of public toilets except for Nelson Mandela block C hostels as shown in Table 1. None of the door handle isolates were ESBL producers. Results obtained also showed that 71% floor isolates and 60% seat isolate from private toilets were ESBL producers. The ESBL production of the *E. coli* was noted from seats of both public and private toilets with 67%. E. *coli* from floor and door handles showed 20% and 33% respectively (Tables 1&2). For the private toilets results obtained showed that ESBL was detected all the isolates from Obicity lodge, it had the highest prevalence of ESBL producing *E. coli* as presented in Table 2.

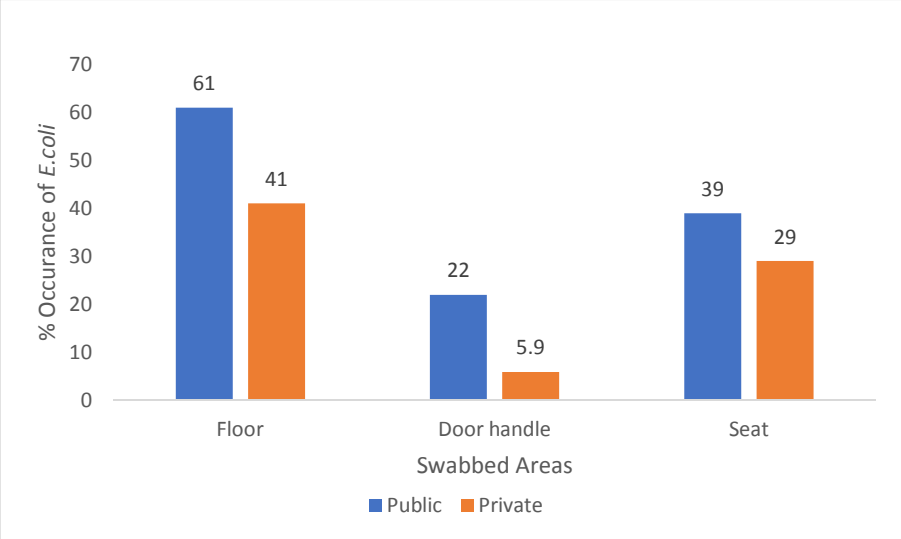

Fig. 1. Percentage occurrence of *E. coli* public and private toilets

Table 1. Resistance profile of *E. coli* in public and private toilets surfaces

S/N	Antibiotics	Nob. of MDR E. *coli* from public toilet	Percentage of MDR *E. coli*
1	CRX-GEN-CXM-OFL-AUG-CPR-CAZ	19	86
2	CRX-GEN-CXM-AUG-CAZ	1	5
3	CRX-GEN-CXM-OFL-AUG-NIT-CPR-CAZ	2	9
S/N	**Antibiotics**	**Nob. of MDR E. *coli* from private toilet**	**Percentage of MDR *E. coli***
1	CRX-GEN-CXM-OFL-AUG-CPR-CAZ	11	85
2	CRX-GEN-CXM-AUG-CAZ	2	15

Key: MDR = Multidrug Resistant

Table 2. Phenotypic detection of extended spectrum beta - lactamase among *E. coli* from public toilets

Isolates code	Double disc synergy diffusion Test (≥22mm)			Modified phenotypic confirmatory test (≥5mm)		ESBL production
	CTX (mm)	AMC (mm)	CAZ (mm)	CAZ (mm)	CAZ/CLAV (mm)	
NDTF01	6	R	12	20	16	-
NDTF02	8	R	24	14	20	+
NCTF14	2	R	22	30	22	-
NCTS14	6	R	20	26	20	-
NATS23	6	R	22	22	32	+
OGTF35	2	R	18	26	28	-
OGTF36	4	R	24	22	10	-
OGTS36	4	R	22	24	30	+

CTX; Ceftriaxone, AMC; Amoxiclav, CAZ; Ceftazidime, CAZ/CLAV; Ceftazidime/Clavulanic acid, R; Resistant, +; Positive, -; Negative

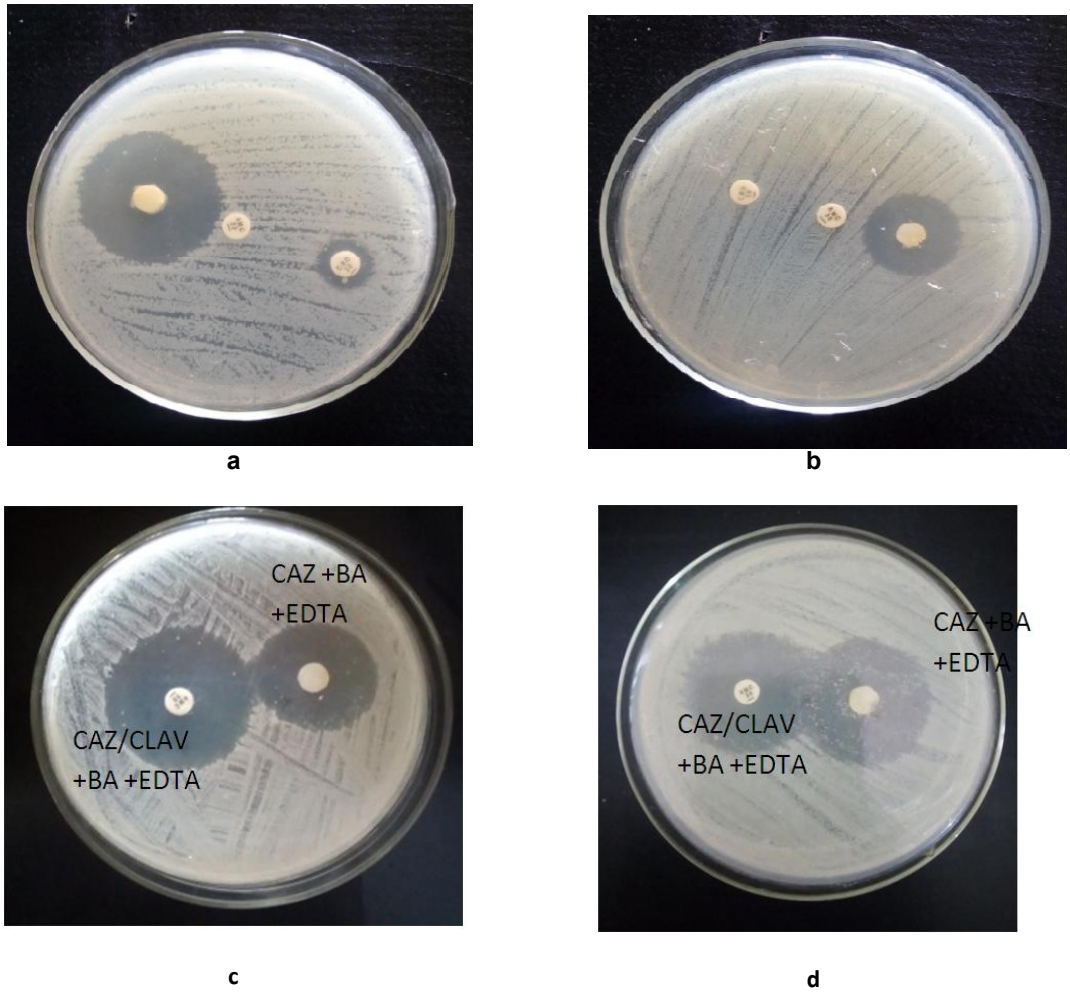

Plate 1. ESBL and non- ESBL producing *E. coli*

Images of ESBL producing and non- ESBL producing E. coli from toilets are presented in plate 1. Isolate (a) NDTF02, indicative of ESBL production and isolate (b) DLAF21, negative for ESBL production. Isolate (c) NDTF02, confirmed ESBL producer and isolate (d) DLAF26, confirmed non-ESBL producer

Table 3. Phenotypic detection of extended spectrum beta- lactamase among *E. coli* from private toilets

Isolates code	Double disc synergy diffusion Test (≥22mm)			Modified phenotypic confirmatory test (≥5mm)		ESBL production
	CTX (mm)	AMC (mm)	CAZ (mm)	CAZ (mm)	CAZ/CLAV (mm)	
OCLF20	6	R	22	14	22	+
OCLS20	2	R	24	26	38	+
OCLS40	6	R	24	22	38	+
MVLF12	2	R	20	24	26	-
MVLF14	2	R	20	18	12	-
MVLF15	4	R	26	24	38	+
DLAF21	4	R	26	28	24	-
DLAS21	2	R	22	16	12	-
DLAF26	6	R	26	12	8	-

CTX; Ceftriaxone, AMC; Amoxiclav, CAZ; Ceftazidime, CAZ/CLAV; Ceftazidime/Clavulanic acid, R; Resistant, +; Positive, -; Negative

4. DISCUSSION

Infection caused by ESBL producing *E. coli* poses a great burden in respect to the control of infectious diseases due to multidrug resistance and the failure of the broad-spectrum antibiotics [18]. Resistant enterobacteria can persist on surfaces in restrooms. Some can be transferred from people that are fecal carrier of ESBL, the restroom environment contained with antibiotic residues exert selection pressure and contribute to the nature of resistant of bacteria associated with restroom surfaces [19]. The present study showed that variety of organisms are associated with restrooms surface, 33% were confirmed to be *E. coli* While 67% were other organisms. This is in agreement with the findings of Erb et al. [20] where 24.3% Enterobacteriaceae were confirmed in 70 latrine samples and 16 *E. coli* identified. The percentage occurrence of *E. coli* in the present study is higher than those reported [20], which might be attributed to sample locations and the number of sampling surfaces within the restrooms. The floors of both the public and private toilets were implicated with the highest carriage of the toilets respectively, followed by the seats having 39% for public toilets and 29% for private toilets. *E. coli* isolates obtained from the door handles of public (22%) and private (5.9%) toilets were the least in occurrence compared to other areas. Reduced number of organisms present on door handles is based on the fact that most individuals wash their hands after using the toilets. Proper hand washing with soap or hand wash cannot be attested to. In private student hostel toilets high level of hygiene was observed because the number of persons residing in such student hostel is reduced unlike the public hostels. The public hostel toilets had high occurrence of organisms based on the number of students allocated to such hostels and the hygiene level is not maintained due to lack of routine cleaning. Some studies reported high occurrence of Enterobacteriaceae in the door handles and floors of the toilet. This can be attributed to the presence of faces or fecal matter on the floor of the restroom, lack of running water or improper washing of hands contributes to the high occurrence of organism in door handles [20]. Gram negative microorganisms demands moist or damp sites for enhanced longevity. Recent reports suggest that *E. coli* and *Klebsiella spp* may survive more than a year in moist surroundings, however *Serratia marcescens* can survive up to two months [21,22]. The prevalence of ESBL from inanimate surface in the hospital environment is higher than those obtained from community sites. Hospitals have patients down with aliments and are on antibiotics, thus resistant strains already exist in the hospital environment. However, these results may vary depending on the studied location and the type of hospital. A study carried out reported 94% multidrug resistance in *E. coli* and *Klebsiella pneumonia* [23]. In their study no resistance to carbapenems was detected, cabapenemase producers are found more among hospital isolates of *E. coli* and *Klebsiella than* community isolates [24]. A point of interest noted in the present study is that all the isolates were resistant to at least 3-5 antibiotics. The resistance was very high to Cefuroxime, Gentamicin, Cefixime, Augmentin, Ceftazidime, Ciprofloxacin, Ofloxacin but were more susceptible to Nitrofurantoin, which is

comparable with the studies of Stoesser [25]. ESBL-producing *E. coli* were detected using several combinations of cephalosporin disks with clavulanic acid disk. Of the four drugs tested, Ceftazidime with clavulanic acid was found to be the best ESBL detector for *E. coli*. Therefore, use of only one disk combination might fail to detect ESBL production resulting in under reporting of prevalence. Simultaneous use of four cephalosporin disks is recommended in screening for ESBL-producing organisms. ESBL production of the isolated *E. coli* was noted from seats of both public and private toilets with 67% for both (2 of 3 isolates) while the floors of the public and private toilets had 20% (1 of 5 isolates) and 33% (2 of 6 isolates) for ESBL production.

The finding in our study indicates the highest-risk areas for contamination with ESBL producing *E. coli* on seats of both public and private toilets and then the floors, suggesting that human feces are the main source for the recovered ESBL producing *E. coli*, as contradictory to the organisms being free-living environmental strains [26]. *E. coli* is the most predominant bacteria isolated from stool, they are found in ESBL producing fecal carriage in stools of healthy people [27]. This explains the presence of ESBL producing Enterobacteriaceae in toilet sits. *E. coli* can adapt and colonize new human hosts, if acquired by toilet users. *E. coli* were recovered from door handles due to lack of washing of hands or improper washing of hands after defecating thus leading to a transfer of fecal matter from some persons to the door handle. This shows that toilets are important reservoirs for ESBL-producing bacteria, given their proven ability to survive on surfaces. Some studies have reported *E. coli* as the most prevalent Enterobacteriaceae in the stools of healthy people. Carbapenemase producers are more prevalent among hospital isolates of *Klebsilella* and *E. coli* than community isolated [24]. In Fezcity a study was carried out to evaluate the efficacy of routine cleaning and disinfection practices of environmental surfaces in healthcare setting. Their study reported the bed rails (100%) were most contaminated with bacteria, this was followed by the bedsides (60%) and the least was reported in the door and room knobs (50%). *E. coli* was among the bacteria identified in the study [28]. Our study can be contrasted with prior related works, which includes three studies that investigated public restrooms [11,29,30]. Most studies carried out on restroom environment reported data mainly for *Staphylococcus* species and for general gut- and skin-associated taxa, providing limited data specifically for *E. coli* [11,30,31]. For prevalence of contamination in relation to site within toilets, these two studies identified toilet-related sites as highest risk and identified contamination. The study also looked at some non-toilet sites (hand dryer systems, inner door surfaces, taps, and soap dispensers) but provided no specific details for *E. coli*. This study identified high prevalence of multidrug resistant *E. coli* associated with toilet surfaces. Resistance of *E. coli* to several antibiotics decreases the effective treatment of infections associated with fecal *E. coli*.

5. CONCLUSION

This study revealed the presence of ESBL producing *E. coli* on floors and seats of private and public toilet samples. Most of the *E. coli* were multidrug resistant and produced ESBL, ESBL- non-producing *E.* coli was higher than ESBL producing *E. coli*. This implies that bet lactamase alone is not the sole cause of resistance of *E. coli* from restroom surfaces to several antibiotics.

6. RECOMMENDATION

It is recommended that further research should be done to type the strains of E*. coli* responsible for resistance in hospital and restroom surfaces. Daily cleaning and disinfection with regular hygiene service are recommended to prevent the spread of fecal *E. coli* present in restroom surfaces which can be released via excreta.

This study discovered the presence resistant *E. coli* from inanimate surfaces in toilet facilities. This is useful in the treatment of infectious diseases caused by fecal *E. coli.* This study will help the researchers to uncover the genes of fecal *E. coli* that are responsible for resistance, thus preventing the spread of resistant strains and infectious diseases through personal hygiene and effective hand washing after using the toilet.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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