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Extended-Spectrum Beta-Lactamase–production in *Escherichia coli* isolated from door handles in Nasarawa State University, Keffi, Nigeria

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ABSTRACT

Serious clinical concern has been raised globally over the continual evolution of pathogenic microorganisms that are resistant to several chemotherapeutic agents, especially the beta-lactam antibiotics. This study investigated ESBL-production in *Escherichia coli* isolated from door handles in Nasarawa State University, Keffi-Nigeria. A total of 200 door handles were sampled and 34 (17.0%) *E. coli* isolates were identified. The bacterial resistance profile to tested antibiotics was: tetracycline 31 (91.18%), cotrimoxazole, ceftazidime, and augmentin with 28 (82.35%). Streptomycin and ampicillin had 26 (76.47%), while ciprofloxacin, chloramphenicol, ceftriaxone, and gentamicin had 16 (47.06%), 14 (41.18%), 12 (35.29%) and 7 (20.59%) resistance profile respectively. Multiple antibiotics resistance index (MARI) $\geq$ 0.3 was recorded in 33 (97.06%) of the isolates. A total of 23 resistant phenotypes were observed in this study. The most common resistant phenotype was AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C with 4 appearances. Fourteen (14) of the isolates were Multidrug resistant (MDR), while 9 were extensively resistant (XDR) isolates. Fifteen (15) ESBL-producers were identified out of which bla_{TEM} was identified in 7 of the isolates, while 10 were carriers of bla_{SHV}, and bla_{CTX-M} gene was not detected in any of the test isolates. This study recommends prompt action by all stakeholders in public health to prevent a potential disease burden from a superbug.

1. Introduction

The discovery of antibiotics in the early twentieth century aroused the optimism that many infectious pathogens have been conquered. This new glimmer of hope was however ephemeral as resistant pathogens were soon detected afterwards. This emergence of resistant microorganisms to antibiotics has now been identified as a major global menace [1, 2, 3, 4, 5, 6, 7]. The major cause of this phenomenon is the spread of the plasmid–encoded Extended-Spectrum Beta-Lactamase (ESBL) genes, conferring resistance to third generation cephalosporins [8]. The spread of ESBL–producing bacteria has been strikingly rapid globally, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Therapeutic options for infections treatment due to ESBL producers have also become increasingly limited [9, 10, 11]. Third generation cephalosporins were introduced into clinical use in the early 1980s to offer effective therapy principally for infections caused by multidrug resistant *Enterobacteriaceae* [12].

Extended Spectrum Beta-Lactamases are derived from genes related to the narrow-spectrum beta-lactamases by mutations that alter the amino acid configuration around the enzyme active site. They are typically encoded by plasmids that can be exchanged readily between bacterial species. These enzymes are most commonly produced by the members of the *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella* species. To date, more than 350 different natural ESBL variants are known that have been classified into nine distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA [13, 14].

Contamination of door handles with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistant determinants can be transferred to other pathogenic bacteria thus, compromising the treatment of severe bacterial infections and enhancing resistance dissemination [15]. Medically important microorganisms such as; Gram–positive *Staphylococcus aureus*, and Gram–negative *Enterobacteriaceae* (which includes *Escherichia coli*, *Klebsiella* species, *Citrobacter*

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Table 1
List of primers for ESBL genes.

Primer	Sequence (5′ – 3′)	Amplicon length (bp)	Reference
bla_{SHV}	F: CGCCTGTGTATTATCTCCCT R: CGAGTAGTCCACCAGATCCT	401	[28]
bla_{CTX-M}	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550–600	[29]
bla_{TEM}	F: TTTCGTGTCGCCCTTATTCC R: ATCGTTGTCAGAAGTAAGTTGG	980	[28]

species, *Salmonellae* species) were found to contaminate various contact surfaces including chairs, tables, windows, door handles, and many other common household fixtures [15, 16, 17, 18, 19, 20, 21, 22]. These microorganisms have been frequently isolated from environmental sources that serve as a relay for the bacteria and plays major role in the spread of infections between different hosts [15]. This study investigated the ESBL-producing *E. coli* isolated from door handles in Nasarawa State University, Keffi-Nigeria.

2. Materials and methods

The study was performed at Nasarawa State University, Keffi, in north–central Nigeria.

2.1. Sample collection

A cross–sectional study was carried out following stratified random sampling technique. A total of 200 door handles were sampled using sterile swab sticks immersed in 0.85% sterile normal saline solution. Collection of samples took place in February, 2018.

2.2. Isolation and identification of Escherichia coli

Samples were cultured on Levine Eosin Methylene Blue (EMB) Agar (HiMedia, India) plates and incubated at 37°c for 24 h. The plates were observed after 24 h incubation; greenish metallic sheen indicates the presence of *Escherichia* spp [23]. API 20E (Biomerieux^{TM}) kit was used for identification of *E. coli* following manufacturer's instructions.

2.3. Antibiotics susceptibility test

The antibiotics susceptibility test of the *E. coli* isolates was carried out using Kirby–Baeur disk diffusion method. The antibiotic disks were firmly placed on the sterile Muiller Hinton Agar (MHA) plates seeded with the tested strains, standardized to 0.5 McFarland's turbidity standard and incubated at 37 °C for 24 h. Diameter of zones of inhibition was then measured to the nearest millimetre and reported in accordance with the antimicrobial susceptibility breakpoint of CLSI [24].

2.4. Determination of multiple antibiotic resistance (MAR) index

The MAR Index was determined according to the method of Krumperman [25] and Paul *et al.* [26]. From the result of the antibiotic susceptibility test, MARI was calculated as:

$$\text{MAR Index} = \frac{\text{No. of antibiotics to which isolate is resistant}}{\text{Total no. of antibiotics tested}}$$

2.5. Phenotypic detection of ESBL

ESBL production was detected by the conventional Double Disc Synergy Test (DDST) using ceftazidime (30μg) and cefotaxime discs (30μg) with or without clavulanic acid (10μg) as recommended by the CLSI. An increase of $\geq$ 5mm in the inhibition zones of either cephalosporin in combination with clavulanic acid compared to the cephalosporin alone was interpreted as ESBL positive. *E. coli* ATCC 25922 was used as standard control strain [24].

2.6. DNA extraction

Bacterial culture was inoculated into Luria-Bertani (LB) broth and incubated at 37^{o}c for 8 h. Five millilitres of the LB broth culture containing the bacterial isolates was spun at 14000rpm for 3 min. The cells were re–suspended in 500μl of normal saline solution and heated at 95 °C for 20 min in the heating chamber. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tubes and

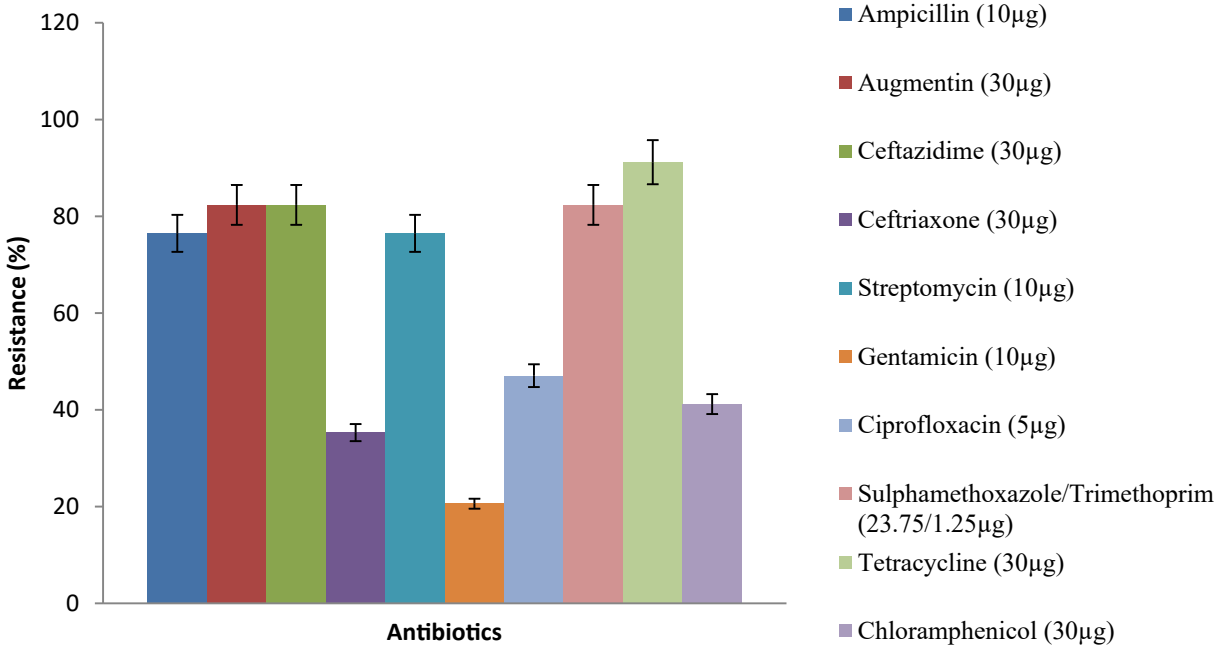

Fig. 1. Antibiotics resistance profile of *E. coli* from door handles in Nasarawa State University, Keffi.

stored at –20 °C for other subsequent experimentations [27].

2.7. DNA quantification

The extracted genomic DNA was quantified using the NanoDrop 1000 spectrophotometer by placing a drop (approximately 2μl) on the sample space and analysed using the NanoDrop 1000 software.

2.8. Amplification of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes

The bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes were amplified using specific primers (Table 1) on thermal cycler (Bio–RAD) at a final volume of 25μl for 35 cycles. The PCR mix included: X2 Dream Taq master mix (Thermo Scientific™), the primers at a concentration of 0.2M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 °C for 5 min; denaturation, 95 °C for 30 s; annealing, 52 °C for 30 s; extension, 72 °C for 30 s for 35 cycles and final extension, 72 °C for 5 min. The product was resolved on a 1.5% agarose gel at 120V for 20 min and visualized on a UV transilluminator. *E. coli* ATCC 25922 was used as standard control strain.

2.9. Statistical analysis

Microsoft Excel™ 2016 was used as statistical tool for computation of percentages, mean and averages of the data obtained.

3. Results

This study recorded the occurrence frequency of 34 (17.00%) *E. coli* isolates out of 200 door handles sampled in Nasarawa State University, Keffi. The bacterial resistance profile to the different tested antibiotics is as shown in Fig. 1 (see Figs. 2 and 3).

4. Discussion

This study recovered 34 (17.00%) *E. coli* out of the 200 door handles investigated. This occurrence of enteric bacteria on door handles entails the rate of personal hygiene of the general populace [30]. It could be asserted hypothetically that a significant number of the people uses toilet without proper hand washing and sterilization afterwards. In recent studies, Nworie *et al.* [30] reported 15% occurrence of *E. coli* from door handles in public conveniences in Abuja, Nigeria. A higher occurrence of 38% was reported in a Nigerian University [31], whereas 24% occurrence was reported by a study from Malawi [32].

Several patterns of antibiotics resistance, especially resistance to beta lactam classes of antibiotics was encountered in this study. These resistance profile are however not strange since previous studies have presented similar reports [2, 3, 10, 19, 21]. The selective resistance to the different antibiotics tested could be alluded to some obvious reasons, such as; inaccessibility to target site of action, decreased absorption of the active substance through efflux action, and the abuse of antibiotics that are commonly accessible – for example, tetracycline is easily accessible and it is administered orally whereas gentamicin is administered via parenteral routes [21]. An important consequence of these resistances is that many bacterial-related diseases have recently been made more expensive and less successful by the emergence and spread of resistant strains [2, 33].

All the 34 *E coli* isolates investigated in this study had multiple antibiotic resistance indices (MARI) ≥0.2 (Table 2). This implies that they were resistant to at least 2 of the tested antibiotics. MARI greater than 0.2 indicates that an organism probably originated from an environment where antibiotics were frequently used [25, 26]. A total of 23 resistant phenotypes were recorded in this study out of which 14 (41.18%) were multidrug resistant (MDR), while 9 (26.47%) were extensively resistant (XDR) phenotypes (Table 3) (see Table 4).

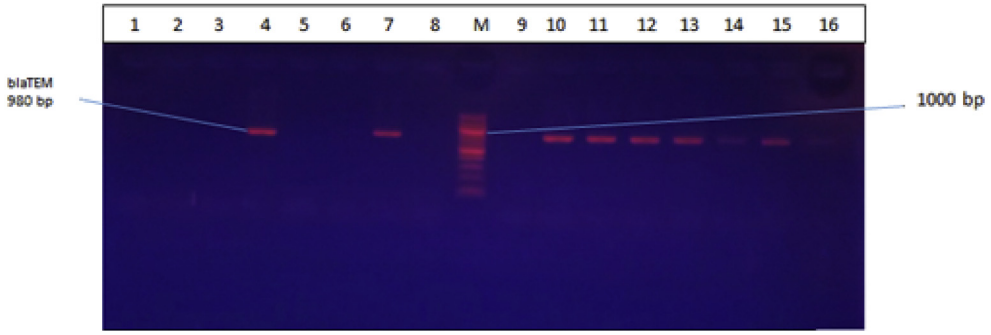

Fig. 2. Agarose gel electrophoresis of the amplified bla_{TEM} genes from the ESBL producing *E. coli* isolates (see supplementary Fig. 1 for full image). Lanes 4, 7, 10–13, and 15 represent the bla_{TEM} bands. Lane M represents the 1000bp molecular marker.

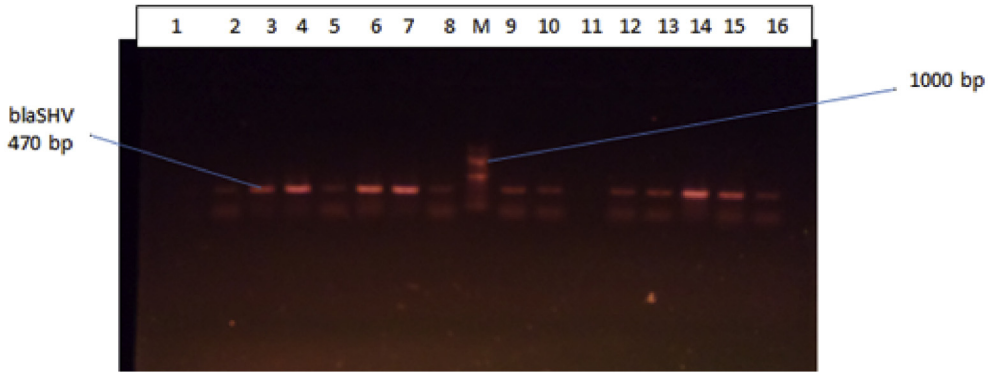

Fig. 3. Agarose gel electrophoresis of the amplified bla_{SHV} genes from the ESBL producing *E. coli* isolates (see supplementary Fig. 2 for full image). Lanes 3, 4, 6, 7, 9, 10, 12–15 represent the bla_{SHV} bands at 470bp. Lane M represents the 1000bp molecular marker.

Table 2
Multiple antibiotics resistance index (MARI) of *E. coli* from Door Handles in Nasarawa State University, Keffi.

Mari	No. (%) of isolates (n = 34)
0.20	1 (2.94)
0.30	2 (5.88)
0.40	4 (11.76)
0.50	5 (14.71)
0.60	5 (14.71)
0.70	6 (17.65)
0.80	4 (11.76)
0.90	5 (14.71)
1.00	2 (5.88)

Table 3
Phenotypic resistance profile of *E. coli* from Door Handles in Nasarawa State University, Keffi.

S/N	Phenotype	No. of Isolates	Occurrence (%) (n = 34)
1	CN-TE	1	2.94
2	CIP-SXT-TE	1	2.94
3	S-SXT-TE	1	2.94
4	AMP-AUG-CAZ-TE	2	5.88
5	AMP-CIP-TE-C	1	2.94
6	S-SXT-TE-C	1	2.94
7	AMP-AUG-CAZ-SXT-TE	1	2.94
8	AUG-CAZ-S-SXT-TE	2	5.88
9	AUG-CAZ–CN–SXT-TE	1	2.94
10	AUG–CN–SXT-TE-C	1	2.94
11	AMP-AUG-CAZ-S-SXT-TE	3	8.82
12	AMP-AUG-CAZ-S-CIP-SXT	2	5.88
13	AMP-AUG-CAZ-CRO-S–CN–TE	1	2.94
14	AMP-AUG-CAZ-CRO-S-SXT-TE	2	5.88
15	AMP-AUG-CAZ-S-CIP-TE-C	1	2.94
16	AMP-AUG-CAZ-S-CIP-SXT-TE	1	2.94
17	AMP-CAZ–CN–S-CIP-SXT-TE	1	2.94
18	AMP-AUG-CAZ-CRO-S-CIP-SXT-TE	1	2.94
19	AMP-AUG-CAZ-CRO-S-SXT-TE-C	2	5.88
20	AMP-AUG-CAZ-S-CIP-SXT-TE-C	1	2.94
21	AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C	4	11.76
22	AMP-AUG-CAZ-S–CN–CIP-SXT-TE-C	1	2.94
23	AMP-AUG-CAZ-CRO-S–CN–CIP-SXT-TE-C	2	5.88

Table 4
Classes of antibiotic resistance of *Escherichia coli* from door handles in Nasarawa State University, Keffi-Nigeria.

Phenotype	Occurrence (%) (n = 34)
MDR	14 (41.18)
NMDR	00 (00.00)
PDR	00 (00.00)
XDR	09 (26.47)

MDR = Multidrug Resistant; NMDR = Non Multidrug Resistant; PDR = Pan Drug Resistant; XDR = Extensively Drug Resistant.

Out of the 34 *E coli* isolates tested, 16 were identified as ESBL producers (Table 5). Beta-lactamase encoding gene bla_{TEM} was identified in 7 isolates (Plate 1), while bla_{SHV} was detected in 10 isolates (Plate 2), and $bla_{CTX\text{-}M}$ was not detected in any of the isolates. This finding corroborates several previous studies about prevalence of bla_{TEM} and bla_{SHV} but negates the studies about increasing prevalence of $bla_{CTX\text{-}M}$ [8, 9, 33, 34, 35].

These results are of paramount significance to public health considering the fact that ESBL genes are plasmid-mediated and are usually associated with transposons and insertion sequences [36, 37, 38, 39],

Table 5
Phenotypic ESBL-production in *E. coli* from door handles in Nasarawa State University, Keffi.

S/N	isolate ID	ESBL Production
1	NUK1	+
2	NUK2	+
3	NUK3	-
4	NUK4	-
5	NUK5	-
6	NUK6	-
7	NUK7	+
8	NUK8	-
9	NUK9	+
10	NUK10	+
11	NUK11	-
12	NUK12	-
13	NUK13	-
14	NUK14	+
15	NUK15	+
16	NUK16	-
17	NUK17	+
18	NUK18	+
19	NUK19	-
20	NUK20	+
21	NUK21	-
22	NUK22	+
23	NUK23	+
24	NUK24	-
25	NUK25	-
26	NUK26	+
27	NUK27	-
28	NUK28	+
29	NUK29	-
30	NUK30	-
31	NUK31	-
32	NUK32	+
33	NUK33	-
34	NUK34	+

which imply that they are easily transmissible to other strains of the same or different species. The exchange of plasmids between bacterial cells and the integration of resistance genes into transferrable genetic elements plays a major role in acquisition and dissemination of antibiotic resistance genes among bacteria isolates [40, 41, 42, 43, 44].

5. Conclusion

The isolation of *E. coli* from door handles and subsequent detection of several resistance patterns indicates a potentially serious public health challenge. Continuous investigation and surveillance will extend our understanding of the transmission dynamics and the evolution of these isolates.

Declarations

Author contribution statement

P.A. Tsaku: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Y.B. Ngwai: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

G.R.I. Pennap: Contributed reagents, materials, analysis tools or data.

D. Ishaleku: Analyzed and interpreted the data.

T. Ibrahim, I.H. Nkene, R.H. Abimiku: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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