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(RESEARCH ARTICLE)

Molecular diversity of multi-resistant and extended–spectrum beta lactamase–producing *Escherichia coli* from door handles in Lafia, central Nigeria

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Abstract

Human hands have been implicated as a major source of dissemination of pathogenic microorganisms through fomites. This study investigated the diversity of ESBL-producing in *E. coli* isolates from door handles using restriction fragment length polymorphism (RFLP). An occurrence of 77(19.25%) of *E. coli* out of 400 samples was recovered from different locations. The study of antibiotics resistance showed that ampicillin, ceftazidime, and streptomycin were predominantly resistant. Multiple antibiotics resistance index of ≥0.3 was recorded in 73(94.81%) of the isolates. A total of 40 resistant phenotypes were observed in this study, with AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C being the commonest. Twenty isolates were characterised as Multidrug resistant (MDR) phenotypes, followed by, pan drug resistance (PDR) and extensive resistance (XDR) phenotypes recorded in 12 and 8 isolates respectively. Thirty-six (36) ESBL-producers were identified out of which 14 harboured bla_{TEM}, while 5 and 9 were carriers of bla_{SHV} and bla_{CTX-M} respectively. Most of the isolates shared a common origin, as revealed by result of the RFLP. The outcome of this study suggests the need for improved personal hygiene and the need for all stakeholders to be proactive in curtailing the spread of resistant pathogens.

Keywords: *Escherichia coli*; Extended-spectrum beta-lactamase; bla_{TEM}; bla_{SHV}; bla_{CTX-M}; Restriction fragment length polymorphism

1. Introduction

Escherichia coli (*E. coli*) are Gram–negative, facultative anaerobic, bacilli (rod–shaped) which constitutes a major microflora of the lower intestines of warm–blooded organisms [1, 2]. Most strains are reportedly non–pathogenic, with some beneficial aspects such as synthesis of vitamins B_{12} and K within the hosts [3, 4], and preventing colonization of the intestine by pathogenic microbes [5, 6]. Nonetheless, some pathogenic strains have also been reported and are capable of causing serious food poisoning in their hosts, and are occasionally responsible for product recall due to food contamination [7, 8]. Faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease.

The microorganism is capable of survival on inanimate surfaces for a limited time period, which makes them suitable indicators to test environmental samples for faecal contamination [9, 10]. Recent research has observed environmentally persistent *E. coli* which can survive for extended periods outside of a host [11]. Cephalosporins, particularly the third and fourth generation cephalosporins are used for treatment of infections caused by enterobacteriaceae such as *E. coli* [12]. Extended spectrum or third generation cephalosporins were introduced into clinical use in the early 1980s to offer effective therapy principally for infections caused by multidrug resistant

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Enterobacteriaceae [13]. Resistance to cephalosporin has emerged globally and numerous types of ESBLs have been detected in several bacterial organisms [14]. Ghafourian *et al*. [15] defined ESBL as "enzymes produced by certain bacteria that are able to hydrolyse extended spectrum cephalosporin. They are therefore effective against beta–lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam." *E. coli* can become resistant to extended spectrum cephalosporins by mutational overproduction of AmpC and/or by expression of acquired ESBLs. The latter emerged in the 1980s as derivatives of *TEM* (named after the patient Temoneira) and Sulfhydryl Reagent Variable (*SHV*) enzyme types [16].

About 300 to 600 different ESBL variants have been Reported [17, 18, 14], with *TEM* and *SHV* as the most common variants. Whereas the *CTX-M* variant have continued to emerge over the past decade, and are now the most frequently reported aside *TEM* and *SHV* [19]. Since ESBLs are normally encoded by genes located on different transferable genetic elements, a diversity of epidemiological situations has been documented, ranging from sporadic cases to large outbreaks [20]. Moreover, ESBL–producing strains are often resistant to antibiotics of other classes (such as, sulfonamides, aminoglycosides, and quinolones) which complicate available treatment strategies in many hospitalized patients [21, 22]. This study therefore determines the Molecular Diversity of ESBL–producing *E. coli* from door handles in Lafia, Central Nigeria.

2. Material and methods

The study was conducted in Federal University, Lafia (FUL) and Nasarawa State Polytechnic, Lafia (NPL). Lafia is a town in central Nigeria. It is the capital city of Nasarawa State and has an approximate population of 330,712 inhabitants according to the 2006 census results. It is the largest town in Nasarawa state.

2.1. Sample collection

A cross–sectional study was carried out following stratified random sampling technique. A total of 400 door handles were sampled (200 each from the two institutions) using sterile swab sticks immersed in 0.85% sterile normal saline solution.

2.2. Isolation and identification of *Escherichia coli*

Samples were cultured on Levine Eosin Methylene Blue (EMB) Agar plates and incubated at 37 °C for 24 hours. The plates were observed after 24 hours incubation; greenish metallic sheen indicates the presence of *Escherichia* spp [23]. API 20E (Biomerieux™) 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 test organisms, standardised to 0.5 McFarland's turbidity standard and incubated at 37 ^{o}C for 24 hours. 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 (30 µg) with or without clavulanic acid (10µg) as recommended by the CLSI. An increase of ≥ 5mm in the inhibition zones of either cephalosporin in combination with clavulanic acid compared to the cephalosporin alone was interpreted as ESBL positive [24].

2.6. DNA extraction

Bacterial culture was inoculated into Luria-Bertani (LB) broth and incubated at 37 °C for 8 hours. Five millilitres of the LB broth culture of the containing the bacterial isolates was spun at 14000 rpm for 3 min. The cells were re–suspended in 500 µl of normal saline 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 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tubes and stored at –20 °C for other subsequent experimentations.

2.7. DNA Quantification

The extracted genomic DNA was quantified using the NanoDrop 1000 spectrophotometer by placing a drop (approximately 2 µl) 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 25 µl 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 ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52 ºC for 30 seconds; extension, 72 ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1.5% agarose gel at 120 V for 20 minutes and visualized on a UV transilluminator.

2.9. Molecular typing of the isolates using Restriction Fragment Length Polymorphism (RFLP) analyses

The method of Lacher *et al*. [27] was adopted for RFLP to identify *E. coli* variants. To achieve species–specific discriminatory patterns, a 5 µl aliquot of PCR products was digested with 10U/µl of *ECO471* (AvaII – ThermoFisher Scientific) in a final volume of 25 µl at 37 °C for 6 hours. The restriction fragments were separated on 2% agarose gel electrophoresis in TBE buffer for about 30 minutes at 120V and visualized by staining with 0.5 µg/ml of ethidium bromide.

Table 1 List of primers for ESBL genes

Primer	Sequence (5' – 3')	Amplicon length(bp)	Reference
bla$_{SHV}$	F: CGCCTGTGTATTATCTCCCT	401	[28]
	R: CGAGTAGTCCACCAGATCCT		
bla$_{CTX-M}$	F: CGCTTTGCGATGTGCAG	550-600	[29]
	R: ACCGCGATATCGTTGGT		
bla$_{TEM}$	F: TTTCGTGTCGCCCTTATTCC	980	[28]
	R: ATCGTTGTCAGAAGTAAGTTGG		
16s rRNA	F: AGAGTTTGATCMTGGCTCAG	27	[30]
	R: CGGTTACCTTGTTACGACTT	1492	

3. Results

In this study, a total 77(19.25%) *E. coli* isolates were recovered from 400 door handles sampled in two different tertiary institutions in Lafia, Nasarawa State, Nigeria. The two institutions are Federal University, Lafia and Nasarawa State Polytechnic, Lafia where the isolation rates were 32(16.00%) and 45(22.50%) respectively (Table 2).

Table 2 Isolation of *E. coli* from door handles in Lafia

Location	No. of Samples	No. of Isolates (%)
FUL	200	32(16.00)
NPL	200	45(22.50)
Total	400	77(19.25)

Key: FUL = Federal University, Lafia
NPL = Nasarawa State Polytechnic, Lafia

The antibiotics susceptibility/resistance profile of the isolated bacteria (as shown in Table 3) indicates that streptomycin 68(88.31%) was the most resistant of the tested antibiotics. Gentamicin 13(16.88%) was however the least resistant among the test antibiotics.

Table 3 Antibiotics susceptibility profile of *E. coli* isolates from door handles in Lafia

Antibiotics	Disc Content (µg)	NO. (%) Resistant		
		FUL (n=32)	NPL (n=45)	Total (n=77)
Ampicillin (AMP)	10	30(93.75)	35(77.78)	65(84.42)
Augmentin (AUG)	30	28(87.50)	22(48.89)	50(64.94)
Ceftazidime (CAZ)	30	30(93.75)	23(51.11)	53(68.83)
Ceftriaxone (CRO)	30	12(37.50)	11(24.44)	23(29.87)
Streptomycin (S)	10	28(87.50)	40(88.89)	68(88.31)
Gentamicin (CN)	10	5(15.63)	7(15.56)	13(16.88)
Ciprofloxacin (CIP)	5	13(40.63)	23(51.11)	36(46.75)
Sulphamethoxazole/Trimethoprim (SXT)	23.75/1.25	27(84.38)	34(75.56)	61(79.22)
Tetracycline (TE)	30	29(90.63)	38(84.44)	67(87.01)
Chloramphenicol (C)	30	14(43.75)	22(48.89)	36(46.75)

Key: FUL = Federal University, Lafia
NPL = Nasarawa State Polytechnic, Lafia

Table 4 Resistant phenotypes of *E. coli* isolates from door handles in Lafia

S/N	Phenotype	No. of Isolates			Class of resistance
		FUL	NPL	TOTAL	
1	AMP-C	-	1	1	MDR
2	S-TE	-	1	1	MDR
3	SXT-TE	-	1	1	MDR
4	S-SXT-TE	-	1	1	MDR
5	AMP-S-TE	1	-	1	MDR
6	S-CIP-TE	-	1	1	MDR
7	AMP-AUG-CAZ-TE	1	-	1	MDR
8	AMP-S-TE-C	1	-	1	MDR
9	CAZ-S-SXT-TE	1	1	2	MDR
10	AMP-AUG-S-SXT	-	1	1	MDR
11	AMP-S-SXT-TE	-	4	4	MDR
12	AMP-S-CIP-TE	-	1	1	MDR
13	CAZ-S-SXT-TE	-	1	1	MDR
14	AUG-CAZ-S-SXT-TE	1	1	2	MDR
15	AMP-AUG-CAZ-SXT-C	-	1	1	MDR
16	AMP-AUG-S-SXT-TE	-	1	1	MDR
17	AMP-CAZ-S-SXT-TE	-	1	1	MDR
18	AMP-S-CN-TE-C	-	1	1	MDR
19	AMP-S-SXT-TE-C	-	1	1	MDR
20	CAZ-S-SXT-TE-C	-	1	1	MDR
21	AMP-AUG-CAZ-S-SXT-TE	7	-	7	PDR
22	AMP-AUG-CAZ-S-CIP-SXT	1	1	2	PDR
23	AMP-AUG-CAZ-CRO-SXT-C	2	1	3	PDR
24	AMP-AUG-S-CN-CIP-SXT	-	1	1	PDR
25	AMP-S-CN-CIP-SXT-TE	-	2	2	PDR
26	AMP-S-CIP-SXT-TE-C	-	1	1	PDR
27	CAZ-S-CIP-SXT-TE-C	-	1	1	PDR
28	AMP-AUG-CAZ-S-CN-TE-C	-	1	1	PDR
29	AMP-CAZ-S-CIP-SXT-TE-C	-	1	1	PDR
30	AMP-AUG-CAZ-CRO-S-SXT-TE	1	-	1	PDR
31	AMP-AUG-CAZ-S-CIP-TE-C	1	-	1	PDR
32	AMP-AUG-CAZ-S-CIP-SXT-TE	1	4	5	PDR
33	AMP-AUG-CAZ-CRO-S-CIP-SXT-TE	3	2	5	XDR
34	AMP-AUG-CAZ-CRO-S-CN-SXT-TE	2	-	2	XDR
35	AMP-AUG-CAZ-S-CN-CIP-TE-C	1	-	1	XDR
36	AMP-AUG-CAZ-S-CN-SXT-TE-C	3	-	3	XDR
37	AMP-AUG-CRO-S-CN-SXT-TE-C	-	1	1	XDR
38	AMP-AUG-CAZ-CRO-S-SXT-TE-C	1	-	1	XDR
39	AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C	2	7	9	XDR
40	AMP-AUG-CAZ-CRO-S-CN-CIP-SXT-TE-C	2	1	3	XDR

Key: FUL = Federal University, Lafia; NPL = Nasarawa State Polytechnic, Lafia; MDR = Multidrug Resistant, PDR = Pan Drug Resistant, XDR = Extensively Resistant

Forty (40) resistant phenotypes was recorded in this study out of which 20 were Multidrug resistant (MDR) phenotype, 12 were Pan drug resistant (PDR) and 8 Extensively resistant (XDR) phenotypes (Table 4).

The Multiple Antibiotics Resistance Index (MARI) indicates that out of the 77 total *E. coli* isolates, only 1(1.30) had 0.00 MARI; implying susceptibility to all tested antibiotics. The remaining 76 isolates had MAR Indices ≥0.2 which implies that they were all resistant to at least 2 of the test antibiotics (Table 5).

Table 5 Multiple Antibiotics Resistance Index (MARI) of *E. coli* isolates from door handles in Lafia

MARI	NO. (%) OF ISOLATES		
	FUL (n=32)	NPL (n=45)	TOTAL (n=77)
0.00	0	1 (2.22)	1 (1.30)
0.20	0	3 (6.67)	3 (3.90)
0.30	1 (3.13)	2 (4.44)	3 (3.90)
0.40	3 (9.38)	8 (17.78)	11 (14.29)
0.50	1 (3.13)	7 (15.56)	8 (10.40)
0.60	10 (31.25)	7 (15.56)	17 (22.08)
0.70	3 (9.38)	6 (13.33)	9 (11.69)
0.80	10 (31.25)	5 (11.11)	15 (19.48)
0.90	2 (6.25)	6 (13.33)	8 (10.39)
1.00	2 (6.25)	0 (0.00)	2 (2.60)

Figures 1, 2 and 3 are gel pictures of TEM, SHV and CTX-M genes extracted from *E. coli* isolates from door handles in Lafia

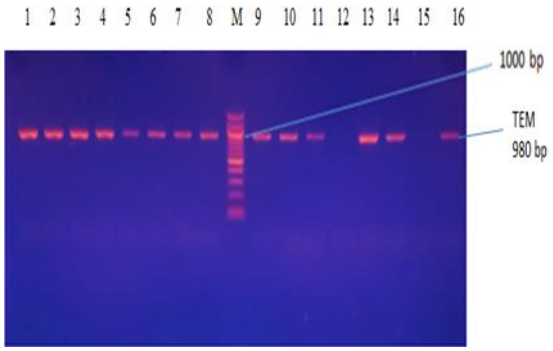

Figure 1 Agarose gel electrophoresis of the amplified *bla*$_{TEM}$ genes from the ESBL producing *E. coli* isolates

Lanes 1-11, 13-14 and 16 represent the *bla*$_{TEM}$ bands. Lane M represents the 100bp molecular marker, while other lanes show no bands.

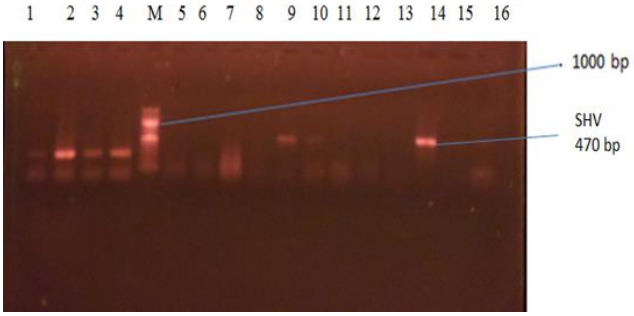

Figure 2 Agarose gel electrophoresis of the amplified *bla*$_{SHV}$ genes from the ESBL producing *E. coli* isolates.

Lanes 2-4, 9 and 14 represent the *bla*$_{SHV}$ bands. Lane M represents the 100bp molecular marker, while other lanes show no bands.

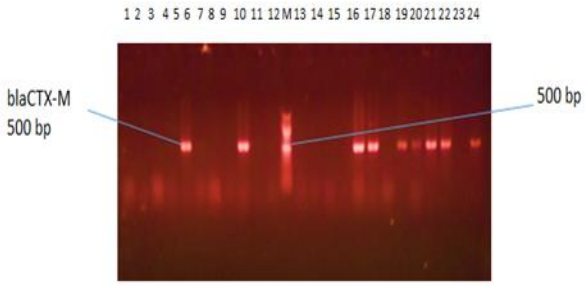

Figure 3 Agarose gel electrophoresis of the amplified *bla*$_{CTX-M}$ genes from the ESBL producing *E. coli* isolates.

Lanes 6, 10, 16-17, 19-22, and 24 represent the *bla*$_{CTX-M}$ bands. Lane M represents the 100bp molecular marker, while other lanes show no bands**.**

4. Discussion

In this study, a significant numbers of *E. coli* isolates was recovered from the door handles studied, which indicates low levels of hygiene practices in the locality of the survey. A total of 77(19.25%) *E. coli* was isolated from 400 door handles sampled in this study. From the two different locations (tertiary institutions) door handles sampled, the isolates obtained were 32(16.00%), and 45(22.50%), representing Federal University Lafia, and Nasarawa State Polytechnic Lafia respectively.

These results should stimulate drastic public health concern, since the presence of *E. coli* on door handles suggests the possibility of contamination from faecal sources. *E. coli* has frequently been isolated from door handles and several other handy surfaces such as bannisters, crevices, and toilet knobs globally [4, 31- 35]. Similarly, enterobacteriaceae such as *Salmonellae, Enterobacter, Proteus, Klebsiella, Citrobacter, Providencia* and *Yersinia* have also been isolated from door handles and other environmental surfaces [31-32, 35-36].

The Antibiotics susceptibility/resistance assessment of the *E. coli* isolates from door handles in this study shows varying forms of antimicrobial resistance to the antibiotics studied. In all, (98.70%) of the isolates were resistant to two or more of the test antibiotics (Table 4), which corroborates the reports of Ajayi and Ekozien, [35] and Opere *et al*. [37]. This observation suggests that the *E. coli* isolates in this study may probably have originated from an environment where antibiotics were often used indiscriminately [38]. Broad-spectrum antibiotics are sometimes reported to be given in place of narrow-spectrum antibiotics as a substitute for culture and sensitivity testing, with the consequent risk of selection of antibiotic-resistant mutants [25, 39- 41].

This study highlights a highly diverse antibiotics resistance rates among the *E. coli* isolates. The general order of antibiotics resistance in the study was: streptomycin (88.31%) > Tetracycline (87.01%) > ampicillin (84.42%) > cotrimoxazole (79.22%) > ceftazidime (68.83%) > augmentin (64.94%) > chloramphenicol (46.75%) = ciprofloxacin (46.75%) > ceftriaxone (29.87%) > gentamicin (16.88%). Similar to previous reports by Tsaku *et al*. [4] and Mohammed *et al*. [36] with similar antibiotics, this study also confirms gentamicin to be more effective than all the tested antibiotics. The effectiveness of gentamicin is possibly due to inaccessibility to the antibiotics and the parenteral routes of administering them. Likewise the massive resistance to antibiotics such as tetracycline and streptomycin could be attributed to their inability to reach target site in the bacteria. The findings from this present study agrees with the work reported by Okonko *et al*. [38], who reported high bacterial isolates resistance to ampicillin, augmentin and co-trimoxazole (60 to 100%). Other researchers have reported bacterial isolates obtained from public surfaces to be resistant to co-trimoxazole [31- 35].

Antibiotic resistance of *E. coli* isolated from door handles in this study may be a reflection of the prevailing resultant effects of self-medication of antibiotics in the schools investigated and the surrounding environments.

A total of forty (40) resistant phenotypes were observed in this study. The most common resistant phenotype was AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C which appeared 9 times. Multidrug resistant (MDR) phenotypes was the most

popular with 20 appearances, whereas, pan drug resistance (PDR) and extensive resistance (XDR) was recorded in 12 and 8 phenotypes respectively. Such multi-antibiotic resistance has important implications for the empiric therapy of infections caused by *E. coli* and other enterobacteriaceae [42, 43]. It has been well documented that Gram negative bacilli harbour series of antibiotic resistance genes like transposons or integrons and R plasmids which can be transferred to other bacteria horizontally [44- 47].

Thirty six (46.75%) of the bacterial isolates were confirmed ESBL producers from the two institutions studied. This result agrees with several reports on the subject area; for example, 48% ESBL-producing *E. coli* was reported from south-east Nigeria [48]. Kamel *et al*. [49], also reported 50.88% ESBL-producing *E. coli* from Egypt, with similar reports from Oyo state, south-western Nigeria [50], Tunisia [51], Morocco [52], and Sudan [53].

An important consequence of these resistances is that many bacterial diseases that could be treated with inexpensive antibiotics, has recently been made more expensive and less successful by the emergence and spread of resistant organisms [38, 54]. However, these multi-antibiotic resistances observed among some of the test *E. coli* isolates from door handles in this study has increased the growing therapeutic failure problem of infectious bacteria that account for most of Africa's disease burden, including respiratory and diarrhoeal diseases [54].

The molecular screening for bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ ESBL genes revealed that 14 out of the 77 isolates harboured bla_{TEM}, while 5 and 9 were carriers of bla_{SHV} and $bla_{CTX\text{-}M}$ respectively. This result unlike most previous reports which indicated that $bla_{CTX\text{-}M}$ was more predominant [53, 55, 56] suggests otherwise. However, more recent reports indicated that bla_{TEM} was most predominant, followed by $bla_{CTX\text{-}M}$ and bla_{SHV} [57].

These results are of significant public health importance considering the fact that ESBLs are plasmid-mediated and are usually associated with transposons and insertion sequences [58], which imply that they are easily transmissible to other potential pathogenic microbes of the same or different species. The exchange of plasmids between bacterial cells and the integration of resistance genes into specialized genetic elements play a major role in acquisition and dissemination of antibiotic resistance genes among bacteria isolates [59- 63].

Molecular typing of the isolates from the different institutions using restriction enzymes, Eco471 (AvaII), revealed similar pattern of cuts; which signifies that the isolates are most probably of the same strain or from the same source. This is an indication of the mobility of the microbes across the geographical location or even further beyond.

This research report is a timely call on all public health stakeholders to be on guard in order to prevent the continuous spread of multiple antibiotics resistant pathogens.

5. Conclusion

There is an occurrence of 19.25% *E. coli* contamination on door handles in Federal University, Lafia and Nasarawa State Polytechnic, Lafia, and 76 out of 77 the isolates are resistant to 2 or more test antibiotics. The most common resistant phenotype was AMP-AUG-CAZ-CRO-S-CIP-SXT-TE-C which appeared 9 times; with 20 MDR phenotypes followed by, PDR (12) and XDR (8).Thirty six (36) ESBL-producers were identified out of 77 *E. coli* isolates. Fourteen (14) isolates harboured bla_{TEM}, while 5 and 9 were carriers of bla_{SHV} and $bla_{CTX\text{-}M}$ ESBL-encoding genes respectively. Most of the isolates shared a common origin, as revealed by result of the RFLP.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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