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Plasmid – mediated Resistance in *Salmonella typhi* Isolates from Door Handles in Nasarawa State, North-central Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAM, PAT, IHN and IKE helped with sample collection and laboratory analysis. Authors VBO and PAT searched for literatures and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

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. This study investigated the antibiotic susceptibility and plasmid profile of *Salmonella typhi* isolated from door handles of two tertiary institutions in Nasarawa State, Nigeria. One hundred door handles from each of the two institutions, making 200 in total were sampled and 36(18.00%) *S. typhi* were isolated. The isolates were 100% resistant to 7 out of the 10 antibiotics tested. Minimum Inhibitory Concentrations (MICs) study on selected multiple antibiotics resistant isolates showed that the isolates were susceptible to the tested antibiotics in the following order: Imipenem = Ciprofloxacin > Ceftazidime > Cefuroxime > Ampicillin > Nitrofurantoin. Fourteen (38.89%) of the multiple antibiotic resistant isolates produced beta lactamase enzymes. Conjugation experiment on the multiple antibiotics resistant *S. typhi* isolates showed that 7(18.42%) of the test *S. typhi* isolates transferred resistance plasmid gene to sensitive *Proteus mirabilis* and the MICs of the recipients increased significantly after

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conjugation. Plasmid profile of the transconjugants and the donors showed the presence of plasmid of different sizes ranging from 1600 to 2500 base pairs in both donor multiple antibiotics resistant *S. typhi* and transconjugants *P. mirabilis*. The transfer of resistant plasmids between bacteria could result in serious epidemics that may be difficult to manage.

Keywords: Salmonella typhi; antibiotics susceptibility; Proteus mirabilis; conjugation experiment; plasmid profile.

1. INTRODUCTION

Salmonellae are the etiologic agent of Salmonellosis causing severe illness in humans. *Salmonella* has been cited as the most common causative agent of food borne illness [1].

The organism has been frequently isolated from environmental sources that serve as a relay for the bacteria and play major role in its spread of infections between different hosts [2]. Typhoid fever, known to be caused by *S. typhi,* has been reported as major public health problem in most developing countries including Nigeria. Recently, there has been a panic across the country about an apparent increase in clinical and laboratory diagnosed of typhoid fever. Although all serovars may be regarded as potential human pathogens, a very limited number of serovars causes the majority of infections. Since this pathogens are transmitted primarily through contaminated food or water, the presence of strains in food animals and ultimately in raw meat products have important public health implication [1]. After a short period of incubation period of few hours to one day, the bacteria multiply in the intestinal lumen, causing an intestinal inflammation. Most people with *Salmonella* develop diarrhoea, fever, vomiting and abdominal cramps 12-72 hours after infection [3]. *Salmonella* infection may spread from intestine to the blood stream and then to other body sites and can cause death unless infected person is treated promptly with antibiotics.

Antibiotics are the mainstay of therapy for of salmonellosis. However, the intensive use of first-line antibiotics, such as ampicillin, chloramphenicol, and cotrimoxazole, has led to the emergence and global spread of multidrug-resistant (MDR) *S. typhi* strains [4]. Due to increasing resistance to the antibiotics used traditionally for therapy, the use of fluoroquinolones, such as ciprofloxacin, for the treatment of salmonellosis has become more common in developing countries. The use of fluoroquinolones has also led to a rapid increase in reduced susceptibility of *S. typhi* to these therapeutics. MDR *S. typhi* with reduced ciprofloxacin susceptibility has become common in Africa and South and Southeast Asia [5-7]. In recent years, ciprofloxacin-resistant *S. typhi* has been reported frequently [8-10].

Multiple antibiotics resistant *S. typhi* is almost exclusively associated with self-transmissible plasmids, which carry a panel of genes conferring resistance to several first-line antibiotics, including ampicillin, augmentin, sulfonamide, streptomycin and trimethoprim [11,12]. A study conducted by Holt et al. [13] indicated that prior to 1995, MDR typhoid was caused by a diverse range of *S. typhi* and MDR plasmids. In this study, we investigated the plasmid profile of multiple antibiotics resistant *S. typhi* isolated from door handles in Nasarawa state, North-central Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa, Nasarawa State, Nigeria. Keffi is approximately 68 Km from Abuja, the Federal Capital Territory and 128 Km from Lafia, the Capital of Nasarawa state Keffi is located between latitude 8°5 N of the equator and longitude 7°8 E and situated on an altitude of 850 M above sea level [14] and Nasarawa is approximately 35 Km South-west from Keffi.

2.2 Sample Collection

Sterile swab sticks were used for the collection of the samples. The swab sticks were immersed in 0.85% sterile normal saline solution and each door handle was swabbed immediately with a single sterile swab stick, and replaced into its cover immediately. The samples were analyzed at the microbiology laboratory of Nasarawa State University, Keffi.

2.3 Isolation of *Salmonella typhi*

Standard microbiological procedures were used for the isolation of *S. typhi* [15].

2.4 Antibiotics Susceptibility Test

The antibiotics were tested against the isolates using standard procedures [16].

2.5 Determination of Minimum Inhibitory Concentrations

The MICs of the antibiotics used was studied using standard agar dilution method following the procedures described in the Clinical Laboratory Standard Institute Manual [16].

2.6 Detection of β-lactamase Enzymes Producing *Salmonella typhi*

Iodometric method [17] and acidometric method [18] were used in the detection of β-lactamase producing species of *S. typhi* from selected multiple antibiotics resistant isolates using standard procedures as described by Samant and PAI [19].

2.7 Conjugation Experiment

The transfer of resistance traits by the resistant isolates of *S. typhi* to ciprofloxacin sensitive *P. mirabilis* was investigated using the methods described by Onaolapo and Klemperer [20] with some modifications.

2.8 Curing Transconjugants

The curing of transconjugants *P. mirabilis* (Rf plasmid) was carried out by treating the *P. mirabilis* transconjugants with acridine orange dye as described by Onaolapo and Klemperer [20].

2.9 Plasmid DNA Analysis of Isolates

2.9.1 Preparation and purification of total DNA using spin-column protocol

The plasmid DNA of transconjugant strains and resistant isolates were isolated following the methods of Bimboim and Doly [21] and Vogelstein and Gillespie [22].

2.9.2 Detection of number and sizes of plasmid DNA (Agarose gel electrophoresis)

Plasmid DNA was detected following the methods of Yah et al. [23].

2.10 Statistical Analysis

Microsoft excel^{TM} 2010 and Smith's Statistical Package (SSP) version 2.8 for analysis were used for computational statistics.

3. RESULTS

Out of 200 samples analyzed, 36(18.00%) *S. typhi* was isolated with 17 of the isolates obtained from Nasarawa state university, Keffi 19 isolated from Federal polytechnic, Nasarawa (Table 1). The distribution of the bacterial isolate from different locations within the two studied institutions (i.e. Nasarawa state university, Keffi and Federal polytechnic, Nasarawa) is represented in Fig. 1 and Fig. 2 respectively. School of general studies (SGS) and school of engineering technology (SET) both in federal polytechnic, Nasarawa accounted for the highest and the lowest prevalence of *S. typhi* with 30% and 0% distribution respectively.

The antibiotics susceptibility test result of the isolated bacteria is shown in Table 2. Table 3 shows the antibiotics susceptibility test result of the bacterial isolates from the two institutions studied. The Multiple Antibiotics Resistance Index (MARI) of the *S. typhi* isolates is shown Table 4.

The antibiotics susceptibility profile of the *S. typhi* isolates based on MIC and peak plasma level is shown in Table 5 and the resistance profile of the bacterial isolates from the two institutions studied based on MIC and peak plasma level is shown in Table 6.

Table 1. Isolation rate of *Salmonella typhi* from door handles of Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

Institution	No. of samples	No. of isolates (%)
NSUK	100	17(17.00)
FPN	100	19(19.00)
TOTAL	200	36(18.00)

$\chi^2 = 0.4512$; P = 0.5017

No statistical difference exist between the values from the two studied institutions, as the p-value is greater than 0.05

Key: NSUK – Nasarawa State University, Keffi; FPN – Federal Polytechnic, Nasarawa

Fig. 1. Distribution of *Salmonella typhi* in door handles from different locations in Nasarawa State University, Keffi

Key: FNAS – Faculty of Natural and Applied Sciences; FARTS – Faculty of Arts; FADM – Faculty of Administration; FSOC – Faculty of Social Sciences; FLAW – Faculty of Law

χ^2 = 1.4648

P = 0.8328

No statistical difference exist between the values from the five study locations, as the p-value is all greater than 0.05

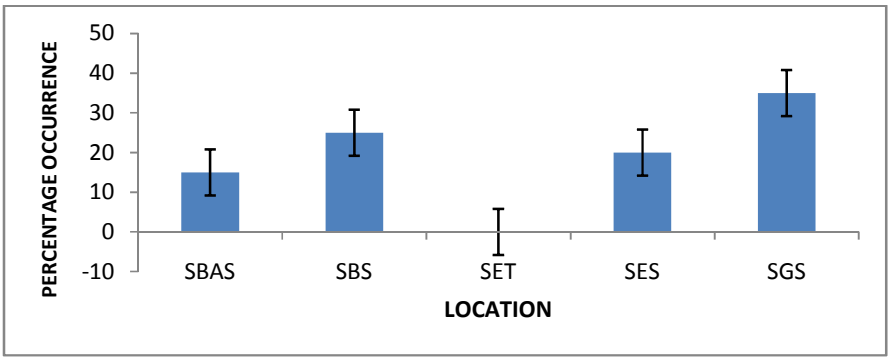

Fig. 2. Distribution of *Salmonella typhi* in door handles from different locations in Federal Polytechnic, Nasarawa

Key: SBAS – School of Basic and Applied Sciences; SBS – School of Business Studies; SET – School of Engineering Technology; SES – School of Environmental Studies; SGS – School of General Studies

χ^2 = 0.5873

P = 0.9645

No statistical difference exist between the values from the five study locations, as the p-value is all greater than 0.05

Table 2. Antibiotic susceptibility results of *Salmonella typhi* Isolates from door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

Antibiotics	Susceptibility (%) (n=36)
Ceftazidime*	0(0.00)
Cefuroxime*	0(0.00)
Gentamicin*	2(5.56)
Ciprofloxacin*	0(0.00)
Ofloxacin*	0(0.0)
Augmentin*	1(2.78)
Nitrofurantoin*	3(8.33)
Ampicillin*	0(0.00)
Sulphamethoxazole/Trimethoprim**	0(0.00)
Imipenem**	0(0.00)

**Antibiotic manufactured by Rapid Lab ltd Basingstoke, UK.*

***Antibiotic manufactured by Oxoid ltd England*

Table 3. Susceptibility profile of *Salmonella typhi* from door handles in the two different institutions studied

Antibiotics	No. susceptible (%)	
	NSUK (n=17)	FPN (n=19)
Ceftazidime*	0(0.00)	0(0.00)
Cefuroxime*	0(0.00)	0(0.00)
Gentamicin*	2(11.76)	0(0.00)
Ciprofloxacin*	0(0.00)	0(0.00)
Ofloxacin*	0(0.00)	0(0.00)
Augmentin*	0(0.00)	1(5.26)
Nitrofurantoin*	1(5.88)	1(5.26)
Ampicillin*	0(0.00)	0(0.00)
Sulphamethoxazole/Trimethoprim**	0(0.00)	0(0.00)
Imipenem**	0(0.00)	0(0.00)

**Antibiotic manufactured by Rapid Lab ltd Basingstoke, UK.*
***Antibiotic manufactured by Oxoid ltd England*
Key: NSUK – Nasarawa State University, Keffi; FPN – Federal Polytechnic, Nasarawa
P = 0.5863
Variation in susceptibility between the two institutions is statistically insignificant since P value is greater than 0.05

Table 4. MAR index of *Salmonella typhi* from door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

MAR index	No. of isolates	Percentage
0.10	6	16.67
0.20	1	2.78
1.00	29	80.56

Table 5. Antibiotics susceptibility profiles of selected isolates based on their M.I.C and peak plasma levels

Antibiotics	Peak plasma level (µg/ml)	No. of isolates	No. (%) resistant
Ceftazidime*	4.0	29	9(31.03)
Cefuroxime*	27.0	29	15(51.72)
Ciprofloxacin*	4.4	29	0(0.00)
Nitrofurantoin*	64.0	29	27(93.10)
Ampicillin*	8.0	29	24(82.76)
Imipenem**	14.0	29	0(0.00)

**Antibiotic manufactured by Rapid Lab ltd Basingstoke, UK.*
***Antibiotic manufactured by Oxoid ltd England*

Table 6. Resistance profile of selected isolates from the two institutions studied based on MIC and peak plasma levels

Antibiotics	Peak plasma level (µg/mL)	No. (%) resistant	
		NSUK (n=13)	FPN (n=16)
Ceftazidime*	4.0	5(38.46)	4(25.00)
Cefuroxime*	27.0	7(53.85)	8(50.00)
Ciprofloxacin*	4.4	0(0.00)	0(0.00)
Nitrofurantoin*	64.0	13(100.00)	14(87.50)
Ampicillin*	8.0	10(76.92)	14(87.50)
Imipenem**	14.0	0(0.00)	0(0.00)

**Antibiotic manufactured by Rapid Lab ltd Basingstoke, UK.*
***Antibiotic manufactured by Oxoid ltd England*
Key: NSUK – Nasarawa State University, Keffi; FPN – Federal Polytechnic, Nasarawa

β-lactamase production experiment was conducted on 29 multiple antibiotics resistant isolates and the result shows that 25 isolates were positive while 4 were non β-lactamase producers (Table 7).

Bacterial conjugation experiment was conducted on 9 multiple antibiotics resistant *S. typhi* isolates and 7 demonstrated the presence of plasmid by transferring same onto *Proteus mirabilis* which was initially susceptible to ciprofloxacin prior to the experiment but showed significant increase in MIC to ciprofloxacin after receiving resistance plasmid from *S. typhi* (Table 8). Acridine orange dye was used to cure the transconjugants and their MICs against ciprofloxacin decreased significantly (Table 9).

Plate 1 shows the plasmid profile of multiple antibiotics resistant *S. typhi* isolates and that of recipient *P. mirabilis* before conjugation while Plate 2 shows the plasmid profiles of the transconjugant *P. mirabilis*.

4. DISCUSSION

Microbiological assessment of door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa showed 36(18.00%) isolation frequency of *S. typhi*. The number of *S. typhi* isolated in this study is similar with other studies such as [24-31,34].

Different microorganisms from the enterobacteriaceae group such as *Enterobacter* spp, *Salmonella* spp, *Proteus* spp, *Klebsilla* spp, *Citrobacter* spp, *Yersinia* spp and *Providencia* spp have been isolated from door handle and other surfaces [32,33] and the presence of *S. typhi* isolated in door handles studied indicates possible faecal contamination [26,34].

Table 7. Beta lactamase enzymes production in selected *Salmonella typhi* isolates

Isolates	Acidometric	Iodometric
FNAS04	+	+
FNAS08	+	+
FNAS14	+	+
FNAS15	+	+
FARTS02	+	+
FARTS16	+	+
FADM04	+	+
FADM16	-	-
FADM17	+	+
FSOC07	-	-
FLAW12	+	+
FLAW17	+	+
FLAW18	+	+
FPN02	+	+
FPN03	+	+
FPN04	+	+
FPN05	+	+
FPN23	-	-
FPN40	-	-
FPN42	+	+
FPN50	+	+
FPN51	+	+
FPN60	+	+
FPN64	+	+
FPN71	+	+
FPN72	+	+
FPN81	+	+
FPN91	+	+
FPN92	+	+

Table 8. Minimum inhibitory concentration (M.I.C) of ciprofloxacin before and after conjugation

Isolates	MIC test antibiotic against donor resistant *S. typhi* (µg/mL)	Recipient characteristics	MIC of test antibiotics against Transconjugants cells (µg/mL)
FNAS14	2.0	+	4.0
FNAS15	0.25	+	4.0
FARTS02	0.063	-	-
FLAW18	4.0	+	4.0
FPN03	0.25	+	2.0
FPN51	0.25	+	1.0
FPN71	1.00	+	1.0
FPN72	1.00	-	-
FPN81	4.0	+	8.0

**MIC of Ciprofloxacin on recipient Proteus mirabilis before conjugation = 0.125 µg/mL*

+ Represents isolate showing pink on MacConkey agar plates.

- Represent isolates that did not transfer resistant traits

Table 9. Minimum inhibitory concentrations (M.I.C.) of ciprofloxacin on Transconjugants

Donor bacteria isolates code	MIC of test antibiotics before Transconjugants cured (µg/mL)	MIC of test antibiotics after Transconjugants *P. mirabilis* were cured (µg/mL)
FNAS14	4.0	2.0
FNAS15	4.0	1.0
FLAW18	4.0	4.0
FPN03	2.0	0.5
FPN51	1.0	0.5
FPN71	1.0	2.0
FPN81	8.0	4.0

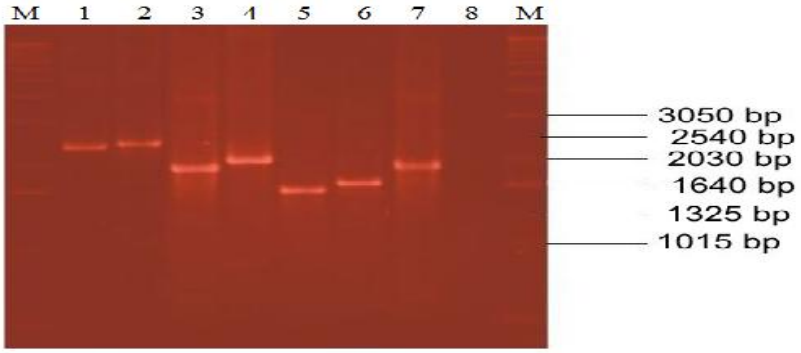

Plate 1. Plasmid profile of multiple antibiotic resistant *Salmonella typhi* Isolates and the recipient *Proteus mirabilis* before conjugation

Lane M: Supercoil Ladder (Marker) composed of DNA fragments (in base pairs).
Lane 1 to 7: Resistant Salmonella typhi (Lab nos FNAS14, FNAS15, FLAW18, FPN03, FPN51, FPN71 and FPN81 respectively).
Lane 8: Recipient Proteus mirabilis before conjugation

The presence of *S. typhi* on door handles is an indication of poor hygiene practices among students and staff of the institutions such as, not washing and cleaning hands with disinfectant after using toilets [35]. Hand washing has been traditionally first line defense in preventing diseases [36]. *S. typhi* is the etiologic agent of typhoid fever.

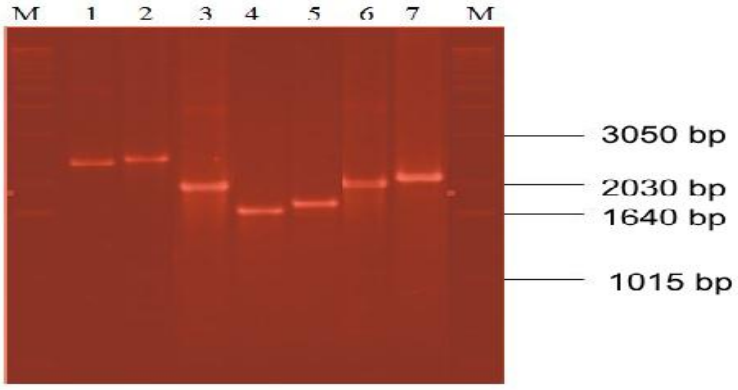

Plate 2. Plasmid profile of Transconjugant *Proteus mirabilis*

Lane M: Supercoil Plus Ladder (Marker) composed of DNA fragments (in base pairs).
Lane 1 to 7: Transconjugant recipients of; FNAS14, FNAS15, FLAW18, FPN42, FPN51, FPN71 and FPN81 respectively

Result of antibiotic susceptibility tests on isolates shows 100% resistance to ceftazidime, cefuroxime, ciprofloxacin, ofloxacin, ampicillin, cotrimoxazole and imipenem. Najwa et al. [25] observed 100% to ampicillin and 50% resistance to ciprofloxacin in *S. typhi* isolates. Also, Adabara et al. [26] observed 100% resistance by *S. typhi* towards cefuroxime, ampicillin, augmentin and ciprofloxacin. This increasing resistance to antibiotics by *S. typhi* suggests that the isolates in this study may probably have originated from an environment where antibiotics are often used indiscriminately [37]. 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 [38-40].

There was a relative susceptibility to gentamicin 2(5.56%), nitrofurantoin 3(8.33%) and augmentin 1(2.78%), this could be as a result of accessibility to the antibiotics and the parenteral routes of administering them.

The multiple antibiotic resistance indices (MARI) give an indirect suggestion of the probable sources of an organism. According to previous researchers, Krumperman [38] and Paul et al. [37], MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used. The overall *S. typhi* isolates from the present study exhibited MAR indices ranging from 0.1 to 1.0 with 80.56% of the isolates having the MAR index of 1.0. This is similar to findings by Thong et al. [41]. It is however higher than the results from the findings of Yoke-Kqueen et al. [42] which show MAR indices ranging from 0.2 to 0.8.

The emergence of multiple antibiotics resistant *S. typhi* isolates in door handles is alarming since it has obvious implications in public health, and multiple resistant limits the possible effectiveness of therapeutic treatments [41].

Beta-lactamase production investigation revealed that 25 out of the 29 resistant *S. typhi* isolates from door handles tested for ß-lactamase enzyme in this study produced beta-lactamase enzyme capable of hydrolysing beta lactam antibiotics. This result is comparable to the reports of Gokul et al. [43-45]. This observation confirmed the high beta-lactam antibiotics resistance that was observed against ampicillin, augmentin, ceftazidime and cefuroxime.

Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance. Resistance genes are often located on extra-chromosomal genetic elements or in segments inserted within the chromosome that originates from other genomes [23,46]. The acquisition of a new gene may occur by genetic transformation or through mobilization by conjugative transfer. The latter may occur at high frequency and efficiency, and several resistance genes can be acquired simultaneously [46].

This study observed the transfer of resistance plasmids in 7 isolates out of 9 multiple antibiotics resistant *S. typhi* isolates. The result of antibiotics susceptibility of the transconjugants using M.I.C method was seen to have increased relatively after conjugation. Changes were observed in the sensitivity pattern of tested transconjugants after curing with acridine dye. Decrease in minimum inhibitory concentration of transconjugants after curing as compared to those before curing revealed that acridine dye was effective curing agent. However, conjugation analysis revealed that apart from plasmids that were transferable by conjugation, other resistance determinants were transferable to sensitive recipient strain of *P. mirabilis* since their M.I.Cs increased. This suggests that these resistance determinants were carried extra-chromosomally on R-plasmids [47]. Indiscriminate use of antibiotic agents and antibiotic sale behaviour (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) has been reported to enhance the development of antibiotic resistance among pathogenic bacteria [48]. In developed countries, the main reservoir for antibiotic resistance in enteric bacteria has been attributed to farm animals such as cattle, sheep, pigs and poultry [49,50]. Contact with these animals or consumption of food products from them such as milk has been the main route of dissemination of resistance into the human populations.

Investigation of the plasmid profiles showed the presence of plasmid of various sizes among the multiple antibiotics resistant isolates ranging from 1600 – 2500 base pairs. The corresponding transconjugants contained plasmids of similar sizes.

According to some researchers such as Carattoli [46] and Yah et al. [23], antibiotic resistance in some bacterial isolates which seem not to possess plasmids was associated with

chromosome and/or transposons. In determining whether the plasmids resistance markers could be transferred to sensitive isolates, the results showed that all the transconjugants expressed plasmid DNA that migrated approximately on agarose gels. Considering the fact that all the *S. typhi* isolates examined in this study had MAR Index of 1.0, meaning, it therefore can be concluded that there is a relationship between possession of plasmid and resistance to antibiotics. Multiple resistance genes clusters in large plasmids are usually associated with transposons and insertion sequences [51]. Plasmid profiles revealed that bacterial isolates with the same resistance profile may differ in their plasmid profiles. This suggests diversity in plasmid contents of bacterial isolates and the contribution of different plasmids in the resistance to a certain antibiotic. 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 [23,46,52,53].

5. CONCLUSION

Findings from this research showed that up to 18% of door handles in the studied area were contaminated with *Salmonella typhi*. Antibiotics susceptibility testing shows that the isolates were highly resistant to the antibiotics tested, being 100% resistant to 7 out of the 10 antibiotics tested. Also, twelve of the isolates are shown to have transferred resistance factors (plasmid) to non-resistant bacteria (*Proteus mirabilis*) through conjugation experiment and the resistant plasmids were visualized using agarose gel electrophoresis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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