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PHENOTYPIC DETECTION OF PLASMID FROM CIPROFLOXACIN RESISTANT *Salmonella typhi* ISOLATED FROM STOOL OF NSUK STUDENTS

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ABSTRACT
Salmonella typhi (*S. typhi*) is a common cause of morbidity and mortality in developing countries. Studies on phenotypic detection of plasmid from ciprofloxacin resistant *S. typhi* isolated from stool of new students Nasarawa State University, Keffi, was carried out. A total of 180 stool samples were collected from the new students. 30 stool samples were collected from each of the six respective Faculties. 29(16.1%) *Salmonella* isolates were recovered and identified by standard Microbiological methods. 21(72.4%) of the isolates were *Salmonella typhi* and 8(27.6%) were *Salmonella enteritidis*. The prevalence of *Salmonella typhi* isolation with respect to Faculties was highest in the Faculties of Art (ART) and Law (LAW) with 6(28.6%) isolates each. On the basis of gender, 15 samples each were collected from both male and female students respectively and the highest prevalence of isolation was 4(8.0%) amongst males in the Faculty of LAW and females in the Faculty of ART, while the least prevalence of isolation was zero (0) amongst males in the Faculty of NAT. On the bases of age, the prevalence of isolation was highest amongst the age group 21-30, 19(10.5%), while age group 51-60 showed zero (0) prevalence of isolation. Antimicrobial Susceptibility was carried out using CLSI method; and 21(72.4%) *Salmonella typhi* isolates were susceptible to Ciprofloxacin, while 8(27.6%) were resistant and the antimicrobial resistance phenotypic pattern of the isolates was also determined. The ciprofloxacin resistance isolates were subjected to "Curing Method" to determine if the resistance was plasmid-mediated or not.

Keywords: *Salmonella typhi*, ciprofloxacin, plasmid, detection, stool

INTRODUCTION
The genus *Salmonella* belongs to the family Enterobacteriaceae whose members are Gram-negative, rod shape, non spore-forming, facultative anaerobes (Ryan *et al.*, 2017). *Salmonella serovars* can be divided into two main groups typhoidal and nontyphoidal *Salmonella* (Ferrari *et al.*, 2019). *Salmonella* can survive well in a variety of foods, which serves as vehicles for it transmission (Fockeret *al.*, 2022), as many infections of *Salmonella typhi* and *Salmonella paratyphi* A, are due to consumption of contaminated food (Ryan *et al.*, 2017), as they have no animal reservoir (Newell *et al.*, 2010).
The antigenic classification of *Salmonella* is based on a number of antigens namely Somatic (O), Flagella (H), and Surface (K) antigens. These antigens are proteins that are heat and alcohol stable (Kauffmann *et al.*, 1966), and are satisfactory for establishing the presence and estimating the prevalence of the infection within a population (Fockeret *al.*, 2022).
Mechanisms of infection of typhoidal and nontyphoidal *Salmonella serovars* differs, due to the difference in their targets site in the body and the different in their symptoms of infection (Ohad *et al.*, 2014). Both groups first entered by crossing the anatomical barrier created by the intestinal cell wall, but once they have passed this barrier, they use different strategies to cause disease.
Typhoidal *Salmonellae* are able to break the intestinal barrier through phagocytosis and trafficking by CD18-positive immune cells, as their key mechanism of infection (Ohad *et al.*, 2014). *Salmonella* cells enter macrophages through macropinocytosis (Kerr *et al.*, 2010). The success of *Salmonella* in causing infection is attributed to two type III secretion systems which play functional role at different times during an infection. One is required for the invasion of nonphagocytic cells, colonization of the intestine, and induction of intestinal inflammatory responses and diarrhea (Jiang *et al.*, 2021). While the other is essential for survival in macrophages and start up of systemic disease (Ohad *et al.*, 2014). These systems bring many genes to work co-operatively to cause infection.
Most cases of invasive nontyphoidal *Salmonella* infection (iNTS) are caused by *Salmonella* serotypes which are strictly adapted to humans or higher primates which include *Salmonella typhi*, *paratyphi* A, *paratyphi* B and *paratyphi* C which are the causes of typhoid fever (Ohad *et al.*, 2014). In the systemic form of the disease, *Salmonellae* pass via the lymphatic system of the intestine to the blood of the patients (typhoid form) and are carried to different organs (liver, spleen, kidneys) to form secondary foci (septic form) (Ohad *et al.*, 2014).
The predominant diagnostic procedure to detect *Salmonella* infections for long time and is still

considered the "gold standard" for *Salmonella* diagnosis is the Microbiological culture techniques (McFadden *et al.*, 2016).

It is recommended that treatment of typhoid begins on the basis of clinical findings, then to definitive diagnosis. Sadly in endermic regions, equipment for definitive diagnosis may be entirely lacking. Supportive measures such as antipyretics, appropriate nutrition, blood transfusion (McFadden *et al.*, 2016), intravenous rehydration therapy discovery in 1960s provided a simple way to prevent many of the deaths of diarrheal disease in general. The introduction of Chloramphenicol in 1948 declined the mortality rate of typhoid from 20 to 1% (McFadden *et al.*, 2016). However, Chloramphenicol does not prevent relapse of *Salmonella* infection unless given for 2-3 weeks, the carrier state is not eradicated nor was it effective against MDR strains (Michael *et al.*, 2021). The duration of treatment has been for 10-14 days, but uncomplicated typhoid fever has shorter courses of treatment (2-3 days) with flouroquinolones (Michael *et al.*, 2021).

The dissemination of resistance genes between members of various bacterial species under natural conditions needed a horizontal transmission by mobilization, conjugation, and transduction. For efficient horizontal gene transfer, two key factors are of importance: (1) a sufficiently high bacterial density, which enables close contact of the partners between which genes are exchanged, and (2) the location of the transferred genes on mobile genetic elements. Such elements include plasmids, transposons, integrons and gene cassettes, and chromosomal genomic islands (Schwarz *et al.*, 2006). The aim of this study was to determine the presence of plasmid (*gyrA*) genes in Ciprofloxacin resistant *Salmonella typhi* isolated from stool of NSUK students.

MATERIALS AND METHODS

Methods

Study area

The study was carried out in Nasarawa State University Keffi, Nigeria. Keffi is located at 8.85º and 7.87º longitudes and is situated at elevation of 321 meters above sea level. Keffi is about 58 km drive from Abuja the Nigerian capital city and 126 km from Lafia the Nasarawa State capital.

Sterilization

Petri dishes, conical flask, and other glass wares were sterilized by autoclaving (Moist heat sterilization at 121º C for 15 min) and by hot air oven (Dry heat sterilization).

Media preparation

Media used are *Salmonella-Shigella* Agar, Bismuth Sulfite Agar, Triple Sugar Agar, Nutrient Broth, and Muller Hinton Agar. These media were prepared according to the manufacturer's specification. Appropriate gram of the powdered agar was weighed and dissolved in appropriate amount of distilled water, followed by shacking and/or heat to boil to dissolve completely (Cheesbrough, 2006). The dissolved agar was sterilized at 121^{o}C for 15 min.

Ethical clearance

Ethical clearance for this work was obtained from Microbiology Department and the school clinic (South Atlantic Medical Centre) in Nasarawa State University Keffi.

Sample collection

A total of 180 stool samples were collected amongst the new students, at the University Clinic (South Atlantic Medical Centre). Thirty (30) stool samples were collected from each of the six (6) Faculties of Nasarawa State University. The stool samples were collected aseptically using sterilized broom stick into sterile, grease free stool container and then transported to the laboratory for the bacteriological examination.

Isolation of *Salmonella typhi*

Samples were collected into clean universal sample bottles and immediately transported to the Microbiology Department laboratory for bacteriological analysis. Stool samples were first enriched in Selenite Fluid broth at 37^{o}C and cultured by streaking on Salmonella-Shigella agar (SSA) plates using an inoculating wire loop. The plates were then incubated aerobically at 37°C overnight. Colonies with a presumptive *Salmonella* morphology on SSA agar were identified by biochemical tests which include; Gram's stain, catalase, oxidase, motility, Triple Sugar Iron agar (TSI), indole, methyl red, Voges-Proskauer, and citrate utilization test were carried to confirm the isolates as *Salmonella* species as described by Cheesbrough (2006).

Biochemical identification of *Salmonella typhi*

Gram-staining

Gram staining of the presumptive *Salmonella* was carried out as described by CLSI (2006). Briefly, a smear of 3 pure colonies of *Salmonella* were made on a drop of normal saline on a clean grease free slide and allowed to air dry. The slide was passed twice over the flame to fix and flooded with crystal violet solution for 30 sec, and rinsed under slow running tap water and flooded with lugols iodine for 30 sec and rinsed under slow tap running water and briefly decolorized with acetone and immediately rinsed under slow running water and counter stained with safranin solution for 60 sec. Examined microscopically using ×100 oil immersion objective.

Indole test

The indole test was carried out as follows: Three (3) pure colonies of *Salmonella* were inoculated into 5 ml of peptone water in bijou Bottles and incubated at 37^{o}C for 24 h. Few drops of Kovac's reagent was added to the 24 h culture of the suspected *Salmonella*, Formation of red ring at the top indicates an indole positive reaction as described by Cheesbrough (2006).

Methyl red test/Voges-Proskeur test

Methyl red/Voges-Proskeur test for the suspected organism was carried out as follows: Three (3) pure colonies of the suspected organism was inoculated into 10 ml of MR/VP medium in Bijou bottles and incubated at 37^{o}C for 72 h. The 72 h culture was

divided into two (2) portions. The first portion, some few drops of methyl red indicator was added and formation of red color indicated Methyl red positive reaction. To the second portion, Ten drops of 10% potassium hydroxide was added, followed by some drops of beta-naphthol. Formation of pinkish red color indicated Voges-Proskeur positive reaction as described by Cheesbrough (2006).

Citrate test

The Citrate test was carried out as follows: Three (3) pure colonies of suspected organism was picked using sterile straight wire and stabbed on Simmon's citrate agar slant and incubated at 37^{o}C for 72 h. Formation of green color indicated citrate positive reaction as described by Cheesbrough (2006).

Antimicrobial susceptibility testing

Antimicrobial Susceptibility test was carried out by the Kirby-Bauer's disc diffusion method on Mueller-Hinton agar (MHA) using commercially available antimicrobial discs (oxoid). Standard suspensions of the isolates was adjusted to 0.5 McFarland Standard, briefly as per CLSI guidelines (2006), 0.5 ml of bacteria suspensions was suspended in 0.95 ml of normal saline to prepare 100 ml McFarland Standard. Immediately after standardization, a sterile cotton swab was immersed into bacterial suspension and a lawn culture was made on the surface of MHA plate. Commercially available antibiotic disc were placed on the surface of the inoculated plates and allowed to stand for pre-diffusion time. The plates were incubated at 37^{o}C overnight. The antibiotics were selected as per CLSI guidelines (CLSI, 2006). After the incubation the zone diameter was measured for each antibiotic and it was compared with the CLSI chart. Thereby the zones of inhibition were interpreted as Susceptible (S), Intermediate (I) and Resistance (R), respectively.

Plasmid curing method

The curing method of testing the presence of plasmid DNA is as briefly described: Add 5µL of Acredine Orange to Luria Bertani (LB) broth, antibiotic resistance *Salmonella* isolates was inoculated into the LB broth containing the Acredine Orange and was incubated at 37^{o}C for 24 h in a shaker incubator. After incubation, this culture was swabbed on Muller-Hinton agar (MHA) plates and Ciprofloxacin Disc was placed on the plates and was incubated at 37^{o}C for 24 h and all the ciprofloxacin resistant *Salmonella* isolates that were resistant became susceptible after the treatment.

RESULTS AND DISCUSSION

The Biochemical Reactions of the suspected isolates from the newly admitted students 2016/2017 session

The biochemical characteristics of the suspected *Salmonella* isolates from stools of newly admitted students 2016/2017 session are as shown in Table 1. From the Table, $S_{63,}$ S_{63} … S_{199} are samples identification number/code, while the negative (-) and positive (+) signs are indicators of negative and positive reactions of the isolates to the various test they were respectively subjected to.

Table 1: Showing Biochemical reactions of the *Salmonella* isolated from newly admitted students 2016/2017 session

S/N	Samples	TSI									
		V.p	Ox	Cit	Mot	Ind	Slop	Butt	H_2S	Gas	inference
1	S_{62}	-	-	-	+	-	**R**	**Y**	+	-	*S. typhi*
2	S_{63}	-	-	-	+	-	**R**	**Y**	-	**+.**	*S. paratyphi A*
3	S_{69}	-	-	-	+	-	**R**	**Y**	-	**d**	Others

VP= Voges-Proskeur; OX= Oxidase; CIT= Citrate; MOT= Motility; IND= I ndole.

Table 2: Showing the cultural, gram reaction and morphological characteristics of *Salmonella* isolated in newly admitted students 2016/2017 session

S/N	Parameters	Characteristics
1	Culture	Colonies are colorless, slightly opaque, dome shaped with central Black spot. Facultative Anaerobes, Temperature of 37^{o}C, pH 7.0
2	Gram Reaction	Gram Negative rod shaped bacillus
3	Morphology	Rod shaped Bacillus

Isolation and identification of *Salmonella*

The cultural, Gram and Morphological characteristics of the *Salmonella* isolated from the newly admitted students 2016/2017 session are as shown in Table 2. This Table shows that *Salmonella* are Gram negative, rod shaped, facultative anaerobes that grows at the optimum temperature 37^{o}C and pH of 7.0

Prevalence of isolation *Salmonella* with respect to faculties of the newly admitted students 2016/2017 session

Out of the one hundred and eighty (180) stool samples of the newly admitted students 2016/2017 session obtained, with respect to their faculties is as shown in Table 3. The faculties are ADM, ART, EDU, LAW, NAT and SOS respectively, with a total number of 30

samples collected from each faculty. The highest prevalence of isolation was recorded in the faculty of ART and LAW with a total of 6 (6.2%) isolates, followed by EDU and SOS each with prevalence of isolation of 5 (29.8%) isolates, while ADM and NAT recorded prevalence of 4 (4.1%) and 3 (3.1%), respectively.

Table 3: Showing the prevalence of *Salmonella* isolation with respect to Faculties of newly admitted students 2016/2017 session

S/N	Faculty	No of samples collected	No (%) of *Salmonella*
1	ADM	30 (16.67%)	4 (4.1%)
2	ART	30 (16.67%)	6 (6.2%)
3	EDU	30 (16.67%)	5 (5.2%)
4	LAW	30 (16.67%)	6 (6.2%)
5	NAT	30 (16.67%)	3 (3.1%)
6	SOS	30 (16.67%)	5 (5.2%)

ADM= Faculty of Administration; ART= Faculty of Art; EDU= Faculty of Education; NAT= Faculty of Natural and Applied sciences; SOS= Faculty of Social sciences.

Table 4: Showing the prevalence of *Salmonella* isolation with respect to gender of newly admitted students 2016/2017 session

S/N	Faculty	No of samples observed Male/female	No of isolates males	No of isolates females
1	ADM	15:15	2 (4%)	2 (4%)
2	ART	15:15	2 (4%)	4 (8%)
3	EDU	15:15	2 (4%)	3 (6%)
4	LAW	15:15	4 (8%)	2 (4%)
5	NAT	15:15	3 (6%)	-
6	SOS	15:15	1 (2%)	4 (8%)

Prevalence of isolation of *Salmonella* with respect to sex of the newly admitted students 2016/2017 session

Out of the 30 samples collected from the various faculties, Fifteen (15) was collected from both sex Males and Females of the newly admitted students 2016/2017 session as shown in Table 4. The highest prevalence of isolation of *Salmonella* was 4(8.0%) which was recorded amongst females in the faculty of ART and males in the faculty of LAW respectively. Males in the faculty of NAT and EDU recorded a prevalence of 3(6%) respectively; males in faculties of ADM, ART, EDU and females in the faculty of ADM had a prevalence of 2 (4%) while males in SOS faculty had prevalence of 1 (2%), respectively. The least prevalence of isolation of *Salmonella* with respect to sex was recorded in males in the faculty of NAT at zero 0(0%).

Prevalence of isolation of *Salmonella* with respect to age of the newly admitted students 2016/2017 session

Out of the 29 positive *Salmonella* isolates that was isolated from a total of 180 stool samples of the newly admitted students 2016/2017 session, with respect to their ages are as shown in Table 5. The ages 21-30 shows the highest prevalence of isolation of *Salmonella* with 19 isolates out of the 29 isolates as such: ADM 1(7.3%), ART 3(14.5%), EDU 3(17.5%) and LAW 4(19.3%) respectively. Followed by ages 15- 20 and 31-40 which recorded prevalence of isolation with 8 isolates each as such: ADM 2 (14.5%), ART 2 (10.0%), NAT 1 (9.6%), SOS 3 (17.5%) and ADM 1 (7.3%), EDU 2 (11.6%), LAW 2 (9.6%), NAT 2 (19.2%), SOS 1 (5.8%), respectively. While ages of 41-50 with total of 2 isolates out of 29 isolates has the least prevalence of isolation as such: ART 1 (4.8%), SOS 1 (5.8%), ages of 51- 60 had zero (0) prevalence of isolation.

Table 5: Showing the prevalence of *Salmonella* isolation with respect to age of newly admitted students 2016/2017 session

S/N	AGE	ADM	ART	EDU	LAW	NAT	SOS
1	11-20	2(14.5%)	2(10%)	-	-	1(9.6%)	3(17.4%)
2	21-30	1(7.3%)	3(14.5%)	3(17.4%)	4(19.3%)	-	-
3	31-40	1(7.3%)	-	2(11.6%)	2(9.6%)	2(19.2%)	1(5.8%)
4	41-50	-	1(4.8%)	-	-	-	1(5.8%)
5	51-60	-	-	-	-	-	-

Table 6: Showing the prevalence of *Salmonella* isolation with respect to stool texture of newly admitted students 2016/2017 session

S/N	Faculty	No of isolates	Formed stool	Semi-formed stool	Unformed stool
1	ADM	4	-	1 (7.25%)	3 (21.8%)
2	ART	6	1 (4.8%)	1 (4.8%)	4 (19.3%)
3	EDU	5	1 (5.8%)	-	4 (23.2%)
4	LAW	6	-	3 (14.5%)	3 (14.5%)
5	NAT	3	-	2 (19.3%)	1 (9.6%)
6	SOS	5	-	1 (5.8%)	4 (23.2%)

Prevalence of isolation of *Salmonella* with respect to stool forms of the newly admitted students 2016/2017 session

The prevalence of isolation of *Salmonella* in stool of newly admitted student 2016/2017 session with respect to stool texture is shown in Table 6. Faculties of ADM, ART, EDU, LAW, NAT and SOS shows 4, 6, 5, 6, 3 and 5 prevalence of isolation, respectively. The highest prevalence of isolation of *Salmonella* was recorded in the unformed stool with ADM 3 (21.8%) out of 4, ART 4 (19.3) out of 6, EDU 4 (23.2%) out of 5 and SOS 4 (23.2%) out of 5 respectively, making a total of (15) isolates. Least prevalence of isolation of *Salmonella* in unformed stools was recorded in NAT 1 (9.6%). Semi-formed is next with prevalence of isolation rate of ADM 1 (7.25%) out of 4, ART 1 (4.8%) out of 6, LAW 3 (14.5%) out of 6, NAT 2 (19.3%) out of 3 and SOS 1 (5.8%) out of 5 respectively, making a total of (8) isolates. Formed stools show least prevalence rate of ART 1 (4.8%) out of 6 and EDU 1 (5.8%) out of 5 isolates, respectively.

Antibiotics susceptibility

The Antibiotic Susceptibility test of the *Salmonella* isolated from newly admitted students 2016/2017 session are as shown in Table 7. The *Salmonella* isolates are more susceptible to Ciprofloxacin 21 (72.4%) and Streptomycin 18 (62.1%), Ofloxacin and Pefloxacin 17 (58.6) each but less susceptible to Amoxicilin/Clavulanate 12 (41.4%), and Norvofloxacin 10 (34.5%), respectively.

Table 7: Antibiotics susceptibility pattern of *Salmonella* isolated from newly student 2016/2017 session

Antibiotics	Disc content (µg)	No. (%) Susceptibility (n= 29)
Nalidicic Acid (NAL)	30	13 (44.3)
Ciprofloxacin (CIP)	5	21 (72.4)
Ofloxacin (OFX)	5	17 (58.6)
Pefloxacin (PEF)	5	17 (58.6)
Norvofloxacin (NOR)	10	10 (34.5)
Gentamycin (CN)	10	12 (41.4)
Streptomycin (S)	30	18 (62.1)
Ampicillin (AMP)	30	15 (51.7)
Amoxycillin/Clavulanate (AMC)	30	12 (41.4)

Table 8: Showing the number and percentages of *Salmonella* isolates in plasmid curing method

No. (%) of isolates	No. (%) of Resistant Isolates	No. (%) of Cured Isolates
29 (100%)	8 (27.5%)	8 (27.5%)

Plasmid curing

The *Salmonella* isolates that show resistance to ciprofloxacin from the stool of the newly admitted students 2016/2017 session were subjected to Curing method using Acredine Orange, as shown in Table 8. Out of the 29 *Salmonella* isolates screened for antibiotic susceptibility pattern, 8 isolates were resistant to ciprofloxacin and was subjected to curing treatment and were all cured 8 (27.5%).

Antibiotic resistance phenotypic pattern of ciprofloxacin-resistant *Salmonella* isolates

Ciprofloxacin resistance *Salmonella* isolated from stool of newly admitted students 2016/2017 session, exhibited different antibiotic resistant phenotypes as shown in Table 9. The commonest Antibiotics resistance phenotypes are NAL, PEF, NOR, CN, AMC; NOR, CN, AMP; NOR, CN, AMP, AMC; NAL, PEF, NOR, CN, S, AMP, AMC which expresses 2 (6.8%) each respectively.

Table 9: Antibiotics Resistance phenotypes of *Salmonella* isolated from newly admitted Students 2016/2017 session

Antibiotics resistance phenotypes	No. (%) *Salmonella* isolates (n=29)
NAL, CIP, AMC	1 (3.4)
NAL, CIP, AMP, AMC	1 (3.4)
CIP, PEF, CN, S, AMP	1 (3.4)
NAL, CIP, OFX, CN, AMC	1 (3.4)
NAL, CIP, OFX, PEF, CN, S	1 (3.4)
NAL, CIP, OFX, S, AMP, AMC	1 (3.4)
CIP, OFX, PEF, NOR, S, AMP, AMC	1 (3.4)

The study investigates phenotypically, the presence of Plasmid in Ciprofloxacin resistant *Salmonella typhi* from stool of new students of Nasarawa State University Keffi (NSUK) 2016/2017 session. It was observed that the detection rate of *Salmonella typhi*in the stool of the new University students was 29(16.1%) and is in agreement with report described by Scherer and Miller (Scherer & Miller, 2001). Up to 10% of untreated convalescent typhoid cases will excrete *Salmonella typhi* in feces for 1 to 3 months and between 1 and 4% become chronic carriers excreting the microorganism for more than one year.

The prevalence of *Salmonella typhi*infection in the new students of the University, with respect to Faculties, shows that out of the 30 samples from each Faculty, that makes up a total of 180 collected, prevalence rate was highest 6 (6.2%) in the Faculties of Art and Law. Meanwhile, prevalence rate of 4 (8%) for both male and females was shown in eachof the Faculties of Law and Art respectively. This showed that there is no significantdifference in the prevalence of *Salmonella typhi*infection with respect to gender. ($p < 0.05$)

Findings in the present study indicated that the prevalence of isolation of *Salmonella yphi* on the bases of age was highest amongst the age group 21-30 with 4 (19.3%) in the Faculty of Law and this result is in agreement with the work earlier reported by Ishaleku (2011) in "Evaluation of the prevalence of *S. typhi* amongst subject attending college of education Health Clinic, Akwanga, Nasarawa State'' from the year 2005 to 2007, who also reported high prevalence rate in age group 21-30 (35.75%).

It was also observed that *Salmonella typhi* isolated from stool of the University students, were susceptible to Ciprofloxacin, Ofloxacin, Pefloxacin and Streptomycin. The highsusceptibility of *Salmonella typhi* to antibiotics mentioned was expected and this may be due to their cost effect and therefore not commonly prescribed and they are not likely tobe abused and this is in agreement with the report by Ngwai *et al.* (2012) that antibiotics that are costly are not likely to be abused. The susceptibility of *Salmonella typhi* isolates to Streptomycin observed in this study is not in agreement with study earlier reported by Ishaleku (2011) in "evaluation of the prevalence of *S. typhi* amongst subject attending college of education Health Clinic, Akwanga, Nasarawa State'' from the year 2005 to 2007. The low level of susceptibility of *Salmonella typhi* isolates to Ampicillin, Amoxycillin/Clavulanate, Norvofloxacin and Nalididcic acid may be due to misuse, abuse and use of substandard antibiotics and the low level of susceptibility of the antibiotics mention above is in agreement with study earlier described by Adabara *et al.* (2012). However, the susceptibility of *Salmonella typhi* to Ciprofloxacin observed in this study disagrees with the work Adabara *et al.* (2012) who reported resistance significant level of resistance to Ciprofloxacin. The occurrence of the Ciprofloxacin resistance *gyrA*genes in *Salmonella typhi* isolated from stool of new students was not surprising and this finding is also in agreement with the reports of Edwards *et al.* (2012), Feasey *et al.* (2012). The rate of occurrence of Ciprofloxacin resistant *Salmonella typhi* isolates in this study was 8 (27.5%) and this might be due to the presence of the Plasmid resistant (*gyrA*) genes that were harbored by these particular *Salmonella typhi* isolates. This study also observed that all the Ciprofloxacin resistance *Salmonella typhi* isolates were Multiple Antibiotic Resistance (MAR), and this finding is not agreement with the work earlier described by

Sohana *et al.* (2023) who reveals highest 85% Multiple Antibiotic Resistance among Nalidixic resistance isolates, in The prevalence of Multi-Drug Resistance *Salmonella typhi* isolated from blood sample. Though the mechanism of resistance to *Salmonella* isolates that are MAR to Ampicillin, Amoxycillin/Clavulanate, Norvofloxacin and Nalididcic acid Lorvofloxacin may not be due to the presence of the plasmid genes but due to other mechanism of antibiotic resistance, such as efflux pump resistance and alteration of drug target site and this is in agreement with the work earlier reported by Friedman *et al.* (2016).

CONCLUSION

*Salmonella typhi*were isolated from stool of the University students and the isolates with resistance to ciprofloxacin were detected but after been subjected to treatment with a curing agent (Acredine Orange), they became ciprofloxacin susceptible. Therefore, this study reveals that the presence of plasmid (*gyrA*) resistance gene might be responsible for the resistance to ciprofloxacin recorded in this study but await confirmation through gene expression assay of all the *gyrA*genes from the isolate.

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