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Antimicrobial susceptibility and plasmid profiles of *Salmonella* Typhi in Sagamu, southwest Nigeria

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Abstract:

Background: *Salmonella enterica* serovar Typhi is the causative agent of typhoid fever, a major public health concern in Nigeria and other low-resource settings. The emergence of antimicrobial resistance (AMR), often plasmid-mediated, poses a serious challenge to treatment. Local surveillance data on resistance and plasmid carriage are critical for guiding effective interventions. This study investigated the antimicrobial susceptibility and plasmid profiles of clinical *Salmonella* Typhi isolates from stool samples in Sagamu, southwest Nigeria.
Methodology: A cross-sectional study of 100 patients with clinical features of typhoid fever and reactive for *S.* Typhi antibody by the Widal agglutination test, was conducted across health facilities in Sagamu, including Olabisi Onabanjo University Teaching Hospital (OOUTH), private hospitals, and selected diagnostic laboratories. Stool samples were collected from each participant and cultured on selenite F broth enrichment, followed by subculture on MacConkey and *Salmonella-Shigella* agar plates. *Salmonella* Typhi was identified by colony morphology, Gram stain reaction, biochemical test scheme of triple sugar iron, citrate, and urease tests, and confirmed by serotyping with agglutination of sample using specific antisera. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Plasmid DNA was extracted by the alkaline lysis method, and electrophoresis was used for plasmid size determination. Mating experiments with *Escherichia coli* K-12 were conducted to assess plasmid transferability. Statistical analysis of data was done using SPSS (version 25), with significance set at $p<0.05$.
Results: Sixty-six isolates were confirmed as *S.* Typhi. Of these, 28.8% (n=19) were multidrug-resistant (MDR), although all isolates were susceptible to ciprofloxacin and ofloxacin. Plasmids with sizes ranging from 4.6 to 5.7kb were detected in 16 of the 19 MDR isolates, 9 (56.3%) of which successfully transferred their plasmids to *E. coli* K-12. A significant association was found between plasmid carriage and MDR (OR=447.86, $p<0.0001$).
Conclusion: The findings of this study suggest that plasmid-mediated resistance plays a major role in the emergence of MDR *S.* Typhi strains in the region, posing a challenge to the effective treatment of typhoid fever. Continuous surveillance and prudent use of antibiotics are necessary to combat the rising threat of AMR in *S.* Typhi population.

Keywords: Antimicrobial resistance; plasmid; mating; transfer; *Salmonella* Typhi

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Sensibilité aux antimicrobiens et profils plasmidiques de *Salmonella* Typhi à Sagamu, sud-ouest du Nigéria

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Résumé:

Contexte: *Salmonella enterica* sérotype Typhi est l'agent responsable de la fièvre typhoïde, un problème de santé publique majeur au Nigéria et dans d'autres pays à faibles ressources. L'émergence de la résistance aux antimicrobiens (RAM), souvent d'origine plasmidique, pose un sérieux défi thérapeutique. Français Les données de surveillance locales sur la résistance et le portage plasmidique sont essentielles pour guider des

interventions efficaces. Cette étude a examiné la sensibilité aux antimicrobiens et les profils plasmidiques d'isolats cliniques de *Salmonella* Typhi à partir d'échantillons de selles à Sagamu, dans le sud-ouest du Nigéria.
Méthodologie: Une étude transversale de 100 patients présentant des caractéristiques cliniques de fièvre typhoïde et réactifs aux anticorps *S.* Typhi par le test d'agglutination de Widal, a été menée dans des établissements de santé à Sagamu, y compris l'hôpital universitaire Olabisi Onabanjo (OOUTH), des hôpitaux privés et des laboratoires de diagnostic sélectionnés. Des échantillons de selles ont été prélevés chez chaque participant et cultivés sur un bouillon d'enrichissement au sélénite F, suivis d'une sous-culture sur des plaques de gélose MacConkey et *Salmonella-Shigella*. *Salmonella* Typhi a été identifiée par morphologie des colonies, réaction de coloration de Gram, schéma de test biochimique des tests de fer triple sucre, citrate et uréase, et confirmée par sérotypage avec agglutination de l'échantillon à l'aide d'antisérums spécifiques. Les tests de sensibilité aux antimicrobiens ont été réalisés à l'aide de la méthode de diffusion sur disque de Kirby-Bauer. Français L'ADN plasmidique a été extrait par la méthode de lyse alcaline et l'électrophorèse a été utilisée pour déterminer la taille du plasmide. Des expériences d'accouplement avec *Escherichia coli* K-12 ont été menées pour évaluer la transférabilité du plasmide. L'analyse statistique des données a été effectuée à l'aide de SPSS (version 25), avec une signification fixée à $p<0,05$.
Résultats: Soixante-six isolats ont été confirmés comme étant *S.* Typhi. Parmi ceux-ci, 28,8% (n=19) étaient multirésistants aux antibiotiques (MDR), bien que tous les isolats soient sensibles à la ciprofloxacine et à l'ofloxacine. Des plasmides d'une taille comprise entre 4,6 et 5,7kb ont été détectés dans 16 des 19 isolats MDR, dont 9 (56,3%) ont transféré avec succès leurs plasmides à *E. coli* K-12. Une association significative a été trouvée entre le portage du plasmide et la MDR (OR=447,86, $p<0,0001$).
Conclusion: Les résultats de cette étude suggèrent que la résistance induite par les plasmides joue un rôle majeur dans l'émergence de souches multirésistantes de *S.* Typhi dans la région, ce qui complique le traitement efficace de la fièvre typhoïde. Une surveillance continue et une utilisation prudente des antibiotiques sont nécessaires pour lutter contre la menace croissante de la RAM dans la population de *S*. Typhi.

Mots-clés: Résistance aux antimicrobiens; plasmide; croisement; transfert; *Salmonella* Typhi

Introduction:

Typhoid fever, caused by the bacterium *Salmonella* Typhi, is a significant public health concern in developing countries, including Nigeria (1). The disease is predominantly transmitted through contaminated food and water, and its prevalence is heightened by poor sanitation and inadequate access to clean water (2). Despite advancements in public health, typhoid fever continues to pose a substantial burden, particularly in regions with limited healthcare resources (1).

The clinical management of typhoid fever has been increasingly complicated by the emergence of multidrug-resistant (MDR) *S.* Typhi strains (3). Resistance to first-line antibiotics such as ampicillin, chloramphenicol, and cotrimoxazole has been widely reported, prompting reliance on fluoroquinolones and third-generation cephalosporins (4). However, the growing resistance to these agents indicates the need for continuous surveillance and a deeper understanding of the underlying mechanisms of resistance.

Plasmids are extrachromosomal DNA elements that play important role in the acquisition and dissemination of antimicrobial resistance (AMR) genes among bacterial populations (5). The presence of transmissible resistance plasmids (R-plasmids) in *S.* Typhi has been identified as a major driver of MDR, posing a significant threat to effective treatment strategies (6).

This study aims to explore the antimicrobial susceptibility patterns and plasmid profiles of *S.* Typhi isolates obtained from various health establishments in Sagamu, southwest Nigeria. By highlighting the resistance patterns and the role of plasmids, the find - ings of this study will contribute to the growing body of knowledge on typhoid fever and its management in endemic regions.

Materials and method:

Study design and sample collection:

This was a descriptive cross-sectional study of 100 patients with clinical features of typhoid fever and reactive for *S.* Typhi antibody by the Widal agglutination test conducted in Sagamu, southwest Nigeria between August and November 2022. Stool samples were collected from each participant recruited from Olabisi Onabanjo University Teaching Hospital (OOUTH), selected private diagnostic laboratories (Synlab, Afriglobal and Dew laboratories) and selected private hospitals (Owokoniran Medical Hospital, Ayotola Specialist Hospital and Rene Medical Center). Informed consent was obtained from each participant prior to sample collection.

Culture isolation and identification of *Salmonella* Typhi:

The stool samples were processed using standard microbiological techniques with initial enrichment in Selenite F, followed by culture on selective media (MacConkey and *Salmonella-Shigella* agar plates), and incubating at 37°C for 24 hours. Presumptive colonies of *Salmonella* were identified by colonial morphology, Gram stain reaction, and biochemical test scheme of triple sugar iron (TSI) agar**,** citrate utilization, and urease tests. Serological confirmation of *S.* Typhi was performed using specific antisera, where agglutination of the sample with the antisera confirmed the presence of *S.* Typhi.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing of the serologically confirmed *S.* Typhi isolates (n=66) was done by the Kirby-Bauer disk diffusion method against 9 selected antibiotics in line with the guidelines of the Clinical and Laboratory Standards Institute (7). The antibiotics tested included ampicillin (10µg), chloramphenicol (30µg), gentamicin (10µg), tetracycline (30µg), cotrimoxazole (25µg), nalidixic acid (30µg), ciprofloxacin (5µg), ofloxacin (5µg) and ceftriaxone (30µg). Zones of inhibition were measured in mm and interpreted as sensitive or resistant in line with the CLSI guidelines (7).

Plasmid DNA extraction and profiling:

Plasmid DNA extraction was carried out using the alkaline lysis method. Briefly, overnight cultures of *S.* Typhi isolates grown in Luria-Bertani (LB) broth were harvested by centrifugation at 10,000 rpm for 10 minutes. The bacterial pellet was re-suspended in 200 µL of a solution containing 50 mM glucose, 25 mM Tris-HCl (pH 8.0), and 10 mM EDTA (Solution I).

The cells were lysed with 200 µL of a lysis buffer containing 0.2 M NaOH and 1% SDS (Solution II), mixed gently, and incubated on ice for 5 minutes. To neutralize the solution, 150 µL of a cold potassium acetate solution (Solution III: 3 M potassium acetate, pH 5.5) was added, followed by gentle mixing and incubation on ice for 10 minutes. The mixture was centrifuged at 13,000 rpm for 10 minutes to pellet cellular debris and chromosomal DNA.

The supernatant containing plasmid DNA was transferred to a fresh tube, and plasmids were precipitated by adding 0.6 vol of isopropanol. After centrifugation at 13,000 rpm for 10 mins, the DNA pellet was washed ed with 70% ethanol, air-dried, and re-suspended in 30µL nuclease-free water.

For profiling, the extracted plasmid DNA was resolved on a 0.8% agarose gel in 1X TAE buffer, stained with ethidium bromide (0.5 µg/mL), and visualized under UV light. A 1 kb DNA ladder and a lambda DNA/HindIII marker were used to estimate the sizes of the plasmids.

Plasmid transfer by mating:

Plasmid transfer experiment was conducted by mating *S.* Typhi isolates with *Escherichia coli* K-12 as the recipient strain. The potential donor strain, carrying the plasmid, was grown overnight in Luria-Bertani (LB) broth, while *E. coli* K-12 was prepared by streaking onto LB agar plates and incubating overnight.

Prior to mating, the *E. coli* K-12 recipient strain was confirmed to be susceptible to ampicillin and chloramphenicol by performing antimicrobial susceptibility testing. The donor and recipient strains were mixed in a 1:1 ratio and spotted onto LB agar plates. After overnight incubation at 37°C, the bacterial cells were scraped from the plate, suspended in saline, and plated onto MacConkey agar containing ampicillin and chloramphenicol to select for transconjugants.

Antimicrobial susceptibility testing of the transconjugants was performed using the disk diffusion method. Successful plasmid transfer was confirmed if the *E. coli* K-12 transconjugants were resistant to ampicillin and chloramphenicol used in the selection media.

Data analysis:

Data obtained from antimicrobial susceptibility testing and plasmid profiling were analyzed using descriptive statistics to summarize the resistance patterns and plasmid profiles of S. Typhi isolates. The distribution of antimicrobial resistance among the isolates was expressed as percentages, and the presence of multidrug-resistant (MDR) strains was noted.

Association between plasmid carriage and multidrug resistance was assessed using Chi-square test to determine statistical significance and p value was set at <0.05 for all analyses. Statistical analyses were conducted using SPSS version 25.0 (IBM Corp, USA).

Results:

A total of 66 confirmed *S.* Typhi were isolated from the stool samples of 100 participants by biochemical and serological identification tests (Table 1).

Table 1: Distribution of *Salmonella* Typhi isolated from the study sites

Institution	No of isolates	No identified as *S.* Typhi (%)
OOUTH, Sagamu	50	31 (62.0)
Private laboratories	40	29 (72.5)
Private hospitals	10	6 (60.0)

OOUTH: Olabisi Onabanjo University Teaching Hospital

All 66 isolates were susceptible to ofloxacin, ciprofloxacin and ceftriaxone, while 65 (98.5%) and 62 (93.9%) were susceptible to nalidixic acid and gentamicin respectively (Table 2). The susceptibility pattern to the commonly used first line antibiotics was variable; cotrimoxazole (54.5%), chloramphenicol (45.5%), ampicillin (34.8%) and tetracycline (27.2%). Only 17 (25.8%) isolates were fully susceptible to all the 9 antibiotics tested while 19 (28.8%) isolates were MDR (resistance to three or more antibiotic classes), and 49 (72.2%) were resistant to at

least one antibiotic.

Of the 19 MDR strains, 8 (42.1%) were resistant to chloramphenicol, ampicillin and tetracycline; 6 (31.6%) to ampicillin, chloramphenicol and cotrimoxazole, and 5 (26.3%) to chloramphenicol, ampicillin, tetracycline and cotrimoxazole (Table 2).

Table 2: Antimicrobial drug resistance patterns of 66 strains of *Salmonella* Typhi

Antibiotic resistant pattern	**Number of resistant *Salmonella* Typhi isolates (%)**
Single drug resistance (n=66)	
Ampicillin	43 (65.2)
Tetracycline	48 (72.7)
Cotrimoxazole	30 (45.5)
Chloramphenicol	36 (54.5)
Gentamicin	4 (6.0)
Nalidixic acid	1 (1.5)
Ciprofloxacin	0
Ofloxacin	0
Ceftriaxone	0
Multidrug resistance (MDR) pattern (n=19)	
Ampicillin, Chloramphenicol, Tetracycline	8 (42.1)
Ampicillin, Chloramphenicol, Cotrimoxazole	6 (31.6)
Ampicillin, Chloramphenicol, Cotrimoxazole	5 (26.3)

The electrophoretic profiles of the isolated plasmid DNA of the 66 *S.* Typhi strains is shown in Table 3. Of the 19 MDR isolates, 16 (84.2%) contained plasmids, with sizes of 4.6 kb (n=5 isolates), 4.9 kb (n=3 isolates), 5.1 kb (n=7 isolates), and 5.7 kb (n=1 isolate). Also, 9 (56.3%) of these successfully transferred their plasmids to *E. coli* K-12. In contrast, none of the MDR-negative isolates contained plasmids, as 47 isolates, including the 17 isolates that were sensitive to all 9 antibiotics, were plasmid-negative

Statistical analysis of the data showed a significant association between plasmid carriage and MDR (x^2=54.24, OR=447.86, 95% CI = 21.935-9144.2, p<0.00001), indicating that plasmid-borne resistance may play a significant role in the dissemination of MDR among *S.* Typhi isolates in the study setting.

Discussion:

This study assessed the antimicrobial susceptibility and plasmid profiles of *S.* Typhi isolates from human stool samples in Sagamu, southwest Nigeria. Our findings highlight a high prevalence of AMR, with a significant association between MDR and plasmid carriage among *S.* Typhi isolates. This research provides valuable insights into the evolving challenges in managing typhoid fever in the region and underscores the importance of continuous surveillance to monitor and combat the spread of resistant strains.

We observed high levels of resistance to commonly used antibiotics, particularly ampicillin, tetracycline, and chloramphenicol. This resistance pattern is consistent with previous reports from Nigeria and other sub-Saharan African countries, where *S.* Typhi has shown increasing resistance to first-line antibiotics. A study from Lagos, Nigeria by Akinyemi et al., (8) reported similar resistance rates to ampicillin and tetracycline respectively among *S.* Typhi isolates. Additionally, a study done in Ghana (9) found that antimicrobial resistance in *S.* Typhi isolates was rising, corroborating our findings. These results are concerning, as they suggest a shift towards the use of second-line drugs, which could have implications for treatment efficacy and healthcare costs in resource-limited settings.

Interestingly, our study found that all *S.* Typhi isolates were susceptible to ciprofloxacin and ofloxacin. This finding aligns with other studies that have reported the retention of susceptibility to fluoroquinolones despite the development of resistance to older antibiotics (8,10). However, it is worth noting that emergence of fluoroquinolone resistance in *S.* Typhi has been documented in other parts of the world. For instance, a study in India reported the emergence of ciprofloxacin-resistant *S.* Typhi, which highlights the

Table 3: Molecular size characteristics of *Salmonella* Typhi plasmid DNA

Plasmid presence/sizes	**Number of MDR isolates (%)**	**Number of non-MDR and sensitive isolates (%)**	**Total isolates (%)**
4.6kb	5	0	5
4.9kb	3	0	3
5.1kb	7	0	7
5.7kb	1	0	1
Plasmid negative	3	47	50
Total	**19 (28.8%)**	**47 (71.2%)**	**66 (100%)**

MDR – Multi-drug resistance; x^2=54.24, OR=447.86, 95% CI=21.935 - 9144.2, p<0.0001

potential for fluoroquinolone resistance to emerge, particularly as its use increase in the treatment of typhoid fever (11). Continuous monitoring of fluoroquinolone resistance is essential to prevent the emergence of resistance in the region.

Our findings also showed a concerning 28.8% prevalence of MDR *S.* Typhi strains. This rate is consistent with those reported in studies from other parts of Nigeria, where MDR *S.* Typhi has become a significant clinical challenge (3,12). MDR *S.* Typhi strains (resistant to three or more classes of antibiotics) pose a major challenge to the treatment regimens of typhoid fever and have been associated with increased morbidity and mortality, especially in regions with limited access to alternative antibiotics (3).

The molecular analysis revealed a strong association between plasmid carriage and MDR. In total, 16 out of 19 MDR isolates carried plasmids, with sizes ranging from 4.6 to 5.7 kilobases. This is in line with previous studies that have identified plasmids as key vectors for the transmission of resistance genes in *Salmonella* species. For example, a study by Owoseni and Onilude (13) demonstrated that MDR *S.* Typhi strains in West Africa were often associated with the presence of plasmids carrying resistance genes. The finding that no MDR-negative isolates carried plasmid elements supports the hypothesis that plasmid-mediated resistance plays a crucial role in the spread of MDR *S.* Typhi.

Plasmid transfer experiments using *E. coli* K-12 as the recipient strain confirmed the transferability of resistance genes. This result aligns with previous research, which showed that plasmids carrying AMR genes can be easily transferred between *Salmonella* strains and other enteric bacteria (14). The ability of *S.* Typhi to transfer resistance plasmids to other bacteria increases the potential for resistance to spread beyond a single pathogen species, exacerbating the overall problem of AMR in the region.

Statistical analysis confirmed a significant association between plasmid presence and MDR in *S.* Typhi isolates (OR=447.86, p <0.0001. This statistical significance supports the conclusion that plasmid-borne resistance is a critical factor in the development and spread of MDR strains in the study area. Previous studies have also found strong associations between plasmid presence and resistance profiles in *S.* Typhi, reinforcing the importance of plasmids in the dissemination of antimicrobial resistance (13,15).

Conclusion:

This study highlights the growing concern of antimicrobial resistance in *S.* Typhi isolates in Sagamu, southwest Nigeria, and emphasizes the role of plasmids in mediating multidrug resistance. A significant proportion of *S.* Typhi isolates were resistant to common antibiotics, with a notable prevalence of MDR strains. Plasmid profiling revealed that plasmid carriage is associated with the resistance phenotypes, particularly in the MDR isolates.

Furthermore, the transfer of resistant plasmids between *S.* Typhi and other enteric bacteria further complicates the management of typhoid fever in the region. These findings underscore the urgent need for enhanced surveillance, effective infection control strategies, and the prudent use of antibiotics to mitigate the spread of resistant *S.* Typhi strains. Additionally, the study advocates for the continuous monitoring of AMR patterns and the development of alternative therapeutic approaches to ensure the effective treatment of typhoid fever.

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Conflicts of interest:

No conflict of interest is declared.

Contribution of authors:

DTO conceived and designed the study, acquired, analyzed and interpreted the data. AOO acquired and interpreted data. Both authors drafted, revised, read and approved the manuscript.

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