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PLASMID PROFILING AND CURING OF TYPHOID (SALMONELLA *TYPHI*) AND NON-TYPHOID (SALMONELLA *ENTERITIDIS*) ISOLATES FROM ENUGU, NIGERIA

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Abstract

Medicine is currently grappling with the issue of antibiotic resistance in bacteria, which has arisen due to the rapid multiplication and widespread dissemination of these organisms, coupled with their ability to effortlessly acquire new genetic material. This work was carried out to profile and cure plasmid of typhoid and non-typhoid Salmonella using thirty-five (35) blood samples taken from students of Godfrey Okoye University. Several analyses such as widal test, morphological analysis, biochemical test, antibiotic sensitivity test, plasmid curing and plasmid profiling were performed. The results showed that among the fifteen isolates obtained that ten were identified as Salmonella typhi and five Samonella enteritidis. Notably, all isolates were resistant to sparfloxacin and cefotaxime, each demonstrating a 90% resistance rate with zones of inhibition less than 13 mm. In contrast, the isolates were susceptible to azithromycin (80%) and levofloxacin (70%), with zones of inhibition exceeding 25 mm. Plasmid curing was performed on eight bacterial isolates that exhibited resistance to more than four antibiotics, followed by a reassessment of their antibiotic sensitivity. Post-curing, these isolates remained highly resistant to sparfloxacin (75%), cefotaxime (62.5%), and augmentin (50%), each with zones of inhibition less than 14 mm. However, resistance to azithromycin and levofloxacin significantly decreased, with only 12.5% of the isolates remaining resistant, and zones of inhibition exceeding 30 mm for these antibiotics.

The curing of plasmids resulted in a shift in antibiotic susceptibility for some isolates, where previously resistant isolates became susceptible, while others maintained their resistance. This change underscores the role of plasmids in conferring antibiotic resistance among the bacterial isolates. Molecular analysis revealed that the majority of isolates carried plasmids with high molecular weights ranging from 3kbp to 10kbp, with S2, S3, S5, and S12 losing their 3kbp plasmids and S11, S14, and N8 retaining theirs.

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1. Introduction

Typhoidal Salmonella poses a severe and potentially life-threatening systemic infection, in contrast to non-typhoidal Salmonella, which typically results in self-limiting gastroenteritis in both humans and animals (Kuhn, *et al.,* 2012). Enteric fever, alternatively known as typhoid fever, is an ailment affecting humans and is instigated by two serovars of Salmonella: *Salmonella typhi* and Salmonella paratyphi A, B, or C (Feasey, *et al.,* 2010). Moreover, it's crucial to highlight that Salmonella infections can be transmitted via contaminated food and water sources, underscoring the significance of adhering to proper food hygiene practices to mitigate the spread of the disease.

In Nigeria, the persistent challenge of typhoid fever remains a substantial health concern. This issue is influenced by various factors, including the rapid increase in urbanization, inadequate access to safe drinking water, substantial migration of workers moving across regions, insufficient infrastructure for proper human waste disposal, strained healthcare delivery systems, and the excessive use of antibiotics. This misuse of antibiotics contributes to the emergence and spread of antibiotic-resistant *S. typhi* (Akinyemi, *et al.,* 2005). Adding to the issue is the lack of a properly organized epidemiological surveillance system, which hampers the accurate assessment of typhoid fever incidence in Nigeria.

Antimicrobial resistance stands out as a formidable obstacle in contemporary empirical treatment approaches for infectious diseases (Salinas, *et al.,* 2019), particularly in the context of addressing infections instigated by Gram-negative bacteria (Aworh, *et al.,* 2021). The examination of plasmids holds paramount significance in the field of Medical Biotechnology because these genetic elements can harbor genes responsible for antibiotic resistance or virulence factors (Bennett, 2008).

Moreover, plasmids play a crucial role as markers for diverse bacterial strains, utilizing a typing system known as plasmid profiling (Ekundayo, 2021). Eliminating or treating a concealed plasmid from a bacterial strain provides a method to validate the association between a genetic characteristic and its existence within that specific plasmid. Despite the persistence of multidrug resistance in bacteria, plasmids carrying these resistance genes can be effectively eliminated through a process known as curing. Within the food industry, plasmid curing emerges as a valuable technique employed to eradicate undesirable genes or traits from bacteria utilized in food fermentation processes, thereby ensuring the purity and safety of the final product. Diverse methods, encompassing both chemical and physical agents, have been devised for plasmid elimination. An example involves treating cell cultures with sodium dodecyl sulfate at a concentration that is insufficient to impede chromosome replication but adequate to hinder plasmid replication (Ekundayo, 2021).

As asserted by Tiwaskar (2019), antibiotic therapy remains the cornerstone for treating typhoid fever. However, the emergence of multidrug-resistant strains in endemic regions has posed a formidable challenge, complicating the treatment of this infectious disease. The absence of a well-coordinated epidemiological surveillance system further complicates the assessment of typhoid fever incidence in the country. The aim of this study is to conduct plasmid profiling and curing of typhoid and non-typhoid *Salmonella*.

2. MATERIALS AND METHODS

2.1. Samples collection, preparation of media and Widal test analysis

Thirty-five samples of blood were taken from students of Godfrey Okoye University using a sterile syringe and dispensed in a sterile container. The samples were labeled according to the time it was collected, and they were subsequently taken to the Godfrey Okoye University Microbiology lab for examination. Salmonella Shigella agar, Muller Hinton agar, Nutrient broth, Peptone water, Citrate agar, Urease agar and TSI (Triple Sugar Iron) agar were all prepared using manufacturers guidance.Widal test was performed with blood samples suspected of containing antibodies against *Salmonella typhi* or paratyphi collected into sterile test tubes and centrifuged to

separate the serum from the plasma. The serum, containing the antibodies were transferred to clean test tubes. Agglutination discs were used to deposit *Salmonella typhi* and paratyphi "O" and "H" antigens onto the discs. These discs were then mixed with the patient's serum and observed for agglutination. Out of 35 samples tested, 15 showed visible clumps of bacteria, indicating agglutination. A positive reaction, indicative of the presence of antibodies against *Salmonella typhi* or paratyphi, was observed in cases where agglutination occurred (Khoharo, *et al.,* 2010).

2.2. Isolation of bacteria and identification of isolates

The fifteen blood samples were cultured using Salmonella Shigella agar and were incubated for 24 hours in an incubator under aseptic measures. A stock solution was then made after the cultivated plates had been incubated for 24 hours at 37^{o}C and checked for bacterial growth (Okike and Ugwu, 2021). The isolates were identified by standard techniques using Faisal, *et al.,* 2017 method. The incubated plates were characterized by identifying the colonies on the media macroscopically via texture, color, shape, microscopically, gram stain reaction and biochemical test. Gram's technique was used to classify the organism into Gram negative rod-shaped. They were further subjected to specific biochemical tests such as citrate utilization, catalase test, indole production, urease production, oxidase test, voges-Proskauer test, hydrogen sulphide test and methyl-red test.

2.3. Antibiotic Sensitivity Test

In this laboratory procedure, an antibiotic sensitivity test was conducted using the Kirby Bauer disc diffusion technique with gram-negative antibiotics namely, Levofloxacin (20µg), Cefotaxim (10µg), Sparfloxacin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg), Augmentin (10µg), Gentamycin (30µg), Pefloxacin (30µg), Tarivid (10µg) and Azithromycin (12µg). Nutrient broth was prepared and inoculated with bacterial colonies, then incubated at 37°C for 24 hours. The turbidity was adjusted to match the 0.5 McFarland standard. Muller Hinton agar plates were prepared and inoculated with bacterial colonies, and sensitivity discs were placed on them. After 24 hours of incubation, zones of inhibition were observed in fifteen samples, and the diameter of each zone was measured. The sensitivity of the isolates was evaluated, comparing results to the Clinical Laboratory Standards Institute guidelines. Samples S2, S3, S5, S6, S11, S12, S14, and N8 showed resistance to more than four antibiotics, prompting selection for plasmid curing.

2.4. Plasmid profiling and curing

A quantity of 0.8g of agarose gel powder was precisely measured and diluted in 100 mL of 1xTAE buffer in a microwavable flask. The mixture was microwaved for 3 minutes until complete dissolution of the agarose was achieved, with careful attention to avoid over-boiling, as excessive evaporation of the buffer could alter the final agarose percentage in the gel. Subsequently, the agarose solution was allowed to cool down to approximately 50 °C, which is the temperature at which one can comfortably touch the flask. To enhance visualization, ten (10) µL of EZ vision DNA stain was then added to the agarose solution. EZ vision, having the ability to bind to DNA, facilitates the visualization of DNA under ultraviolet (UV) light. Following the addition of EZ vision DNA stain, the agarose solution was carefully poured into a gel tray, ensuring the well comb was properly positioned. The freshly poured gel was then subjected to two options for solidification: it was left at room temperature for 20-30 minutes (Abraham, *et al.,* 2019). In the plasmid curing process described, eight organisms showing resistance to more than four antibiotics underwent treatment with a sub-inhibitory concentration (10%) of sodium dodecyl sulphate (SDS), also known as sodium lauryl sulphate. Following a method by Ehiaghe et al. (2013), 4.5ml of nutrient broth was inoculated with an overnight broth culture, and 0.5ml of 10% SDS was added. The mixture was then incubated at 37°C for 48 hours, inducing stress on the bacterial cells to promote plasmid loss. After this initial incubation, a fresh batch of nutrient broth was prepared, and 0.5ml of the previously treated culture was

added. This mixture underwent an additional 24-hour incubation at 37°C. The resulting culture was then stored in a refrigerator until further use.

2.5. Loading Samples and Running an Agarose Gel

In this laboratory procedure, purified plasmid DNA samples were prepared for analysis. Loading dye was mixed with the extracted plasmid samples, and the mixture was loaded onto an agarose gel. The gel was carefully placed into an electrophoresis unit and covered with 1xTAE buffer to facilitate the electrophoresis process. A molecular weight ladder was loaded into the first lane of the gel to serve as a reference for estimating DNA fragment sizes. The purified plasmid DNA samples were loaded into the remaining wells of the gel. Electrophoresis was carried out at 100V for 80 minutes. After completion, power was turned off, and the electrodes were disconnected. The gel was then removed from the unit, and purified plasmid fragments were visualized under UV Trans-illumination to identify the bands harboring the plasmid (Ehiagh *et al.,* 2013).

2.6. Antibiotic Sensitivity test after plasmid Curing

Cured isolates were tested for antibiotic resistance to determine if the plasmid curing agent successfully removed the resident resistance plasmids. Bacterial colonies from the cured isolates were inoculated into nutrient broth and incubated at 37°C for 24 hours. The turbidity was adjusted to match the 0.5 McFarland standard. Muller Hinton agar plates were prepared, and bacterial colonies were streaked onto the plates. Gram-negative sensitivity discs were placed onto the streaked plates using forceps cleaned with alcohol. The plates were allowed to dry for five minutes to ensure proper absorption of the inoculum. After incubating the plates upside down at 37°C for 24 hours, the zones of inhibition around the antibiotic discs were measured using a metric ruler, accounting for the disc diameter. Results were reported as susceptible (S), intermediate (I), or resistant (R).

3. RESULTS

Thirty-five (35) blood samples were collected from students of Godfrey Okoye University located at Jideofor Street, Thinkers Corner Enugu State, in the southeastern region of Nigeria. Out of the thirty-five samples, fifteen samples were confirmed with the presence of the target organisms (Table 1). Ten (10) isolates were *Salmonella typhi* while the remaining five isolates were *Salmonella enteritidis*. *Salmonella typhi* were labelled as S1, S2, S3, S5, S6, S7, S11, S12, S14 and S15 while *Salmonella enteritidis* were labelled as N1, N2, N3, N8 and N10.

Antibiotic susceptibility profile of uncured plasmid bacterial isolates.

Table 3 shows the zones of inhibition measured in millimeters and compared to the Clinical Laboratory Standards Institute Chart. Salmonella typhi exhibited high resistance to sparfloxacin (90%), cefotaxime, amoxicillin, and augmentin (70%), with the lowest resistance observed against Levofloxacin and Azithromycin (20%). Salmonella enteritidis showed high resistance to cephalothin (100%) and sparfloxacin (80%), while amoxicillin, augmentin, and pefloxacin demonstrated zero resistance. The Multiple Antibiotic Resistance Index (MARI) was calculated, with isolate S11 having the highest index (1.0), followed by S6 and S12 (0.7 each). Isolates S15, N2, N3, and N10 had the lowest MARI (0.2 each). Antibiotic resistance percentages were also calculated, with isolate S11 having the highest (67%), and isolates S15, N2, N3, and N10 having the lowest (13% each).

The antibiotic susceptibility pattern of cured plasmid bacterial isolates.

Bacterial isolates with more than four resistances to certain antibiotics underwent plasmid curing and subsequent antibiotic sensitivity testing to confirm the removal of plasmids causing resistance (Table 4). After curing, the isolates exhibited high resistance to Sparfloxacin (75%), followed by cefotaxime (62.5%) and augmentin (50%), while the lowest resistance was observed against azithromycin and levofloxacin (12.5% each). The Multiple Antibiotic Resistance (MAR) index was calculated, with isolate S11 having the highest index (0.9), followed by S14 (0.7) and N8 (0.5), and the lowest indices observed in isolates S2 and S6 (0.1 each). Isolate S11 also showed

the highest antibiotic resistance percentage (113%), while isolates S2 and S6 exhibited the lowest resistance percentages (13% each).

Plasmid resistance genes

The results revealed that not all isolates showed elimination of plasmid resistance genes (Table 5). Specifically, sample S2 lost resistance to four antibiotics after curing, S3 lost resistance to three antibiotics, S5 lost resistance to three antibiotics, S6 lost resistance to six antibiotics, S11 remained unchanged with no lost resistance, S12 lost resistance to five antibiotics, S14 gained resistance to three antibiotics, and N8 lost resistance to only one antibiotic.

Plasmid profile of cured and uncured isolates using gel electrophoresis.

Initially, all isolates exhibited plasmids of high molecular weight ranging from 3kbp to 10kbp (Plate 1 and 2). After plasmid curing, isolates S2, S3, S5, S6, and S12 lost their 3kbp plasmid, which initially measured 13kbp, while retaining the 10kbp plasmid (Table 5). On the other hand, isolates S11, S14, and N8 retained plasmids ranging from 3kbp to 10kbp without losing any plasmid.

Table 4.1: Morphological Characterization of Bacterial Isolates

Key

S – *Salmonella typhi*

N – *Salmonmella enteritidis*

Table 4.2: Antibiotic Susceptibility Pattern of Uncured Plasmid Bacterial Isolates

Bacterial Isolates	Antibiotic Agents (µg)														
	LEV	CF	SP	CPX	AM	AU	CN	PEF	OFX	AZ	CH	SXT	S	MAR Index	%
S1	S	S	R	S	R	R	S	S	S	S	-	-	-	0.3	20
S2	S	R	R	I	S	I	R	I	R	R	-	-	-	0.5	33
S3	R	R	R	R	I	S	S	S	R	S	-	-	-	0.5	33
S5	S	R	R	R	R	R	R	S	S	S	-	-	-	0.6	40
S6	S	R	R	R	R	R	R	R	S	S	-	-	-	0.7	47
S7	S	S	S	S	R	R	R	S	S	S	-	-	-	0.3	20
S11	R	R	R	R	R	R	R	R	R	R	-	-	-	1	67
S12	S	R	R	R	R	R	R	R	S	S	-	-	-	0.7	47
S14	S	R	R	R	R	R	S	S	R	S	-	-	-	0.6	40
S15	-	-	R	S	S	S	S	S	S	-	R	S	S	0.2	13
N1	-	-	R	I	S	S	S	S	S	-	R	S	R	0.3	20
N2	-	-	R	S	S	S	S	S	S	-	R	S	S	0.2	13
N3	-	-	R	S	S	S	S	S	S	-	R	S	S	0.2	13
N8	-	-	R	R	S	I	R	S	R	-	R	I	R	0.6	40
N10	-	-	S	S	S	S	S	S	R		R	S	S	0.2	13

Key

R – Resistant, S – Susceptible, I – Intermediate, LEV – Levofloxacin, CF – Cefotaxim, SP – Sparfloxacin, CPX – Ciprofloxacin, AM – Amoxacillin, AU – Augmentin, CN – Gentamycin, PEF – Pefloxacin, OFX – Tarivid, AZ

– Azithromycin, CH – Cephalothin, SXT – Sulfamethoxazole, S – Streptomycin, MAR- Multiple antibiotic resistance.

Table 4.3: Antibiotic Susceptibility Profile of Cured Plasmid Bacterial Isolates

Bacterial Isolates	Shape	Texture	Colour	Microscopy	Characterization	Probable organism
S1	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S2	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S3	Circular	Smooth	Colourless with black center	Pink-rod shape	Gram-negative	*Salmonella typhi*
S5	Circular	Smooth	Colourless with black center	Pink-rod shape	Gram-negative	*Salmonella typhi*
S6	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S7	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S11	Circular	Smooth	Colourless with black center	Pink-rod shape	Gram-negative	*Salmonella typhi*
S12	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S14	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
S15	Circular	Smooth	Colourless with black center	Red-rod shape	Gram-negative	*Salmonella typhi*
N1	Circular	Smooth	Pink	Red-rod shape	Gram-negative	*Salmonella enteritidis*
N2	Circular	Smooth	Pink	Red-rod shape	Gram-negative	*Salmonella enteritidis*
N3	Circular	Smooth	Pink	Red-rod shape	Gram-negative	*Salmonella enteritidis*
N8	Circular	Smooth	Pink	Red-rod shape	Gram-negative	*Salmonella enteritidis*
N10	Circular	Smooth	Pink	Red-rod shape	Gram-negative	*Salmonella enteritidis*

Bacterial Isolates	Antibiotic Agents (µg)														
	LEV	CF	SP	CPX	AM	AU	CN	PEF	OFX	AZ	CH	SXT	S	MAR Index	%
S2	S	S	R	S	S	I	S	S	S	S	-	-	-	0.1	13
S3	S	R	S	R	S	S	S	S	I	S	-	-	-	0.2	25
S5	S	R	R	I	R	S	S	S	S	S	-	-	-	0.3	38
S6	S	S	S	S	I	R	I	S	S	S	-	-	-	0.1	13
S11	R	R	R	S	R	R	R	R	R	R	-	-	-	0.9	113
S12	S	R	R	I	S	I	S	S	S	S	-	-	-	0.2	25
S14	S	R	R	I	R	R	R	R	R	S	-	-	-	0.7	88
N8	-	-	R	R	S	S	R	S	I	-	R	S	R	0.5	63

Key : R – Resistant,S –Susceptible, I - Intermediate, LEV – Levofloxacin, CF – Cefotaxim, SP – Sparfloxacin, CPX – Ciprofloxacin, AM – Amoxacillin, AU – Augmentin, CN – Gentamycin, PEF – Pefloxacin, OFX – Tarivid, AZ – Azithromycin, CH – Cephalothin, SXT – Sulfamethoxazole, S – Streptomycin, MAR- Multiple antibiotic resistance.

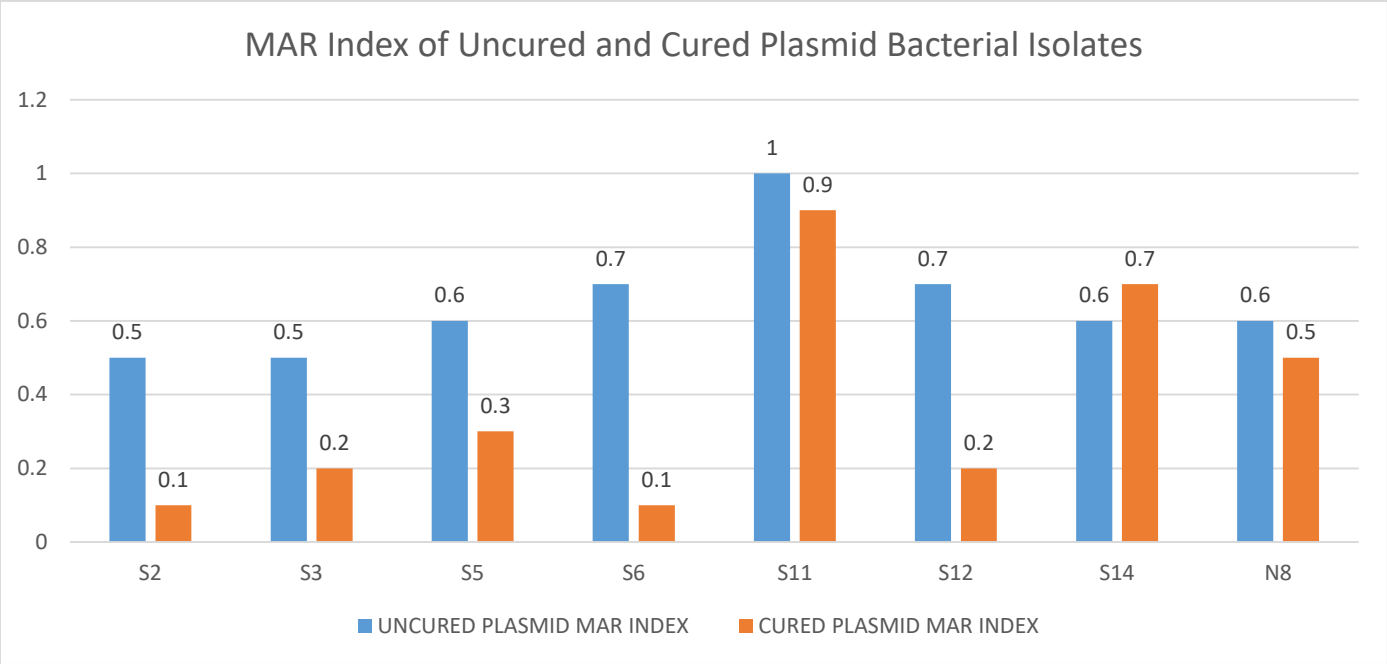


Fig 1: Comparison between MAR Index of Uncured and Cured Plasmid Bacterial Isolates

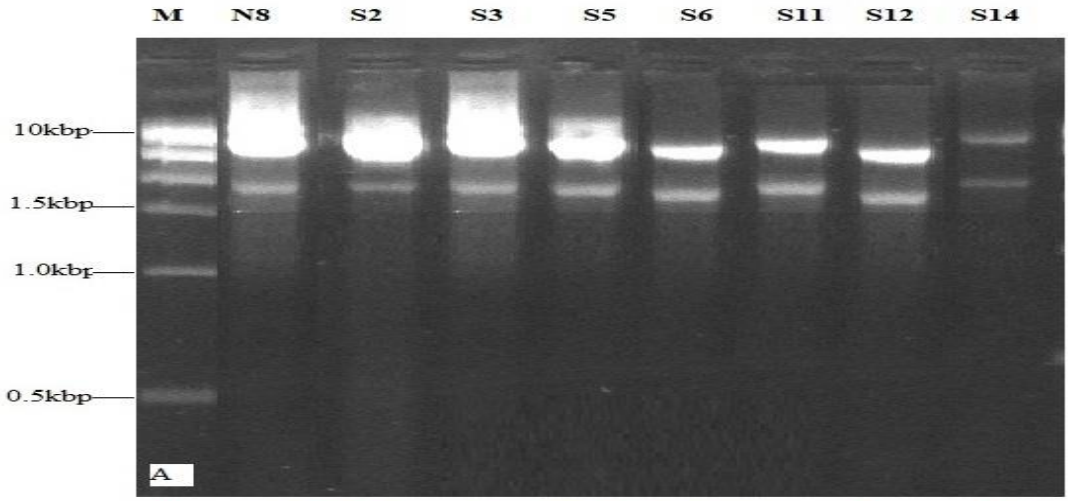
Table 4.4: Resistance Profile of Bacterial Isolates of Cured and Uncured Plasmid.

Bacterial Isolates	Resistant Profile Before Curing	Resistant Profile After Curing	Probable Organism
S2	5	1	*Salmonella typhi*
S3	5	2	*Salmonella typhi*
S5	6	3	*Salmonella typhi*
S6	7	1	*Salmonella typhi*
S11	10	10	*Salmonella typhi*
S12	7	2	*Salmonella typhi*
S14	6	9	*Salmonella typhi*
N8	6	5	*Salmonella enteritidis*

Table 4.5: Gel Electrophoresis Plasmid Profile of Plasmid-cured and uncured Isolates

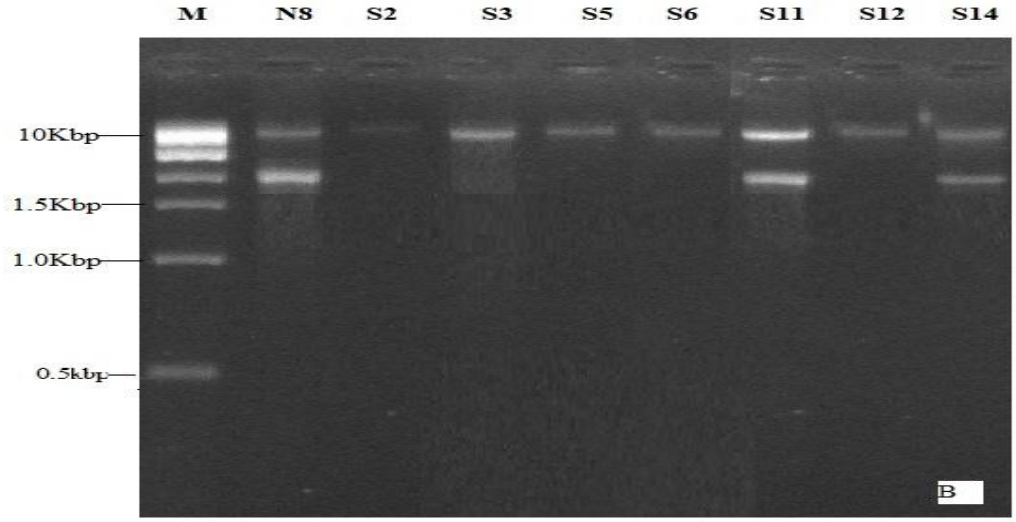
Lane in gel	Molecular Weight (Kbp) of Uncured Plasmid	Molecular Weight (Kbp) of Cured Plasmid	Probable Organism	Sample
S2	3 – 10	10	*Salmonella typhi*	Blood
S3	3 – 10	10	*Salmonella typhi*	Blood

S5	3 – 10	10	*Salmonella typhi*	Blood
S6	3 – 10	10	*Salmonella typhi*	Blood
S11	3 – 10	3 – 10	*Salmonella typhi*	Blood
S12	3 – 10	10	*Salmonella typhi*	Blood
S14	3 – 10	3 – 10	*Salmonella typhi*	Blood
N8	3 - 10	3 – 10	*Salmonella enteritidis*	Blood



Plasmid profiling showing plasmids of high molecular weight between 3Kbp to 10Kbp. M is a 1Kbp DNA ladder

Plate 1: Plasmid profile of plasmid-uncured isolates by gel electrophoresis



Gel image showing cured plasmids of 3Kbp for isolate S2, S3, S5 S12 following curing using 10% SDS. M is a 1Kbp DNA ladder.

Plate 2: Plasmid profile of plasmid cured isolates by gel electrophoresis

4. DISCUSSIONS

The study findings suggest that the resistance exhibited by the bacterial isolates is not solely plasmid-mediated, indicating that some of the bacterial isolates' resistance mechanisms are chromosomally mediated. The efficacy of the plasmid curing process was assessed by subjecting the cured isolates, cultivated in nutrient broth, to antibiotic resistance testing. This crucial step aimed to determine whether the plasmid curing agent successfully eliminated the resident resistance plasmids initially present in the antibiotic-resistant isolates. Following the plasmid curing process, Sparfloxacin emerged as the antibiotic with the highest resistance rate at 75%. This indicates that even after plasmid elimination, a considerable proportion of bacterial isolates maintained resistance to Sparfloxacin. This was in line with Ehiagh *et al.,* 2013 stating that *pseudomonas aeruginosa* was resistant to sparfloxacin in their research. Cefotaxim exhibited the second-highest resistance rate at 62.5%, suggesting a moderate success in reducing resistance through plasmid curing. This finding is against Kuehn, *et al.,* 2012 who reported that cefotaxime was susceptible to certain bacteria isolates and can be used to treat typhoid. Augmentin followed with a 50% resistance rate, indicating a notable reduction compared to the pre-curing resistance.

Interestingly, Azithromycin and Levofloxacin demonstrated the lowest post-curing resistance rates at 12.5%. This suggests a more successful removal of plasmids associated with resistance to these antibiotics. The lower resistance rates for Azithromycin and Levofloxacin post-curing indicate a potential effectiveness of the plasmid elimination process in rendering these antibiotics more clinically viable against the bacterial isolates. This finding has been reported by (Ali, *et al*., 2011; Effa and Bukirwa, 2011) it was stated that levofloxacin which a fluoroquinolone was found to be the most effective drug in both oral and injectable form of treatment of uncomplicated typhoid while Azithromycin was effective therapy for typhoid fever in an endemic region.

The outcome of this result reflect the impact of plasmid elimination on the antibiotic resistance patterns of these isolates, revealing a nuanced response across different samples. Sample S2 demonstrated a notable reduction in antibiotic resistance, losing resistance to four antibiotics after plasmid curing. This outcome suggests a successful elimination of plasmids associated with resistance in this particular sample. Similarly, S3 and S5 displayed positive responses to plasmid curing, losing resistance to three antibiotics each. In contrast, S6 exhibited a remarkable improvement, losing resistance to six antibiotics after plasmid curing. This substantial reduction suggests that the plasmid elimination process was highly effective in this sample. On the other hand, S11 showed no change, indicating that plasmid curing had no impact on the resistance profile of this particular isolate. Sample S12 displayed a significant reduction in resistance, losing resistance to five antibiotics after plasmid curing. However, S14 demonstrated an unexpected result, gaining resistance to three antibiotics after plasmid elimination. This discrepancy underscores the complexity of bacterial responses to plasmid curing, with variations observed even within the same strain.

For *Salmonella enteritidis,* sample N8 experienced a reduction in resistance, losing resistance to one antibiotic after plasmid curing. This result indicates a moderate success in eliminating plasmids associated with antibiotic resistance in this particular isolate.

Figure 1 illustrates the Multiple Antibiotic Resistance (MAR) index of bacterial isolates before and after plasmid curing. The MAR index is a measure of resistance, with higher values indicating greater resistance to antibiotics. The MAR index of bacterial isolates before plasmid curing represents their resistance profile with plasmids present. Plasmids often carry antibiotic resistance genes, contributing to higher MAR indices. After plasmid curing, which involves the removal or deactivation of plasmids, a significant decrease in the MAR index was observed in most isolates. This indicates that the plasmids were responsible for the antibiotic resistance in these isolates. Unlike other isolates, isolate S14 showed an increase in the MAR index by 0.1 after plasmid curing. This unusual result suggests that the resistance in S14 is not primarily plasmid-mediated. Other mechanisms, such as

chromosomal mutations or efflux pumps, might be contributing to its antibiotic resistance. However, the exception of isolate S14 highlights the complexity of resistance mechanisms, emphasizing the need for comprehensive analysis beyond plasmid curing to fully understand antibiotic resistance in bacteria.

The findings outlined in Table 4.5, provide a nuanced perspective on the effectiveness of plasmid curing across different bacterial isolates. The varied responses observed highlight the heterogeneity in plasmid content and the diverse mechanisms underlying antibiotic resistance in these strains. The plasmid profiles of both plasmid-cured and uncured bacterial isolates, employing gel electrophoresis as the analytical technique facilitated the visualization of plasmids based on their molecular weights, offering a comprehensive understanding of the impact of plasmid curing on the isolates.

The plasmid profiling for the eight bacterial isolates initially revealed the presence of plasmids within the high molecular weight range of 3kbp to 10kbp. This suggests a diverse array of plasmid sizes among the isolates, reflecting the genetic heterogeneity within the bacterial population. This was in line with Ekundayo, 2021 who reported different plasmid weights of Enterobacteriaceae.

Following the plasmid curing process, specific alterations in the plasmid profiles were observed. Isolates S2, S3, S5, S6, and S12 exhibited a notable change by losing their 3kbp plasmids while retaining the 10kbp plasmids. This outcome indicates a targeted impact of plasmid curing on the lower molecular weight plasmids, potentially associated with antibiotic resistance, while preserving the larger plasmids.

Conversely, isolates S11, S14, and N8 retained plasmids within the 3kbp to 10kbp range without losing any plasmid during the curing process. This suggests that plasmid elimination did not significantly impact the plasmid content in these isolates within the observed molecular weight range. This finding was also reported by (Ekundayo, 2021 and Ebele, *et al.,* 2022) stating that antibiotic resistant from isolates could not be fully treated by curing their plasmid.

The molecular analysis results presented in the project, particularly those from plate 1 and 2 using gel electrophoresis, offer crucial insights into the impact of plasmid curing on the molecular weight of plasmids within bacterial isolates. The observed variation in molecular weights after plasmid curing is indicative of the heterogeneous nature of plasmids among the isolates.

The finding that some isolates lost molecular weight while others remained the same underscores the diverse responses to plasmid curing. This suggests that the sodium dodecyl sulphate, employed as the plasmid curing agent, did not uniformly eliminate plasmids across all bacterial isolates. The existence of unchanged molecular weights in some isolates indicates resistance to the plasmid curing process, highlighting potential variations in plasmid stability or the presence of other mechanisms contributing to antibiotic resistance. This corresponds with Ehiaghe, *et al.,* 2013 and Ebele, *et al.,* 2022 who reported in their findings that multidrug resistance were not fully plasmid mediated.

Moreover, the acknowledgment that not all isolates that are resistant to certain antibiotics exhibit plasmid-mediated resistance is a significant observation. This implies that resistance can also be mediated by chromosomal factors. The coexistence of plasmid bands in all bacterial isolates, even after plasmid curing, suggests the possibility of chromosomal elements contributing to antibiotic resistance. This adds complexity to the understanding of antibiotic resistance mechanisms, emphasizing the need for a holistic approach that considers both plasmid and chromosomal factors.

Conclusion

The plasmid profile and curing analysis contribute valuable insights to the complex interplay between plasmids, possible chromosomal elements, and antibiotic resistance. The existence of unchanged molecular weights in some

isolates indicated resistance to the plasmid curing process, highlighting potential variations in plasmid stability or the presence of other mechanisms contributing to antibiotic resistance.

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