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Antimicrobial Resistance and Plasmid-Mediated Multidrug Resistance in *Salmonella* Isolates from Clinical and Veterinary Sources in Owerri, Imo State, Nigeria

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Abstract **Original Research Article**

The increasing prevalence of multidrug-resistant (MDR) *Salmonella* constitutes a major public health challenge, particularly in regions with extensive antibiotic use in animal production. This study investigated antimicrobial resistance patterns and plasmid - mediated mechanisms in *Salmonella* isolates from clinical and veterinary sources in Owerri, Imo State, Nigeria. A total of 250 samples were collected from poultry and pigs, as well as blood samples from a specialist hospital. *Salmonella* isolates were identified using standard bacteriological methods, and antimicrobial susceptibility was determined by the Kirby–Bauer disc diffusion method against ten commonly used antibiotics. Isolates resistant to at least three antibiotics were subjected to plasmid curing with sodium dodecyl sulphate (SDS), post-curing susceptibility testing, and plasmid profiling by agarose gel electrophoresis. High resistance rates were observed for ampicillin (96%) and β-lactam antibiotics, the cefotaxime (87%), and ceftazidime (78.8%), with notable resistance to carbapenems. Plasmid curing reduced resistance in some isolates, indicating plasmid-mediated resistance, while some isolates did not show reduced resistance, indicating that resistance can also be chromosomally mediated. Plasmid profiling revealed plasmids ranging from 4–10 kbp. The findings demonstrate widespread MDR *Salmonella* in both clinical and veterinary settings and underscore the need for strengthened antibiotic stewardship and alternative antimicrobial strategies.

Keywords: *Salmonella*, antimicrobial resistance, plasmid curing, multidrug resistance, veterinary and clinical isolates.

1.1.Introduction

Salmonella species are among the most significant foodborne pathogens worldwide, causing salmonellosis in humans and animals. Infections are commonly associated with contaminated food products of animal origin, especially poultry and pork. In humans, *Salmonella* infections can lead to gastroenteritis, septicaemia, meningitis, and other severe systemic illnesses, posing a substantial public health burden.

Salmonella is a rod-shaped, Gram–negative bacterium belonging to the family of Enterobacteriaceae and the causative agent of

Salmonellosis (Grunberg, 2022).Salmonellosis in warm blooded vertebrates is most commonly associated with serovars of *Salmonella* enterica. It affects most animal species as well as humans and is a major public health concern responsible for a variety of human illnesses, including urinary tract infections, gastroenteritis, meningitis, septicaemia and pneumonia (Encyclopedia. Com, 2019; Grunberg, 2022).

Antimicrobial drugs (antibiotics) are crucial in combating various forms of infectious diseases in all parts of the world. However, research indicates the emergence of multidrug resistant strains of pathogenic bacteria (Ashneal *et al*., 2023), which has become a leading problem in the medical industry and calls for investigations and discovery of new antibiotics. The persistent increase in the antibiotic resistant strains have directed researchers towards the use of nontoxic and more biocompatible herbal materials (Afrah *et al.,* 2023)

The widespread and often indiscriminate use of antibiotics in human medicine, veterinary practice, and livestock production has contributed significantly to the emergence of antimicrobial-resistant *Salmonella* strains. Of particular concern is the increasing resistance to β-lactam antibiotics and other critical drug classes which are frequently used for the treatment of severe infections.

Antibiotic resistance in *Salmonella* is often mediated by plasmids, which are mobile genetic elements capable of transferring resistance genes between bacteria. Plasmid-mediated resistance facilitates the rapid dissemination of multidrug resistance across bacterial populations, especially in environments such as farms and hospitals where antibiotic pressure is high.(Partridge *et al* ., 2018)

Given the close interaction between humans, animals, and the environment, veterinary settings serve as important reservoirs for resistant *Salmonella* strains that can be transmitted to humans. This study therefore aimed to evaluate the antimicrobial resistance profiles of *Salmonella* isolates from clinical and veterinary sources in Imo State, Nigeria, and to determine the role of plasmids in mediating observed resistance patterns.

2.0. MATERIALS AND METHODS

2.1. Sample types and collection

Salmonella isolates were recovered from poultry, pig farms and specialist hospital. The chicken cloacal swab and chicken faeces were collected from Imo State University Poultry farm, using sterile containers. The method described by Ejikeukwu *et al.* (2017) was adopted for the sample collection. The method of Amaechi & Nwankwo (2015) was adopted for sample collection of Pig rectal swab and pig faeces using sterile spatula and placed inside 20mls sterile plastic sampling bottles while Blood samples were collected from patients suspected of having typhoid fever from the Imo State Specialist Hospital, Umuguma, Owerri. The patients recruited were both male and female, 5-45 years of age. Ten antibiotics belonging to Aminoglycoside, carbapenem, penicillins, fluoroquinolone, cephalosporins and monobactams classes were tested and they include Gentamicin (10µg), Imipenem (10µg), Ampicillin (10µg), Levofloxacin (5µg), Ceftazidime (30µg), Aztreonam (30µg), Ceftaxime (30µg), Ciprofloxacin (5µg), Meropenem (10µg) and Ertapenem (10µg)

The breakpoints in µg/ml for resistant isolates according to Clinical and Laboratory Standards institutes (CLSI) (2019) are Gentamycin ≤ 12, Ertapenem ≤ 19, Imipenem ≤19, Meropenem ≤19, Ciprofloxacin ≤15, Levofloxacin ≤ 13, Ceptazidime ≤ 17, Aztreonam ≤ 17, Cefotaxime ≤ 22 and Ampicillin ≤ 13.

2.2. METHODS

2.2.1. Isolation and characterization of *Salmonella* isolates

MacConkey and eosin *Salmonella* agar were used for primary isolation of *salmonella* after enrichment with brain heart infusion (BHI) broth for 24 hours at 30°C. Subsequently, 2 or 3 distinct colonies from these primary plates with characteristic colonies were selected and purified on nutrient agar plates to obtain pure colonies. The isolates were confirmed using Gram stain reaction and IMViC tests for *Salmonella* classification. Cheesbrough, M. (2003).

2.2.2. Antimicrobial resistant test

Antimicrobial resistant test was carried out by the Kirby –Bauer technique according to Cheesbrough,M.(2003). Briefly, isolates are standardized to 0.5 McFarland standards and were spread on the surface of already prepared and dried Muller Hinton agar plates using swab sticks. The antimicrobial discs were placed on the agar surface using sterile forceps, the bacterial films on the agar was left to dry briefly and plates subsequently incubated at 35°C for 24 hours. After incubation, the zones of inhibition were measured using a meter rule and designated as resistant or susceptible according to already established breakpoints.

2.2.3. Plasmid curing and profiling of resistant isolates

2.2.3.1. Plasmid curing

In the plasmid curing process. Five isolates showing resistance to more than two 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.

2.2.3.2. Plasmid profiling

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 for solidification, it was left at room temperature for 20-30 minutes (Abraham *et al.*, 2019).

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 (Ehiaghe *et al.*, 2013).

2.2.3.3. 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. (Cheesbrough, M. 2003).

Method of Data Analysis

Data analysis was performed using IBM-SPSS statistics version 23 (SPSS Inc.Chicago, USA).Descriptive statistics was used to summarize the data. The frequency distribution of the variable characteristics was computed and presented in a table of distribution which was also expressed as percentage of the distribution.Chi- square and ANOVA test (LSD) was used to separate the means in which $p < 0.05$ was considered significant.

3. RESULTS

Table 1. Comparative resistant rates (%) of the isolates from the five different clinical and veterinary samples.

Antibiotics	Chicken Cloacal Swab (n = 50)	Chicken faeces (n=50)	Pig Rectal swab (n=50)	Pig faeces (n=50)	Blood (n=50)	Total n =250
Ampicillin	98(19.6)	100(20.0)	98(19.6)	98(19.6)	86(17.2)	480(96.0)
Cefotaxime	94(18.8)	96(19.2)	98(19.6)	100(20.0)	48(9.6)	436(87. 0)
Ceftazedime	72(14.4)	94(18.8)	88(17.6)	100(20.0)	40(8.0)	394(78.8)
Imipenem	76(15.2)	86(17.2)	96(19.2)	74(14.8)	22(4.4)	354(70.8)
Meropenem	80(16.0)	86(17.2)	88(17.2)	76(15.2)	12(2.4)	342(68.4)
Azetreonam	80(16.0)	52(10.4)	100(20.0)	90(18.0)	10(2.0)	332(66.4)
Ertapenem	52(10.4)	96(19.2)	88(17.6)	28(5.6)	22(4.4)	286(57.2)
Ciprofloxacin	68(13.6)	74(14.8)	48(9.6)	38(7.6)	36(7.2)	264(52.8)
Gentamycin	70(14.0)	54(10.8)	8(1.6)	22(4.4)	18(3.6)	172(34.4)
Lavafloxacin	36(7.2)	39(7.8)	40(8.0)	40(8.0)	12(2.4)	167(33.5)
SD	**18.31**	**21.87**	**31.75**	**31.47**	**23.36**	**103.20**
X^2	**41.55; p < 0.001**	**55.39;p < 0.001**	**120.66;p < 0.001**	**133.82;p < 0.001**	**160.54;p < 0.001**	**297.02;p < 0.001**
Overall X^2	**225.74; p < 0.001**					
ANOVA LSD	**10.47**	**12.5**	**18.16**	**17.99**	**13.36**	**59.01**

Standard Deviation (SD) and Chi-Square (X^2) Results

Standard Deviation (SD)

- Total SD = **103.20**, indicating high variability in resistance rates across antibiotics and sources.
- Pig-related samples (rectal and faeces) show the highest SD (31.75 and 31.47), reflecting greater fluctuations in resistance patterns.

Chi-Square (X^2) Analysis

- All X^2 values are highly significant ($p < 0.001$).
- This means there is a statistically significant difference in resistance rates among the different sample types.
- The highest X^2 in the total sample (297.02) confirms strong variation across antibiotics and sources.

Table 2. Plasmid profile of cured and uncured isolates using gel electrophoresis.

Salmonella Isolates	Before Curing	After Curing	Probable Organism
1(blood)	5	1	*Salmonella*
5 (pig rectal swab)	5	2	*Salmonella*
2 (chicken cloacal swab)	10	10	*Salmonella*
3(chicken faeces)	6	9	*Salmonella*
4(pig faeces)	6	5	*Salmonella*
SD	**2.073**	**4.037**	
X^2	**2.69 ;p = 0.611**	**12.07;p = 0.017**	
Overall X^2	**4.25;p = 0.373**		
ANOVA LSD	**1.301**	**2.534**	

Statistical Analysis

Standard Deviation (SD)

- Before curing: SD = 2.073 (moderate variability in resistance across isolates).
- After curing: SD = 4.037 (greater variability post-curing, likely due to differential plasmid loss among isolates).

Chi-square (X^2)

- Before curing: $X^2 = 2.69$; $p = 0.611$ → Not statistically significant, indicating resistance distribution among isolates was relatively uniform prior to curing.
- After curing: $X^2 = 12.07$; $p = 0.017$ → statistically significant, indicating plasmid curing caused differential changes in resistance among isolates.
- Overall $X^2 = 4.25$; $p = 0.373$ → No significant difference when combining all data, suggesting variability in plasmid contribution to resistance is isolate-dependent.

ANOVA LSD

- Before curing: 1.301 → Small differences between isolates not statistically meaningful.

After curing: 2.534 → Differences between isolates are now more meaningful, reflecting the variable impact of plasmid loss.

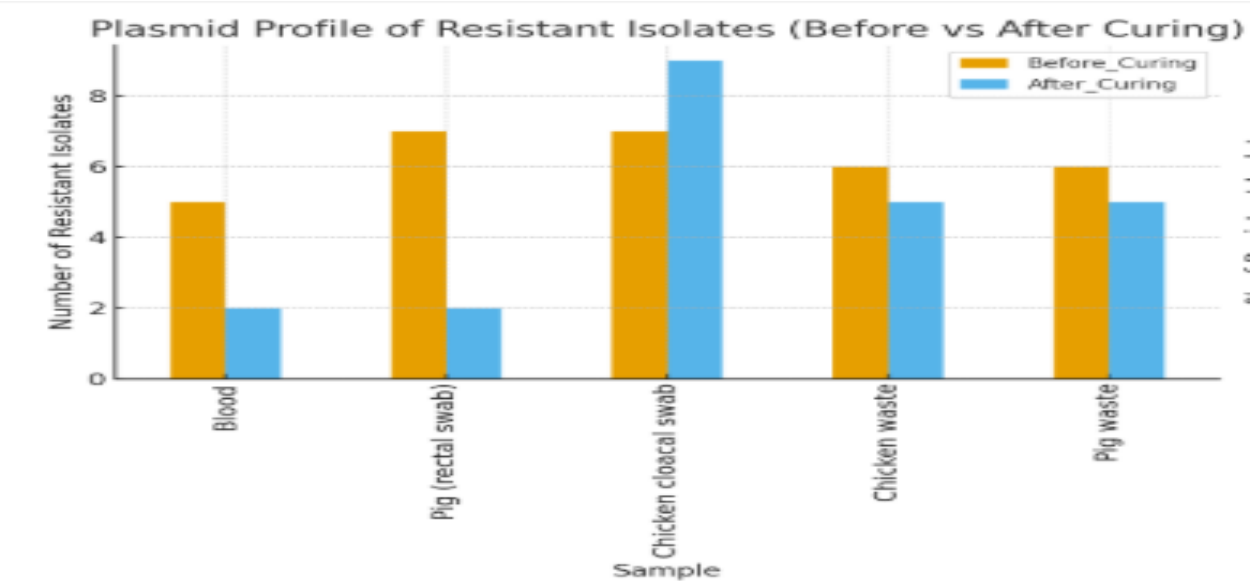

Fig 1

Table 3. Gel Electrophoresis Showing Molecular Weight Of Plasmid Profile of cured and uncured Isolates

Lane in gel	**Molecular Weight (Kbp) of Uncured Plasmid**	**Molecular Weight (Kbp) of Cured Plasmid**	**Probable Organism**	**Sample**
1	4 – 10	10	*Salmonella*	Blood
5	4 – 10	10	*Salmonella*	pig rectal swab
2	4 – 10	4 – 10	*Salmonella*	Chicken cloacal swab
3	4 – 10	4 – 10	*Salmonella*	chicken faeces
4	4 - 10	10	*Salmonella*	Pig faeces

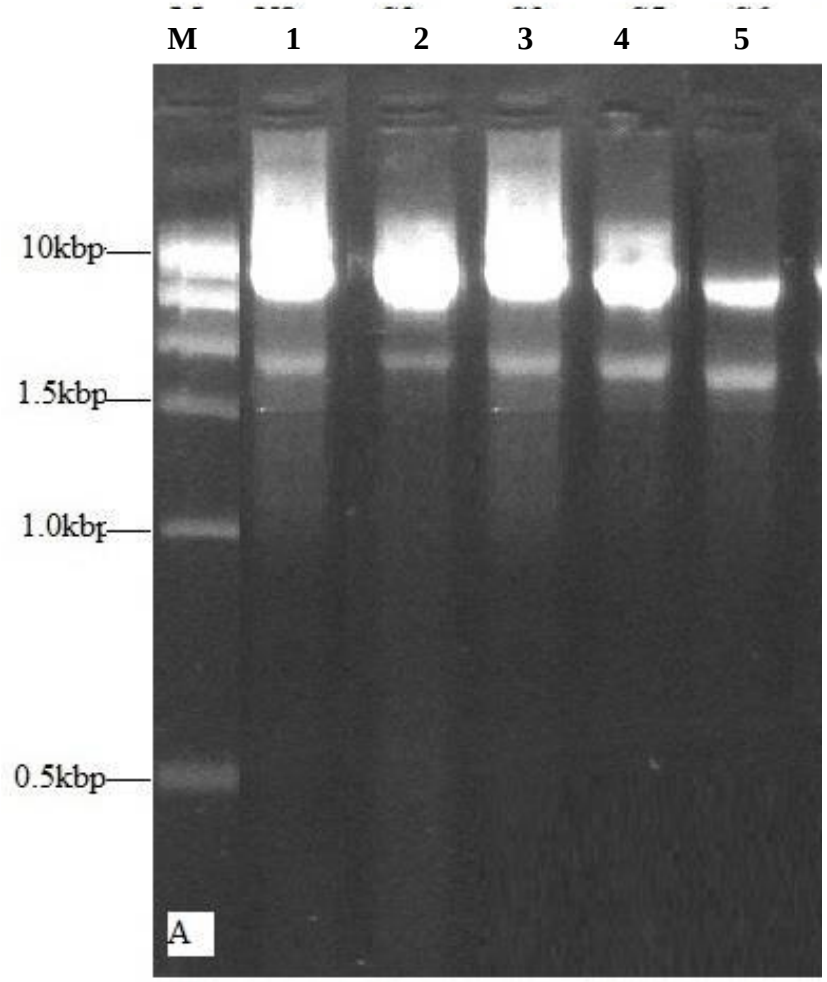

Fig 2. Plasmid profile of the *Salmonella* isolates

Isolate 1: Blood (from specialist hospital), Isolate 2: chicken cloacal swab, Isolate 3: chicken faeces, Isolate 4: pig faeces, Isolate 5: pig rectal swab.

Table 4. Antibiotic Susceptibility Analysis of Resistant cured isolates

ANTIBIOTICS	**No. (%) of Resistant Isolates *Salmonella***
Ampicillin	37(74)
Cefotaxime	31(62)
Ceftazidime	29(58)
Ciprofloxacin	21(42)

Imipenem	27(54)
Meropenem	26(52)
Aztreonam	25(50)
Gentamycin	9(18)
Ertapenem	11(47)
Lavofloxacin	6(12)
SD	**10.28**
X^2	**42.86;p < 0.001**
ANOVA LSD	**5.88**

Statistical Analysis

- **Standard Deviation (SD = 10.28)**

  Moderate variability in resistance among antibiotics.

- **Chi-square ($X^2 = 42.86$; $p < 0.001$)**
  - Highly significant, confirming that resistance differs significantly across antibiotics after plasmid curing.
  - β-lactams and carbapenems remain more resistant compared to fluoroquinolones and aminoglycosides.
- **ANOVA LSD = 5.88**
  - Differences in mean resistance greater than 5.88% are statistically meaningful.
  - Confirms that the reduction in resistance post-curing is significant for some antibiotics

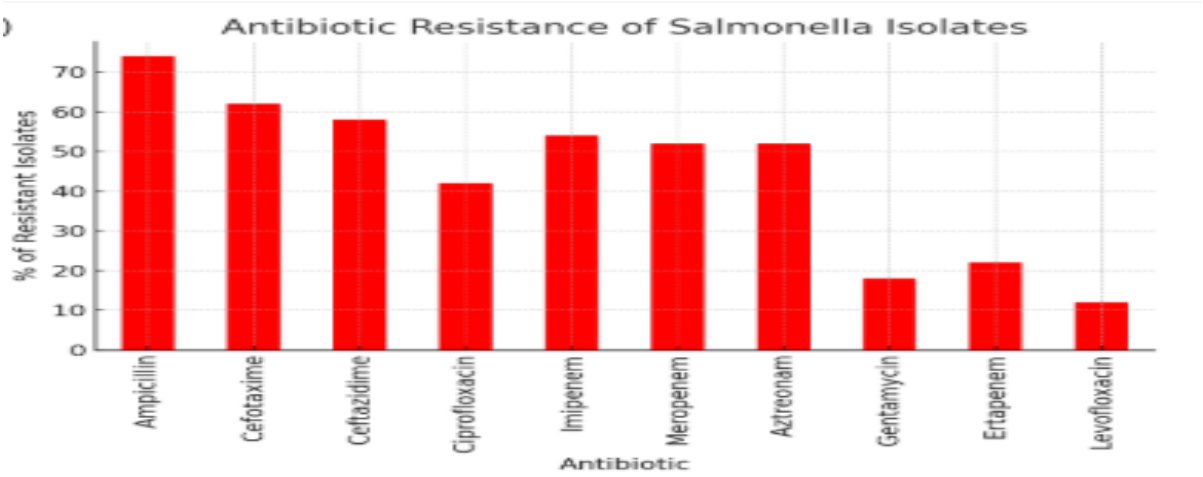

Fig 3

DISCUSSION

The findings of this study revealed a high prevalence of antimicrobial resistance among *Salmonella* isolates obtained from both veterinary and clinical sources. Resistance to commonly used antibiotics such as ampicillin, cefotaxime, and ceftazidime, carbapenems were exceptionally high, indicating prolonged exposure of *Salmonella* populations to β-lactam and carbapenems antibiotics. These results are consistent with reports from other developing regions where unrestricted access to antibiotics is common.(Threlfall, 2002; WHO, 2017).

Among different classes of antibiotics, the highest comparative resistance percentage was against Ampicillin (96%), Cefotaxime (87%), Ceftazedime (78%),Imipenem (70.8%), Meropenem (68%) and Aztreonam (66%).The moderate percentage was seen among Ertapenem (57%), Ciprofloxacin (52%).On the other hand, the lowest resistance percentage was in Gentamicin(34%) followed by Levofloxacin (33%) (Table 1). These resistance rates are in agreement with the results reported from United States, reporting 63% and 26% resistance to tetracycline and streptomycin, respectively (Velasquez *et al.*, 2018).

Given that Ampicillin and third generation cephalosporins are classified by the World Health Organization (WHO, 2019) as "critically important" antibiotics used in treating serious human infections and diseases caused by organisms transmitted from non-human sources or capable of acquiring resistance genes, the resistance levels noted in this study, particularly to Ampicillin, cefotaxime , Ceftazedime and carbapenems raised concerns .

The detection of substantial resistance to carbapenems, including imipenem and meropenem, is particularly alarming. Carbapenems are considered last-line antibiotics for treating severe Gram-negative infections, and resistance to these agents suggests the emergence of highly resilient *Salmonella* strains.

Veterinary isolates, especially those from pig and poultry sources, generally exhibited higher resistance rates compared to clinical blood isolates, underscoring the role of animal production systems as reservoirs of resistant bacteria. More than 60.0% of isolates were resistant to third-generation cephalosporins. In line with the current results, Saeed *et al*., 2020 from Pakistan, reported resistance rates of 96.3%, and more than 50.0% against carbapenems, trimethoprim-sulfamethoxazole, and third-generation cephalosporins, respectively. El-Tayeb *et al.,* 2017 from Saudi Arabia, revealed a high resistance rate of *Salmonella Typhi* isolates against nitrofurantoin as a result of nitrofurans being used as a feed supplement or treatment by veterinary professionals, particularly those working in the poultry industry, *Salmonella* has exhibited high resistance to this antibiotic.

The high resistant of these antibiotics is probably due to wide use of the antibiotics as growth promoter, prophylaxis and therapy in poultry industry, pig farms and discharge of waste from households, drug manufacturing units, hospitals. These antibiotics may have led to evolution of bacterial strains that are resistant to antibiotics which then grow in numbers and spread in the environment and subsequently leaching of the antibiotics into the environment are contributing to the increased AMR in bacteria.

Bacteria resistant to antibiotics like β-lactam, multidrug /efflux are highly abundant in the poultry environment, contaminated poultry products represent the primary transmission vector for *Salmonella*. Therefore, monitoring antibiotic resistance in *Salmonella* originating from poultry is essential (Ince & Akan, 2023). AMR rates are extremely high across all sample sources.

Veterinary isolates, especially from pigs and chickens, show the highest levels of resistance, including to last-resort antibiotics with exceptionally high resistance to β-lactams. Clinical isolates exhibit lower but still concerning resistance levels. Statistical analysis confirms significant differences in resistance patterns across sample types. The findings underscore the urgent need for antibiotic stewardship, particularly in animal production systems.

The statistical values (SD, Chi-square, ANOVA-LSD) confirm that the resistance differences across antibiotics are significant. These findings signal serious public health concerns and emphasize the need for strict antibiotic regulation, better biosecurity measures, and routine AMR surveillance in poultry farms.

Plasmid curing experiments demonstrated a reduction in antibiotic resistance in some isolates following SDS treatment. This confirms that resistance in these isolates was at least partially plasmid-mediated. However, resistance persisted in certain isolates even after curing, suggesting the involvement of chromosomal resistance mechanisms or incomplete plasmid elimination.

In Table 2, Sample 1 (blood) demonstrated a notable reduction in antibiotic resistance, losing resistance after plasmid curing. This outcome suggests a successful elimination of plasmids associated with resistance in this particular sample. Similarly, sample 5 (pig rectal swab) displayed positive responses to plasmid curing, losing resistance to antibiotics too. On the other hand, Sample 2 (chicken cloacal swab) showed no change, indicating that plasmid curing had no impact on the resistance profile of this particular isolate. However, Sample 3 (chicken faeces) demonstrated an unexpected result, gaining resistance to antibiotics after plasmid elimination. This discrepancy underscores the complexity of bacterial responses to plasmid curing, with variations observed even within the same strain. Sample 4 (pig faeces) experienced a reduction in resistance, losing resistance to antibiotic plasmid curing. This result indicates a moderate success in eliminating plasmids associated with antibiotic resistance in this particular isolate. Variations in resistance patterns following plasmid curing, including resistance loss, no observable change, or unexpected resistance gain, have been widely reported and may be attributed to chromosomal resistance genes or plasmid mediated, compensatory mutations, or stress-induced adaptive responses (Carattoli, 2003; Andersson & Hughes, 2010).

Agarose gel electrophoresis revealed plasmids ranging from 4–10 kbp, which are consistent with plasmid sizes known to harbor antimicrobial resistance genes in enteric bacteria.

The plasmid profiling for the five *Salmonella* isolates (Table 3) initially revealed the presence of plasmids within the high molecular weight range of 4kbp 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 1 (blood), 5 (pig rectal swab) and 4 (pig faeces) exhibited a notable change by losing their 4kbp 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 2 (chicken cloacal swab) and 3 (chicken faeces) retained plasmids within the 4kbp 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 & Okoye *et al.*, 2022) stating that antibiotic resistant from isolates could not be fully treated by curing their plasmid.

The molecular analysis results (Table 3) presented in this project using gel electrophoresis, offer crucial insights into the impact of plasmid curing on the molecular weight of plasmids within *Salmonella* 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; Okoye *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 *Salmonella* 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. 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 plasmids

Five isolates on which plasmid analysis were carried out contained plasmid band, this might be before the initial antibiotic resistance exhibited by the isolates before plasmid analysis in this study while the resistance observed in other isolates might have been chromosomal mediated and this is in agreement with the findings of Kroll *et al.,* 2010 who submitted that plasmid have encoded genes that provide resistance to occurring antibiotic in competitive environmental niche.

The curing antibiotics sensitivity analysis (Table 4) carried out on the MDRIs bacterial isolates revealed that the test isolates were susceptible to those antibiotics that they were previously resistant to. This implies that the presence of the plasmids in the five isolates were responsible for the multiple antibiotic resistance pattern exhibited by the isolates initially. This finding agrees with the work done by Afolami et *al* 2018 who reported that plasmid-mediated mechanisms might increase spread of antibiotic resistances in bacteria. More so, efflux pump mechanisms or other factors like mutation of gene encoding ribosomal protein, which decreases permeability of the cell envelope in enteric bacteria might also be responsible for antibiotic resistances (*Pharmaceuticals, 2025; BMC Microbiology, 2024*).

In the present study, Occurrence of plasmid was more in isolates from food animal origin compared to those from humans. This is in line with the findings of Akinyemi & Iwalokun, 2018 stating that Food/animal sources may have higher plasmid diversity and carriage due to ongoing antibiotic pressure in animal rearing environments and serve as reservoirs for resistant plasmid-bearing bacteria. The study findings also suggested 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, to antibiotic susceptibility 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, Ampecillin emerged as the antibiotic with the highest resistance rate at 74%. This indicates that even after plasmid elimination, a considerable proportion of bacterial isolates maintained resistance to Ampecillin. Ceftazedime, imipenem, meropenem, Aztreonam followed with a 74%, 58%, 54%, and 50 %. Ertapenem and Ciprofloxacin followed with a 47% and 42% resistance rate, indicating a notable reduction compared to the pre-curing resistance. Interestingly, Gentamicin and Levofloxacin demonstrated the lowest post-curing resistance rates at 18% and 12%. This suggests a more successful removal of plasmids associated with resistance to these antibiotics. The lower resistance rates 18% and 12% for Gentamicin and Levofloxacin post-curing indicate a potential effectiveness of the plasmid elimination process in rendering these antibiotics more clinically viable against the *Salmonella* isolates. This finding has been reported by (Ali *et al*., 2011), it was stated that levofloxacin, which is a fluoroquinolone, and

gentamicin is an aminoglycoside was found to be the most effective drug in both oral and injectable form of treatment of uncomplicated typhoid. (Fasema *et al*., 2024)

The statistical analysis showed significant differences in resistance patterns among sample sources and antibiotics tested, further emphasizing the widespread and heterogeneous nature of antimicrobial resistance in *Salmonella* populations within the study area. Statistically, significant differences across antibiotic classes highlight varying contributions of plasmids to AMR.

Conclusion

This study demonstrates a high prevalence of multidrug-resistant *Salmonella* isolates from poultry, pig, and human clinical sources in Imo State, Nigeria. Resistance to multiple antibiotic classes, including β-lactams presents a serious public health concern.

The plasmid profiling and curing results indicate that plasmids play a significant role in the dissemination of antimicrobial resistance among *Salmonella* isolates. Veterinary environments appear to serve as important reservoirs for resistant strains, with potential transmission to humans through the food chain and environmental exposure. Plasmid curing reduced resistance in several antibiotics, confirming that plasmids play a significant role in mediating multidrug resistance. Residual resistance indicates chromosomal determinants or curing-resistant plasmids, particularly for β-lactams. The study underscores the complexity of *Salmonella* resistance mechanisms and the importance of integrated One Health approaches to control AMR.

Recommendations

Antibiotic Regulation: Strict control and regulation of antibiotic use in livestock production and human healthcare settings should be enforced to reduce selective pressure for resistance.

Surveillance Programs: Continuous monitoring of antimicrobial resistance in both clinical and veterinary environments is necessary to detect emerging resistance trends early.

Public and Farmer Education: Awareness programs should be implemented to educate farmers, veterinarians, and healthcare workers on the dangers of indiscriminate antibiotic use.

Alternative Therapies: Further research should explore non-antibiotic treatment options, including plant-based antimicrobials and other novel therapeutic approaches.

Molecular Characterization: Advanced molecular studies should be conducted to identify specific resistance genes and plasmid types involved in multidrug resistance.

Competing Interests

Authors have declared that no competing interest exist.

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