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Antimicrobial Susceptibility Profile of *Salmonella* Isolates from Veterinary Specimens in Owerri, Imo State, Nigeria

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Abstract **Original Research Article**

The antimicrobial susceptibility profile of *Salmonella* isolates obtained from veterinary specimens in Owerri was investigated. Chicken and pig faecal samples were aseptically collected, cultured, and identified using standard microbiological techniques, including morphological and biochemical characterization. Antimicrobial susceptibility testing against ten antibiotics was performed using the Kirby–Bauer disk diffusion method, while plasmid detection was carried out using the alkaline lysis method. A total of 100 *Salmonella* isolates were recovered from both sources. Isolates from chicken faeces exhibited higher resistance rates compared to those from pig faeces. Specifically, chicken isolates showed high resistance to Ampicillin (100%), Cefotaxime (96%), Ceftazidime (94%), Imipenem (86%), Meropenem (86%), Ciprofloxacin (74%), Ertapenem (96%), and Levofloxacin (78%), with moderate resistance to Aztreonam (53%) and Gentamycin (54%). In contrast, pig isolates showed high resistance to Ampicillin (98%), Cefotaxime (100%), Aztreonam (90%), Imipenem (96%), Ceftazidime (100%), and Meropenem (76%), moderate resistance to Ciprofloxacin (38%), and low resistance to Gentamycin (22%) and Levofloxacin (28%).Multidrug-resistant (MDR) isolates were subjected to plasmid profiling and curing. Twenty-five non-typhoidal *Salmonella* isolates were analyzed. Post-curing results indicated incomplete elimination of plasmid-mediated resistance. Notably, chicken isolates gained resistance to three antibiotics after curing, while pig isolates lost resistance to only one antibiotic. These findings suggest partial success in plasmid elimination and highlight the complexity of resistance mechanisms. The increasing antimicrobial resistance observed among *Salmonella* isolates poses a significant public health threat and underscores the need for continuous surveillance and development of effective antimicrobial strategies.

Keywords: Antimicrobials, Kirby–Bauer technique, *Salmonella*, veterinary specimens.

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INTRODUCTION

Salmonella is a rod-shaped, Gram-negative bacterium belonging to the family *Enterobacteriaceae* and is the causative agent of salmonellosis (Grunberg, 2022). In warm-blooded vertebrates, infections are predominantly associated with *Salmonella enterica* serovars, affecting both animals and humans. It remains a major public health concern, causing diseases such as gastroenteritis, septicemia, meningitis, pneumonia, and urinary tract infections (Encyclopedia.com, 2019; Grunberg, 2022).

Antimicrobial agents play a critical role in the treatment of infectious diseases worldwide. However, the emergence of multidrug-resistant (MDR) bacterial strains has become a major global health challenge (Ashneal *et al*., 2023), necessitating continuous research into novel therapeutic options.

β-lactam antibiotics represent a broad class of antimicrobial agents categorized based on their chemical structures. The emergence and global dissemination of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Salmonella* strains since the early 2000s have raised significant concerns (Mulvey *et al.*, 2003; Tzouvelekis *et al*., 2002; Winokur *et al*., 2000). Screening for multidrug-resistant *Salmonella* is typically conducted using susceptibility testing based on established breakpoint values (Nordmann *et al*., 2011).

Veterinary environments are recognized as important reservoirs of antimicrobial-resistant bacteria, facilitating transmission to humans through the food chain, direct contact, and environmental exposure. The indiscriminate use of sub-therapeutic antibiotic doses as growth promoters in animal husbandry contributes significantly to this problem. Therefore, this study aimed to evaluate the antimicrobial susceptibility profile of *Salmonella* isolates from veterinary specimens in Owerri, as well as to assess plasmid-mediated resistance through profiling and curing techniques (Akinyemi *et al*., 2018)

MATERIALS AND METHODS

Sample Collection

Chicken faecal samples (n = 50) and pig faecal samples (n = 50) were aseptically collected from the Imo State University poultry farm using sterile containers. Samples were transported promptly to the microbiology laboratory for analysis.

Isolation and Identification of *Salmonella*

Primary isolation was carried out using MacConkey agar and Eosin *Salmonella-Shigella* agar following enrichment in Brain Heart Infusion (BHI) broth at 30°C for 24 hours. Distinct colonies were subcultured on nutrient agar to obtain pure isolates. Identification was confirmed using standard biochemical tests, including IMViC reactions (Cheesbrough, 2003).

Antibiotics Tested

Ten antibiotics from different classes were evaluated: Gentamycin, Imipenem, Ampicillin, Levofloxacin, Ceftazidime, Aztreonam, Cefotaxime, Ciprofloxacin, Meropenem, and Ertapenem. Breakpoints were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2019) guidelines.

Antimicrobial Susceptibility Testing

The Kirby–Bauer disk diffusion method was employed (Cheesbrough, 2003). Standardized inocula (0.5 McFarland) were spread on Mueller-Hinton agar plates, and antibiotic discs were applied. Plates were incubated at 35°C for 24 hours, and zones of inhibition were measured and interpreted.

Plasmid Profiling and Curing

Plasmid extraction was conducted using the alkaline lysis method. Agarose gel electrophoresis (0.8%) was used for plasmid visualization following staining with EZ Vision

DNA dye (Abraham *et al*., 2019). For plasmid curing, selected MDR isolates were treated with 10% sodium dodecyl sulfate (SDS) following the method described by Ehiaghe *et al*. (2013). Treated cultures were incubated and subsequently re-tested for antibiotic susceptibility.

Post-curing Antibiotic Sensitivity Testing

Cured isolates were re-evaluated for antibiotic susceptibility using standard Kirby–Bauer procedures to determine the impact of plasmid elimination.

RESULTS

A total of 100 *Salmonella* isolates were obtained. Chicken derived isolates exhibited higher resistance rates compared to pig-derived isolates. Overall, multidrug resistance was prevalent. Plasmid profiling revealed high molecular weight plasmids (4–10 kbp). Post-curing analysis showed partial plasmid elimination, with variable effects on resistance patterns. Unexpectedly, some isolates gained resistance after curing, suggesting alternative resistance mechanisms such as chromosomal mutations or gene regulation changes e.g. unregulation of efflux pumps or mutations in target sites (Andersson & Hughes 2010)

Table 1. Antimicrobial Resistant Profile of *Salmonella* Isolated from Chicken Feces Samples

ANTICIOTICS	**No(%) of Resistant Isolates**
	Salmonella
	(n=50)
Ampicillin	50(100)
Cefotaxime	48(96)
Ceftazidime	47(94)
Ciprofloxacin	37(74)
Imipenem	43(86)
Meropenem	43(86)
Aztreonam	26(52)
Gentamycin	27(54)
Ertapenem	48(96)
Lavofloxacin	39(78)

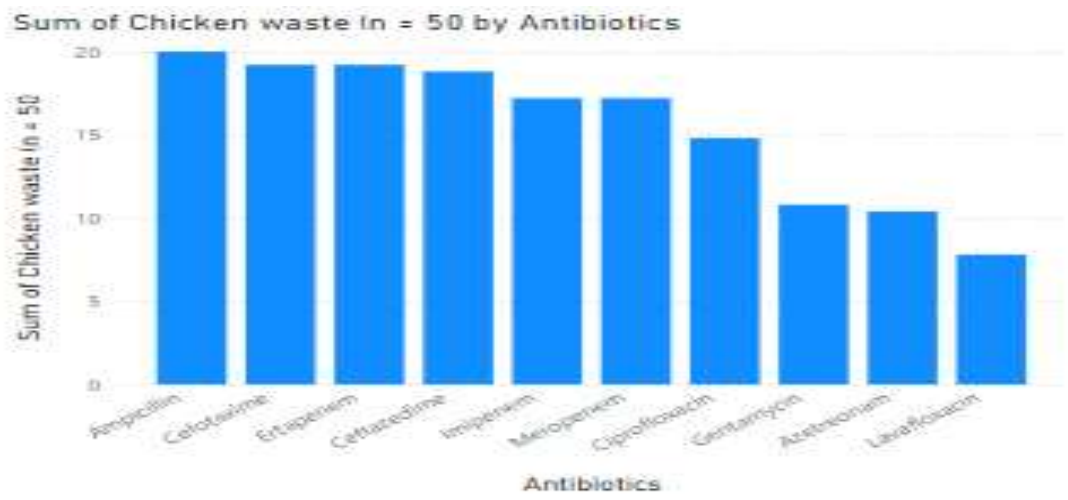

Figure 1: Sum of Chicken Faeces (N 50 by Antibiotics).

Table2. Antimicrobial Resistant Profile of *Salmonella* Isolated from Pig Feces Samples

ANTIBIOTICS	**No(%) of Resistant Isolates**
	Salmonella
	(n=50)
Ampicillin	49(98)
Cefotaxime	50(100)
Ceftazidime	50(100)
Ciprofloxacin	19(38)
Imipenem	37(74)
Meropenem	38(76)
Aztreonam	45(90)
Gentamycin	11(22)
Ertapenem	14(28)
Lavofloxacin	20(40)

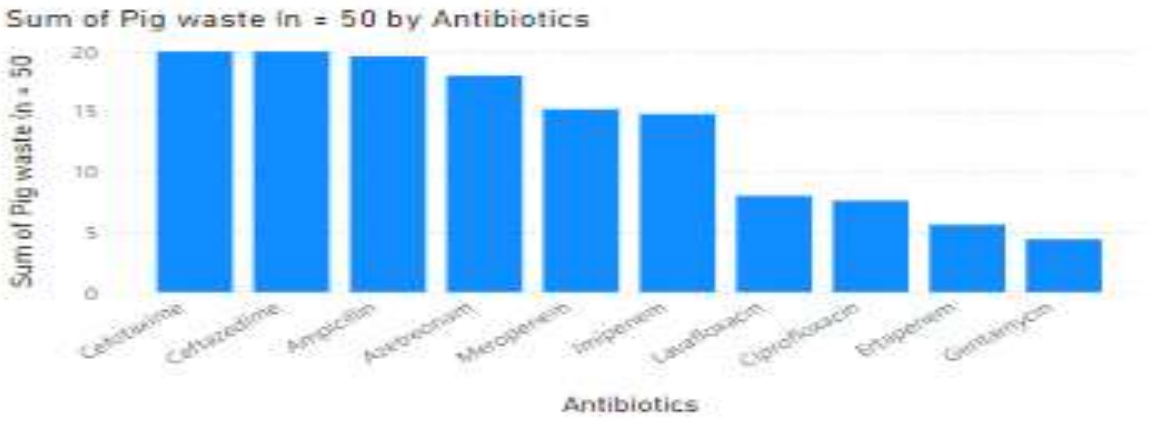

FIG 2.The Bar Chart Displays the Sum of Pig Feces (N =50) By Antibiotics

Table 3: Plasmid Profile of Cured and Uncured Isolates Using Gel Electrophoresis.

Salmonella Isolates After Curing	Resistant Profile	Resistant Profile Before Curing	Probable Organism
1.(chicken waste)	6	9	*Salmonella*
2.(pig waste)	6	5	*Salmonella*

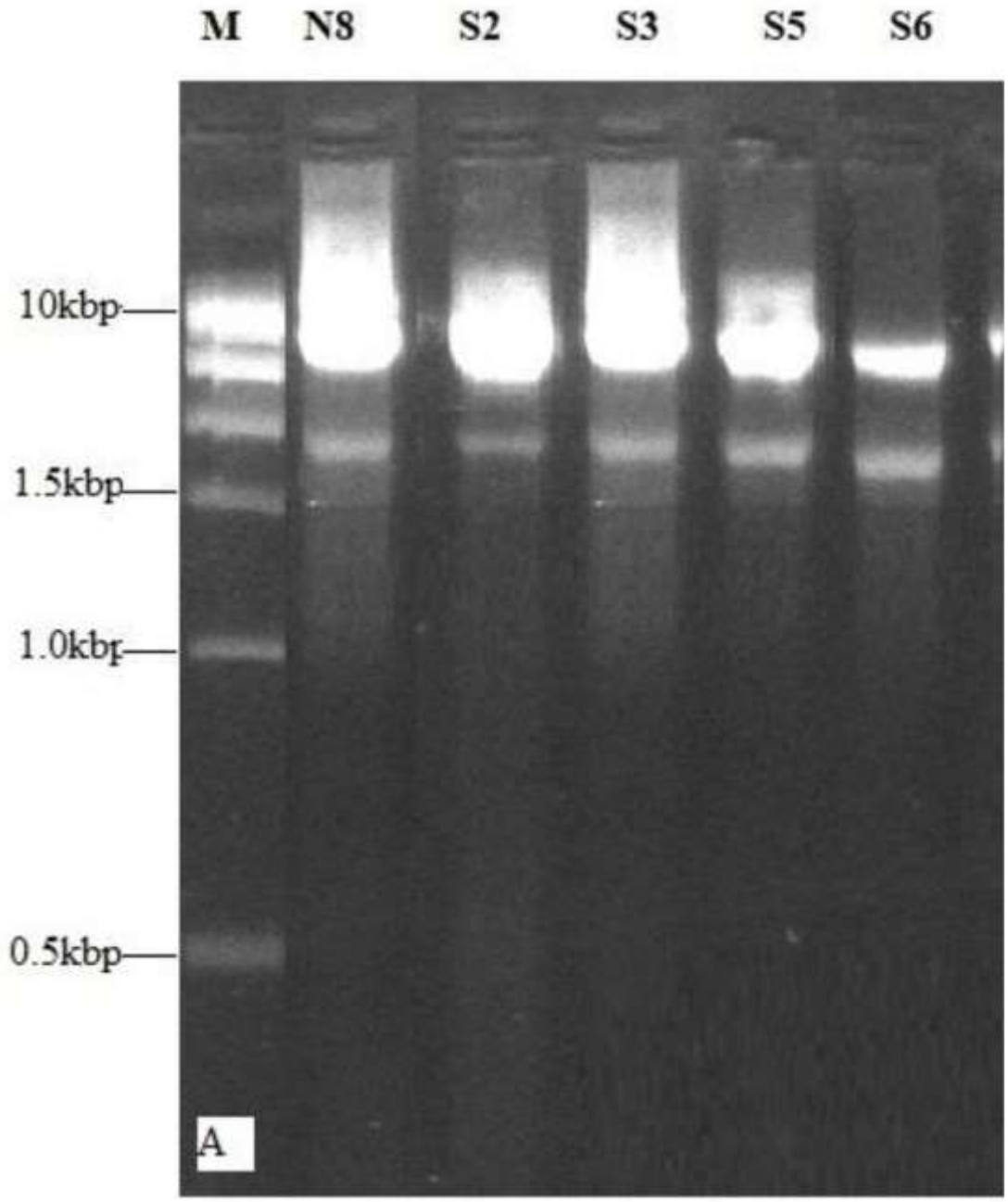

Figure 3: Plasmid Profile of the Salmonella Isolates.

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)

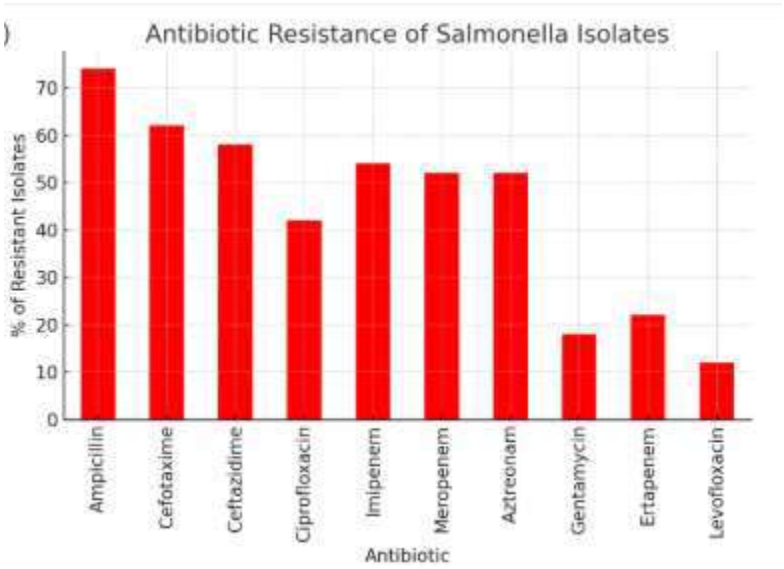

FIGURE 4: Antibiotic Susceptibility Analysis of Resistant Cured Isolates.

DISCUSSION

The findings of this study demonstrate a high prevalence of antimicrobial resistance among *Salmonella* isolates from veterinary sources. High resistance rates were observed for Ampicillin, Cefotaxime, and Ceftazidime, consistent with global trends (Velasquez *et al.*, 2018). The high resistance to critically important antibiotics, as classified by the World Health Organization (WHO, 2019), is particularly concerning. Similar findings have been reported in Pakistan, where high resistance to cephalosporins and carbapenems was documented (Saeed *et al.*, 2020).

The persistence of resistance following plasmid curing suggests that not all resistance mechanisms are plasmid-mediated. This aligns with previous reports (Ehiaghe *et al.*, 2013; Okoye *et al.*, 2022), indicating the role of chromosomal resistance determinants. The presence of plasmids within the 4–10 kbp range reflects genetic diversity among isolates

(Ekundayo, 2021). The incomplete elimination of plasmids further suggests variability in plasmid stability and curing efficiency. Additionally, mechanisms such as efflux pumps, gene mutations, and reduced membrane permeability may contribute to resistance (Richard *et al*., 2016).

CONCLUSION

Salmonella remains a significant foodborne pathogen with serious public health implications. The presence of multidrug-resistant strains, particularly those harboring transferable plasmids, poses a major threat to effective treatment. The high resistance rates observed in this study highlight the urgent need for rational antibiotic use, improved infection control practices and enhanced surveillance systems. The continued emergence of multidrug-resistant *Salmonella* strains could severely limit therapeutic options, particularly in developing countries. Therefore, stricter regulation of antibiotic use in animal production and improved hygiene practices are strongly recommended.

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