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# Antibiotic Resistance Profile of Porcine Salmonella in Nasarawa state, North Central Nigeria

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## Abstract

Salmonellosis is among the important zoonosis attracting global attention recently. The burden of this pathogen is rising due to antibiotics resistance threatening food safety, therapeutic outcomes and manpower productivity mostly in developing nations. The aim of this study was to determine the Antibiotic resistance profile of *Porcine Salmonella* in Nasarawa State, Nigeria. A total of 637 freshly voided fecal samples were collected and subjected to culture and isolation according to methods described by Office International des epizooties (OIE). Antibiotic susceptibility patterns of the isolates were determined against mostly used antibiotics in veterinary and human medicine. The Antimicrobial susceptibility testing revealed that all the isolates were resistant to Erterpenem (100%),some highly resistant to Penicillin G (96.7%) and Erythromycin (90.0%) but susceptible to Cefepime (96.7%), Sulphamethoxazole (83.3%), Chloramphenicol (80.0%), Ceftriaxone (76.7%), Ampicillin (60.0%), Cefoxitin (73.3%), Imipenem (53.3%), Nitrofurantoin (43.3%), Cefotaxime (33.3%), Ciprofloxacin (30.0%), Pefloxacin, Amikacin and Gentamicin (23.3%), Tetracycline and Amoxicillin/Clavulanic acid (13.3%) while susceptibility for Erythromycin and Penicillin G was 3.3%. Intermediate resistance ranged from 43.3% for Amoxicillin/Clavulanic acid, 36.7% for Amikacin, 33.3% for Ciprofloxacin, 30% for Nitrofurantoin, 26.7% for Cefotaxime and Tetracycline, 20.0% for Ceftazidime and Gentamicin,16.7% for Ampicillin and Imipenem, 10.0% for Chloramphenicol and Cefoxitin while Sulphamethoxazole/Trimethoprim, Ceftriaxone and Erythromycin had 6.7%. The MAR index ranged from 0.21 to 0.97 indicating high levels of environmental contamination with antimicrobials. This study confirms pigs as reservoirs of resistant *Salmonella* highlighting the need for One-Health approach to safeguard the health of the populace.

**Keywords**: Antibiotic Resistance, Porcine Salmonella, Nasarawa State

## Introduction

*Salmonella* belongs to the genus of bacteria well-known for its significant role as a causative agent of foodborne illness globally, particularly in humans. It includes various serotypes, with *Salmonella enterica* serotype Typhimurium *and Salmonella enterica* serotype Enteritidis being the most commonly associated with human infections (Centers for Disease Control and Prevention [CDC], 2020). Clinical manifestations of salmonellosis ranges from mild gastroenteritis to severe systemic infections including typhoid fever often leading to hospitalization and in some cases even death (Majowicz et al., 2010). While antibiotics are often used in the treatment of severe cases, there is still rising resistance among *Salmonella* strains even to last resort antibiotics presenting significant public health challenge,

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complicating treatment options and increasing morbidity and mortality rates associated with these infections (Rowe & Gleeson, 2019).

Antibiotic resistance profiles of *Salmonella* present a daunting challenge for healthcare systems globally. The complex interplay of various resistance mechanisms poses significant threats to effective treatment strategies necessitating continuous research and surveillance. Antibiotic resistance in *Salmonella* is multifaceted and stems from various factors including indiscriminate and or overuse of antibiotics in human medicine, livestock and agriculture, poor sanitation practices as well as the natural genetic adaptability of bacteria (Lamichhane, et al., 2024; McEwen & Fedorka-Cray,2002). Recently, the World Health Organization (WHO) report alarming and prevalent trends of the emergence of multi-drug resistant (MDR) strains (WHO,2014) complicating existing treatment protocols and driving the need for alternative therapeutic strategies (Magiorakos et al.,2012). The mechanisms of antibiotic resistance in *Salmonella* are diverse involving various genetic factors. Plasmids, transposons, and chromosomal mutations which further contribute to the spread and persistence of resistance genes particularly extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases have been identified as key enzymes driving resistance to multiple classes of antibiotics including penicillins and cephalosporins (Wang et al., 2015). Additionally, efflux pumps and changes in membrane permeability additionally serve as routes by which *Salmonella* can evade the action of antibiotics (Li et al., 2015; Oliveira & Pires, 2019). Furthermore, horizontal gene transfer among bacterial populations further allows for resistance traits to disseminate widely thereby affecting not only *Salmonella* species but also other commensal and pathogenic bacteria (Alekshun & Levy, 2007; Sanchez et al., 2010).

The alarming trends of resistance by *Salmonella* underscores the need to monitor resistance profiles of *Salmonella* species. This study was therefore conducted in order to provide *Porcine Salmonella* antibiotic resistance profile data and evidence towards appropriate antibiotic usage, stewardship and other integrative approaches including enhanced surveillance systems and public health policies alongside educational campaigns targeting both the medical community and the general public which are critical to managing public health threat (Molbak, 2003; Hossain et al., 2018).

## Materials and methods

Freshly voided fecal samples were collected from pig farms from January to September of 2024 (n=637) from urban, sub-urban and rural settlements in all the Local Government Areas of Nasarawa State. Samples were stored in sterile zip lock bags and placed at $4^{O}$C then transported to the laboratory for microbial analysis within five hours.

### Sample processing for *Salmonella* isolation

All the samples collected were processed according to standard methods for *Salmonella* isolation described by ISO 6579 (2020) and OIE Terrestrial Manual (2018) for diagnostic test and vaccines for domestic and terrestrial animal's standard protocol in duplicates. For pre-enrichment, one gram (1g) of freshly voided fecal samples was added into 10 mL of Buffered Peptone water (BPW) and incubated at between $37^{O}$C for 24 hours. For enrichment, 1 mL of the broth culture was added to 9 mL of enrichment media, Muller -Kauffmann Tetrathionate Novobiocin (MKTTn) broth and incubated at $37^{O}$C for 24 hours. A quantity of 0.1ml of the culture was then inoculated onto the surface of freshly prepared Xylose Lysine Deoxycholate (XLD) agar using streak plate method, then incubated again at $37^{O}$C for 24 hours. After incubation, typical colonies of *Salmonella* on XLD agar had a black center and a lightly transparent reddish zone. The suspected *Salmonella* colonies were transferred onto nutrient agar plates, incubated at $37^{O}$C for 18-24 hours and subsequently subjected to preliminary

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identification; oxidase and indole test, Triple Sugar Iron (TSI) test for gas and hydrogen sulphide ($H_2S$) production, citrate and urease utilization, sugar fermentation (glucose, lactose, sucrose, maltose, dulcitol, mannitol, inositol, ramnose, sorbitol, mannose, arabinose, malonate, and trehalose) and lysine decarboxylation. The *Salmonella* isolates were stored at -80$^{O}$C for further characterization.

The antibiotic susceptibility testing of the *Salmonella* isolates was carried out according to standard methods of analysis as described by the Clinical and Laboratory Standard Institute (CLSI) M100 (2020) and the WHO (2018) Standard Operating Procedures for Antimicrobial Susceptibility Testing. Briefly, the isolates to be tested were purified by sub culturing on nutrient agar plates and incubated at 37$^{O}$C for 18-24 hours. A sterile loop was used to pick small portion of a well- isolated colony and transferred to a tube of sterile distilled water. The inoculum was emulsified and standardized to 0.5 McFarland which equals approximately to $10^8$CFU/mL using a Nephelometer.

Using a sterile cotton swab, the standardized inoculum of the organism was spread evenly unto Muller Hinton agar, and the plates were allowed for 5 – 15 minutes for the suspension to be adsorbed into the agar. Using a disc dispenser, Sulphamethozazole/Trimethoprim (STX; 25 µg), Ceftriaxone (CRO; 30 µg), Ampicillin (AMP; 10 µg), Imipenem (IPM;10 µg), Ceftazidime (CAZ;30 µg),Tetracycline (TE; 30 µg), Amoxicillin/Clavulanic acid (AMC; 30 µg), Cefoxitin (FOX; 30 µg), Cefepime(FEP; 30 µg), Ciprofloxacin(CIP; 1 µg), Nitrofurantoin (F; 300 µg), Cefotaxime(CTX; 30 µg), Chloramphenicol (C;30 µg), Pefloxacin (PEF; 5 µg), Gentamicin (CN;10 µg), Ertapenem (ETP; 10 µg), Amikacin (AK;30 µg), Erythromycin (E;15 µg) and Penicillin G (P;10 µg) antibiotics discs were dispensed onto the agar surface. The inoculated plates were incubated aerobically at 35$^{O}$C for 16-18 hours. Interpretation of the diameter zones of inhibition were determined using the CLSI M100 (2020) standards.

**Result and Discussion**

The phenotypic antibiotic susceptibility profile of *Porcine Salmonella* isolates is presented on Table 1. There was absolute resistance to Erterpenem (100%), high resistance to Penicillin G (96.7%) and Erythromycin (90.0%) but susceptibility to Cefepime (96.7%), Sulphamethoxazole (83.3%), Chloramphenicol (80.0%), Ceftriaxone (76.7%), Ampicillin (60.0%), Cefoxitin (73.3%), Imipenem (53.3%), Nitrofurantoin (43.3%), Cefotaxime (33.3%), Ciprofloxacin (30.0%), Pefloxacin, Amikacin and gentamicin (23.3%), Tetracycline and Amoxicillin/Clavulanic acid (13.3%) was recorded while susceptibility for Erythromycin and Penicillin G was 3.3% respectively. Intermediate resistance was observed in most of the antibiotics examine and ranges from 43.3% for Amoxicillin/Clavulanic acid, 36.7% for Amikacin, 33.3% for Ciprofloxacin, 30% for Nitrofurantoin, 26.7% for Cefotaxime and Tetracycline, 20.0% for Ceftazidime and Gentamicin,16.7% for Ampicillin and Imipenem, 10.0% for Chloramphenicol and Cefoxitin. Intermediate resistance observed for Sulphamethoxazole/Trimethoprim, Ceftriaxone and Erythromycin was 6.7% respectively.

The phenotypic antimicrobial susceptibility profile *of Porcine Salmonella* isolates in this study demonstrates an alarming pattern of resistance particularly against critical antibiotics such as Ertapenem, Penicillin G, and Erythromycin. As observed in this study, the absolute resistance to Ertapenem (100%) is alarming due to the significance of this antibiotic as a part of the carbapenem class, which is often considered the last resort for treatment of multi-drug resistant infections (Elipsha et al., 2024). High resistance to mostly used veterinary antibiotics like Penicillin G (96.7%) and Erythromycin (90.0%) further underscore the rise and persistence of antibiotic resistance in livestock. The implications for both animal health and food safety is overwhelming (Gao et al., 2023). Meanwhile, higher susceptibility rates observed for Cefepime (96.7%), Sulphamethoxazole (83.3%), and Chloramphenicol (80.0%) indicate some remaining

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effectiveness of these agents against *Porcine Salmonella*. However, existence of intermediate susceptibility and a significant number of isolates that exhibited multi-drug resistance (MDR) poses a threat to therapeutic options. For instance, intermediate resistance levels noted in commonly used antibiotics such as Ciprofloxacin (33.3%) and Nitrofurantoin(30%), suggest that these agents may soon lose effectiveness if the trends of resistance persist (Tadesse et al., 2017).

**Table 1. Percentage Antimicrobial Susceptibility patterns of Porcine Salmonella isolated from fecal samples (n=30)**

| Antimicrobial agents | Resistance (%) | Intermediate (%) | Susceptible (%) |
|---|---|---|---|
| Sulphamethoxazole/ Trimethoprim (STX) | 3(10.0) | 2(6.7) | 25(83.3) |
| Ceftriaxone (Cro) | 5(16.7) | 2(6.7) | 23(76.7) |
| Ampicillin (Amp) | 7(23.3) | 5(16.7) | 18(60.0) |
| Imipenem (Ipm) | 9(30.0) | 5(16.7) | 16(53.3) |
| Ceftazidime (Caz) | 20(66.7) | 6(20.0) | 4(13.3) |
| Tetracycline (Te) | 18(60.0) | 8(26.7) | 4(13.3) |
| Amoxicillin/Clavulanic acid (Amc) | 13(43.3) | 13(43.3) | 4(13.3) |
| Cefoxitin (Fox) | 5(16.7) | 3(10.0) | 22(73.3) |
| Cefepime (Fep) | 1(3.3) | 0(0.0) | 29(96.7) |
| Ciprofloxacin (Cip) | 11(36.7) | 10(33.3) | 9(30.0) |
| Nitrofurantoin (F) | 8(26.7) | 9(30.0) | 13(43.3) |
| Cefotaxime (Ctx) | 12(40.0) | 8(26.7) | 10(33.3) |
| Chloramphenicol (C) | 3(10.0) | 3(10.0) | 24(80.0) |
| Pefloxacin (Pef) | 23(76.7) | 0(0.0) | 7(23.3) |
| Gentamicin (Cn) | 17(56.7) | 6(20.0) | 7(23.3) |
| Ertapenem (Etp) | 30(100) | 0(0.0) | 0(0.0) |
| Amikacin (Ak) | 12(40.0) | 11(36.7) | 7(23.3) |
| Erythromycin (E) | 27(90.0) | 2(6.7) | 1(3.3) |
| Penicillin G (P) | 29(96.7) | 0(0.0) | 1(3.3) |

The Multi-Drug Resistance (MDR) and Multiple Antibiotic Resistance (MAR) Index of *Porcine Salmonella* isolates is presented on table 2. The isolates showed high levels of resistance to various classes of antimicrobial agents. The MAR index ranged from 0.16 (isolates resistant to 3 antibiotics) to 0.79 (isolates resistant to 15 antibiotics). The isolates exhibited Multidrug resistance to three or more different classes of antibacterial agents.

The identification of MDR phenotype in 100% of the isolates in this study raises profound concerns on potential transmission of resistant strains from animals to humans particularly through the food supply chain. The MAR index findings which indicated some isolates resistant to up to 15 antibiotics underscore the severity of MDR. The higher MAR index (0.79) reflects the accumulation of selective pressure due to the inappropriate and excessive use of antibiotics in animal husbandry (Lopez-Aparicio et al., 2020). From similar studies, there are empirical evidences that confirms the indiscriminate use of antibiotics in food animals contributing significantly to the prevalence of AMR bacteria in both animal and human populations (Van Boeckel et al., 2015). As such, the high levels of resistance observed in this study are potentially reflective of farming practices that favor the routine use of prophylactic and therapeutic antibiotics that promote the emergence and dissemination of resistant strains of *Porcine Salmonella* (Khachatryan et al., 2023). This rapid rise in resistance rates among *Salmonella* isolates necessitates a holistic approach involving both veterinary and human health

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sectors (One Health approach). Various initiatives such as implementing stricter regulations concerning antibiotic use in agriculture, improving biosecurity measures and enhancing surveillance programs is necessary to mitigate antibiotic resistance crisis (Aarestrup, 2015).

**Table 2. Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of individual Salmonella isolated from Porcine fecal samples**

| Isolate ID | Antibiotics Resistance Profile | Number of Antibiotics | MAR Index |
|---|---|---|---|
| NEG45 | Caz, Te, Pef, Etp, Ak, E, P | 7 | 0.37 |
| AND40 | Caz, Te, Pef, Cn, Etp, Ak, E, P | 8 | 0.42 |
| KR13 | Caz, Te, Amc, Cip, F, Pef, Cn, Etp, E, P | 10 | 0.53 |
| WC12 | Amp, Caz, Te, Amc, Fox, Pef, Cn, Etp, Ak, E, P | 11 | 0.58 |
| AND11 | Ipm, Caz, Te, F, Cn, Etp, E, P | 8 | 0.42 |
| AK55 | Caz, Te, Amc, F, Ctx, Pef, Etp, Ak, E, P | 10 | 0.53 |
| AND48 | STX, Amp, Te, Amc, Cip, Ctx, Pef, Cn, Etp, Ak, E, P | 12 | 0.63 |
| KR26 | Ipm, Caz, Cip, Pef, Etp, Ak, P | 7 | 0.37 |
| NEG77 | Cro, Amp, Ipm, Caz, Te, Amc, Fox, Cip, Ctx, Pef, Cn, Etp, E, P | 14 | 0.74 |
| AND10 | Cro, Caz, Te, Amc, Cip, F, Ctx, Pef, Cn, Etp, Ak, E, P | 13 | 0.68 |
| AW46 | Caz, F, Ctx, Cn, Etp, E, P | 7 | 0.37 |
| AND49 | Caz, Pef, Cn, Etp, Ak, E, P | 7 | 0.37 |
| NEG85 | Amp, Ipm, Caz, Te, Amc, Fox, Pef, Cn, Etp, E, P | 11 | 0.58 |
| WS22 | Stx, Te, Cip, C, Pef, Cn, Etp, E, P | 9 | 0.47 |
| AND21 | Ipm, Amc, Pef, Etp, Ak, E, P | 7 | 0.37 |
| KR27 | Cip, Pef, Etp | 3 | 0.16 |
| NEG87 | Ipm, Caz, Te, Cip, Ctx, Pef, Cn, Etp, E, P | 10 | 0.53 |
| WS5 | Cro, Amp, Ipm, Caz, Te, Amc, Fox, Ctx, Pef, Cn, Etp, Ak, E, P | 14 | 0.74 |
| AK23 | Stx, Cro, Amp, Ipm, Caz, Te, Amc, Cip, F, Ctx, C, Pef, Etp, E, P | 15 | 0.79 |
| WW3 | Te, Amc, Fep, F, Ctx, Cn, Etp, E, P | 9 | 0.47 |
| NEG70 | Cro, Ipm, Caz, Ctx, Pef, Cn, Etp, E, P | 9 | 0.47 |
| AK3 | Caz, Pef, Etp, E, P | 5 | 0.26 |
| AND42 | Caz, Etp, E, P | 4 | 0.21 |
| AK17 | Amc, Pef, Etp, Ak, E, P | 6 | 0.32 |
| AND38 | Caz, Etp, E, P | 4 | 0.21 |
| WC25 | Amp, Te, Amc, Pef, Etp, P | 6 | 0.32 |
| AW56 | Te, Cip, Pef, Cn, Etp, E, P | 7 | 0.37 |
| NEG46 | Etp, Ak, E, P | 4 | 0.21 |
| WC26 | Fox, F, Ctx, C, Etp, E, P | 7 | 0.37 |
| AK40 | Caz, Te, Cip, Ctx, Pef, Cn, Etp, Cn, E, P | 10 | 0.53 |

Stx: sulphamethozazole/Trimethoprim; Cro: Ceftriaxone; Amp: Ampicilin; Ipm: Imipenem; Caz: Ceftazidime; Te: Tetracycline; Amc: Amoxycilin/Clavulanic acid; Fox: Cefoxitin; Fep: Cefipime; Cip: Ciprofloxacin; F: Nitrofurantoin; Ctx: Cefotaxime; C: Chloramphenicol; Pef: Pefloxacin; Cn: Gentamicin; Etp: Ertapenem; Ak: Amikacin; E: Erythromycin; P: Penicillin G.

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## Conclusion

The antimicrobial resistance profile of *Porcine Salmonella* in Nasarawa State has been established. All the isolates were resistant to Enterpenem and susceptible Cefepime. A high multiple antibiotic resistance (MAR) index was established, indicating a significant rate of resistance to the classes of commonly used antibiotics for treating infections in humans and animals. These findings elucidate a significant challenge in the management of antimicrobial resistance in *Porcine Salmonella* isolates. With high the levels of resistance observed across multiple antibiotic classes, a robust response involving policy changes, antibiotic stewardship and enhanced as well as coordinated AMR surveillance systems is necessary in order to mitigate and curtail antibiotics resistance for public health safety.

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## Conflict of Interest

All the authors declare that they have no competing interest.

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