

ISOLATION AND CHARACTERIZATION OF MULTIDRUG RESISTANT *SALMONELLA* SPECIES FROM ABATTOIR WASTEWATER IN ABIA STATE, NIGERIAEdward, K.C.^{1,2}, Ibekwe, V.I.², Amadi, E.S.² and Umeh, S.I.³

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ABSTRACT

Over the years, zoonotic bacteria of the genus *Salmonella*, have acquired antimicrobial resistance properties, with a wide variety of resistance genes and resistance-mediating mutations being identified. This study isolated and characterized multiple drug resistant *Salmonella* species isolated from abattoir wastewaters in Abia State, Nigeria. Seven hundred wastewater samples from three abattoirs: Aba (300), Ubakala (250) and Lokpa (150) samples were analyzed from 2016 to 2019. Standard microbiological procedures were followed in isolation and identification of the *Salmonella* spp isolates. The antibiotic susceptibility test was done using the Kirby Bauer disk diffusion method. The results showed moderate but significant prevalence of *Salmonella* spp in the three abattoir locations; 135(45.0%) from Aba, 108 (43.2%) from Ubakala and 74 (49.3%) from Lokpa. The antibiotic susceptibility pattern of the *Salmonella* spp isolates show that the organisms were highly susceptible to Ofloxacin (84.4% in Aba, 89.8% in Ubakala and 82.4% in Lokpa) and highly resistant to Ampicillin (97.0% in Aba, 91.7% in Ubakala and 98.6% in Lokpa). Most isolates recorded Multiple Antibiotic Resistance (MAR) index greater than (>0.2). This result emphasizes the urgent need for regulation in the use of antibiotics in man and animals and their subsequent disposal into the environment.

Keywords: wastewater, antibiotic, resistance, *Salmonella* spp, abattoir

INTRODUCTION

Antimicrobial resistance (AMR) transmission can be spread between people, animals and the environment through different routes (Woolhouse *et al.*, 2015) with the environment acting as a bridge between animals to compost to soil to water to sediments to sewage (Fouz *et al.*, 2020). The environment serves the dual role of a reservoir and mobile genetic elements (MGEs) mixer that interact and diffuse into other parts or into human and animal host or the environment acts as the reservoir as well as work simultaneously to mix MGEs that interact (Woolhouse *et al.*, 2015). The situation of Antimicrobial resistance genes (ARGs) is complex as they cannot be degraded and can be spread through horizontal gene transfer among microbial communities especially enterobacteriaceae (Stecher *et al.*, 2012).

The genus *Salmonella* are normally found in the intestinal tract of various warm and cold blooded animals and humans (Sterzenbach *et al.*, 2013). There are about 2600 serotypes that have been identified using the standard Kauffman-White scheme and most serotypes can adapt within a variety of animal hosts including humans (Eng *et al.*, 2015). Based on the differences in their 16S rRNA sequence analysis, the genus *Salmonella* is classified into two species, *Salmonella enterica* and *Salmonella bongori* (Dhama *et al.*, 2013). *Salmonella enterica* is further divided into six subspecies which are denoted with roman numerals: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica* based on their biochemical properties and genomic relatedness (Eng *et al.*, 2015). Among all the subspecies of *Salmonella*, *S. enterica* subsp. *enterica*(I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals. In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Brenner *et al.*, 2000). More than 150 serotypes is incriminated in foodborne salmonellosis (Dhama *et al.*, 2013).

Salmonella is one of the leading causes of infection in food producing animals such as poultry, cattle and swine (Magwedere *et al.*, 2015) and can be present in the faeces of these animals and maybe transferred to raw foods of animal origin during slaughtering and processing. Most of the *Salmonella* infections in humans cause mild gastroenteritis, however life-threatening systemic infections are common especially among high risk groups (Fagbamila *et al.*, 2017). *Salmonella* normally has long periods of latent carriage with occasional faecal shedding in food producing animals, which is the leading source of contamination of food, water and environment.

Salmonella infections remain a major public health concern worldwide, being responsible for the economic burden of both developed and developing countries as a result of cost associated with its surveillance, prevention and treatment. (Eng *et al.*, 2015). The high prevalence and dissemination of Multidrug resistant (MDR) *Salmonella* have become a growing public health concern with increasing

number of *Salmonella* isolates being resistant to clinically important antimicrobials such as fluoroquinolones and 3rd generation cephalosporins used in the treatment of life threatening conditions in humans (Amera *et al.*, 2017). The high prevalence is linked to the use of antimicrobials in Public health and Veterinary sectors as well as the environment (Addis *et al.*, 2011).

Humans are the sole reservoir of the typhoidal *Salmonella* (TS) while non typhoidal *Salmonella* (NTS) are predominantly found in animal reservoirs and have been isolated from the gastrointestinal tract of birds and mammals (Thomas *et al.*, 2020). It has been reported that despite improvements in hygiene in both industrialized and developing countries like Nigeria, non typhoidal *Salmonella* infections are on the increase (Majowicz *et al.*, 2010) with NTS being endemic in underdeveloped countries especially in Africa. NTS transmission to humans can occur through the ingestion of food and/ or water contaminated with infected animals' waste, direct contact with infected animals or consumption of infected food animals (Eng *et al.*, 2015). *Salmonella* infection outbreaks are associated with incomplete cooking of food products, improper storage and direct contact with raw ingredients (Eng *et al.*, 2015). Infection can also occur as a result of contact with environment contaminated with animal faeces and subsequent accompanying ingestion of pathogens (Thomas *et al.*, 2020).

NTS infections though usually self limiting in healthy humans can lead to complications if not properly managed especially in children, the elderly and in immunocompromised persons like those with some diseases like HIV/AIDS and cancer who are taking certain immunosuppressive drugs; and those with inherited diseases that affect the immune system (UN, 2017). Bacteraemia and immunological syndromes such as reactive arthritis have been linked to NTS (Thomas *et al.*, 2020).

Sources and modes of transmission of non-typhoidal *Salmonella* are not well understood in Africa due to absence of coordinated natural epidemiological surveillance systems (Kagambega *et al.*, 2013). Hence, only few African countries report surveillance data on *Salmonella* resulting in limited information being available on *Salmonella* isolation in this continent. *Salmonella* species is variable from time to time so updated information on their resistance patterns are needed for proper selection and use of antimicrobial agents in a setting (Amera *et al.*, 2017). The present study therefore assayed the prevalence and antibiotic susceptibility pattern of multidrug resistant *Salmonella* species from abattoir wastewater in Abia State, Nigeria.

MATERIALS AND METHODS

Sample collection

Abattoir wastewater was collected aseptically in sterile screw capped bottles from three abattoirs in Abia State (Aba, Ubakala and Lokpa with samples sizes, 300, 250 and 150 respectively between May, 2017 and February, 2019. Samples were transported immediately and processed within 2h of collection.

Isolation and Identification

One (1) ml of well homogenized abattoir wastewater samples were inoculated into 9 ml of sterile peptone water and incubated at 37°C for 18-24h and then sub-cultured on *Salmonella-Shigella* (SS) agar and *Salmonella* Chromagar plus (Chromagar Id, France) using streak method and incubated aerobically at 37°C for 24-48h. After incubation, colonies were picked based on colonial morphology (the colonies which appear colourless with black centre on SS agar and violet on Chromagar plus) and sub-cultured on nutrient agar to obtain pure colonies which are then transferred into nutrient agar slants and stored in the refrigerator for further studies. Isolated organisms were identified biochemically in a systemic way following standard methods (Chesbrough, 2006).

Antimicrobial susceptibility testing

After isolation and identification of the isolates, in vitro antimicrobial testing was performed on Mueller-Hinton agar (Rapid Labs, UK) using the standard Kirby-Bauer disc diffusion method (Bauer et al., 1966). About 4-6 colonies from an overnight culture were suspended in 5 ml nutrient broth and incubated for 4h at 37°C. Turbidity of the broth culture was adjusted to match the 0.5 McFarland standard. This suspension was used to uniformly inoculate the surface of Mueller Hinton agar using sterile swab stick. Commercially standardized antibiotic discs containing 30 µg Ceftazidime (CAZ), 30 µg Cefuroxime (CRO), 10µg Gentamicin (GEN), 5µg Ciprofloxacin (CPR), and 5µg Ofloxacin (OFL), 10 µg Ampicillin (AMP), 30 µg Augmentin (AUG), 300 µg Nitrofurantoin (NIT), 30 µg Aztreonam (ATM), 30 µg Chloramphenicol (CHL), 10 µg Imipenem (IMP) and 25 µg Cotrimoxazole (COT) from Rapid Labs and Oxoid, UK were placed on the inoculated agar and allowed to stand for 30mins face up to enhance diffusion and then incubated at 37°C for 18-24h. The diameter zones of inhibition around the discs were measured after incubation and were interpreted as sensitive, intermediate and resistant according to clinical Laboratory Standard Institute guidelines (CLSI, 2014).

RESULTS

Table 1 shows the prevalence of *Salmonella* spp isolates from the three abattoirs sampled. *Salmonella* spp. had a prevalence of 135/300 (45.0%), 108/250 (43.2%) and 74/150 (49.3%) from Aba, Ubakala and Lokpa abattoirs respectively. The overall prevalence of the *Salmonella* isolates is 317/700 (45.3%).

Antibiotic susceptibility pattern

The antibiotic susceptibility pattern of the *Salmonella* spp. isolates from the three abattoirs is presented in Table 2. The results show that the organisms were highly susceptible to Ofloxacin (84.4% in Aba, 89.8% in Ubakala and 82.4% in Lokpa) and highly resistant to Ampicillin (97.0% in Aba, 91.7% in Ubakala and 98.6% in Lokpa) while the highest intermediate reaction was recorded against Ciprofloxacin ((57.8%, 71.2% and 75.7%) in Aba, Ubakala and Lokpa abattoirs respectively).

Multiple Antibiotic Resistance (MAR) Index

The percentage MAR index of the *Salmonella* spp isolates was shown in Figure 1. Most of the isolates (135(100%) from Aba, 108(100%) from Ubakala and 73(98.6%) from Lokpa) were found to have a MAR index greater than 0.2. Most Aba isolates 27 (20.0%) had a MAR index of 0.5 and 0.8 respectively. For Ubakala isolates, 33 (30.6%) had a high MAR index of 0.8 and in Lokpa, most of the isolates 24 (32.4%) recorded a MAR index of 0.7.

Multiple drug resistance

Among the 317 *Salmonella* spp isolated in this study, none of these isolates was susceptible to all the antibiotics used and none was resistant to only one antibiotic. Only one isolate (0.3%) was resistant to two antibiotics (MAR index= 0.2). 24 (7.6%) *Salmonella* isolates were resistant to 3 or 4 antibiotics (MAR index = 0.3), 40 (12.6%) were resistant to 5 antibiotics (MAR index=0.4), 45(14.2%) isolates were resistant to 6 antibiotics (MAR index= 0.5) while 207(65.3%) isolates were resistant to 7 or more antibiotics (MAR index = ≥0.6). The overall result of the multiple drug resistance (resistance to ≥3 antibiotics classes) is 316/317(99.7%).

Table 1 Prevalence of *Salmonella* spp. in waste water samples from the three abattoirs

Location	Total no of samples N (%)	No of positive samples N (%)		No of negative samples N (%)		Total N (%)
		Individual abattoirs	Overall	Individual abattoirs	Overall	
Aba	300 (42.9)	135 (45.0)	135 (42.6)	165 (55.0)	165 (43.1)	300(100)
Ubakala	250 (35.7)	108 (43.2)	108 (34.1)	142 (56.8)	142 (37.1)	250(100)
Lokpa	150 (21.4)	74 (49.3)	74 (23.3)	76 (50.7)	76 (19.8)	150(100)
Total	700 (100)		317 (100)		383(100)	700 (100)

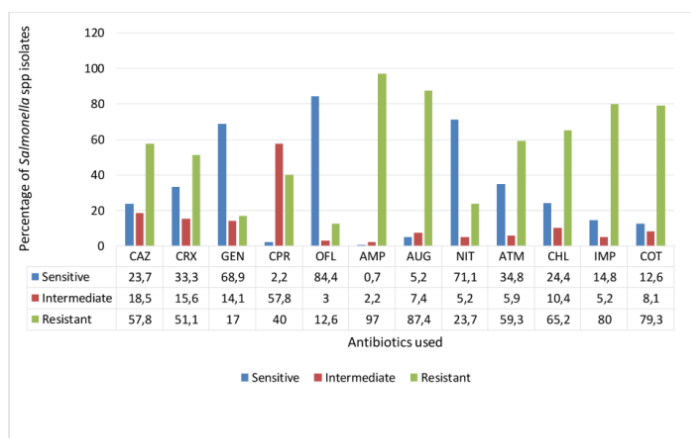


Figure 1 Antibiotic resistant profile of *Salmonella* spp isolates from Aba

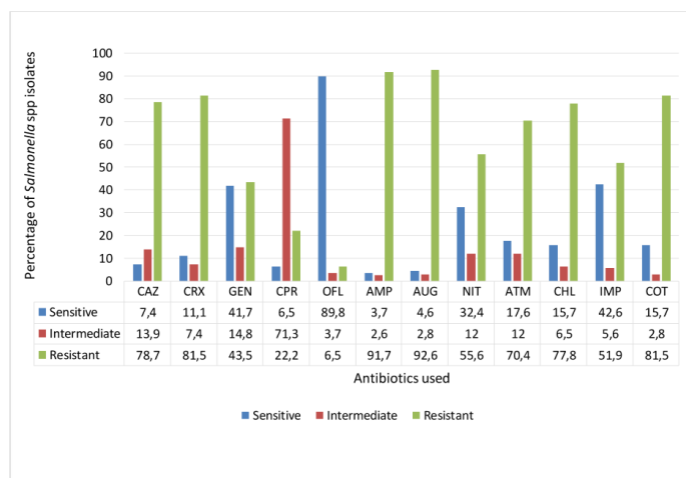


Figure 2 Antibiotic resistant profile of *Salmonella* spp isolates from Ubakala

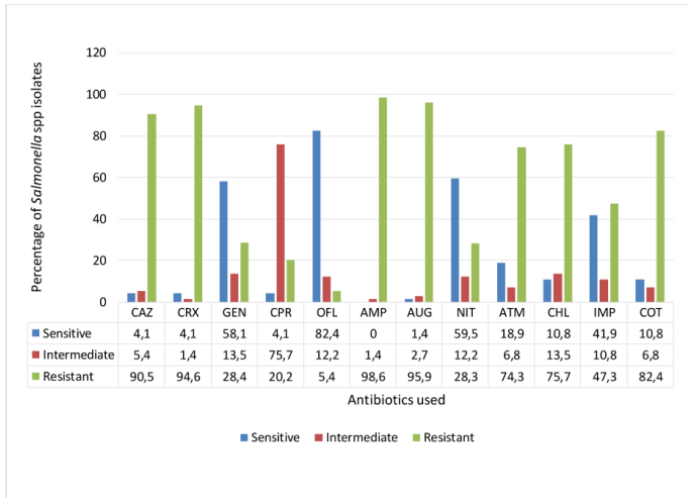


Figure 3 Antibiotic resistant profile of the *Salmonella* spp isolates from Lokpa

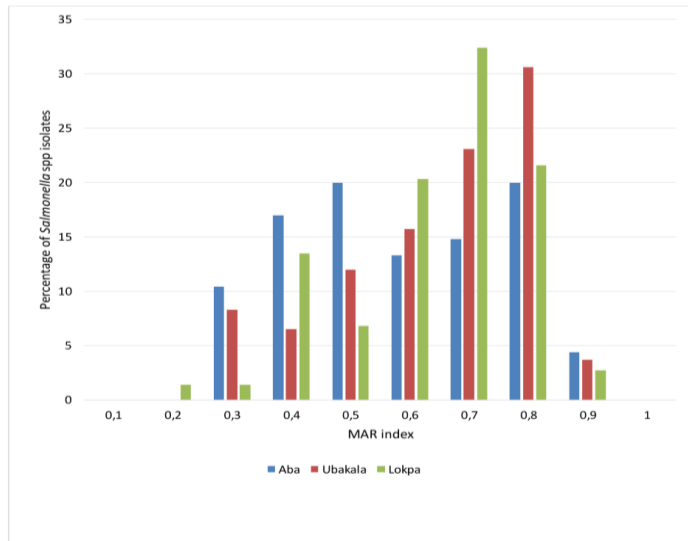


Figure 4 Percentage MAR index of the *Salmonella* spp. isolates from the three abattoirs.

DISCUSSION

Food and waterborne infections remain a continuous threat to human and animal health due to poor technology and unhygienic practices in abattoirs found in developing countries (Nwanta et al., 2010). *Salmonella* species are the dominant members of *Enterobacteriaceae* causing food and waterborne infections. The emergence and expansion of antibiotic resistance in *Salmonella* species is also an emerging public health hazard due to the compromised efficacy in the treatment of infectious diseases (Eng et al., 2015; Amera et al., 2017).

This study isolated and characterized multidrug resistant *Salmonella* spp. isolated from waste water from three abattoirs in Abia State, Nigeria. The high prevalence of *Salmonella* spp. recorded is not surprising as it is reported to be an environmentally relentless pathogen capable of living and multiplying in diverse surroundings (Winfield and Groisman, 2003). It is also known to be the most extensively spread organism that has the ability to cause foodborne infections and can be transmitted to man through water and cow (Egualle et al., 2018). Studies by Kupriyanoy et al. (2010) revealed that *Salmonella* can persist in farm surrounding for long period of time owing to movement of animals and humans inside the farm and from livestock excrement, soil and plants. *Salmonella* has been identified as a major food contaminant and the leading bacterial agent responsible for foodborne outbreaks in many countries (Majowicz et al., 2010). This study showed an overall prevalence rate of 45.3% for *Salmonella* species. This was found to be higher than 16.04% that was recorded by Gautam et al.

(2019), 22.64% and 30.19% recorded by Omeregbe et al. (2018), 31% by Adzitey, (2015) and 33.3% reported by Iroha et al., (2016) from abattoir effluents in Ogbete. In contrast, Ayogu et al. (2018) reported a high prevalence rate of 60% from meat market and Ogoja Road abattoir effluents in Abakiliki metropolis while Onuoha et al. (2016) recorded an even higher prevalence (64%) from Afikpo. The findings of Iyer et al. (2013) (45%) however, corroborates this study.

The *Salmonella* spp. isolates were found to have high resistance to most of the antibiotics used in this study. Similar results were obtained by Tesfaye et al. (2019). The problem of antibiotic resistance bacteria occurring in the environment is the serious threat it poses to public health, the higher disease burden it adds and the fact that the antibiotics effectiveness is reduced leading to increased mortality rate (Amaya et al., 2012; Igwaran et al., 2018).

The antibiotic susceptibility tests showed that *Salmonella* spp. were highly susceptible to Ofloxacin but showed varied susceptibility to Gentamicin, Nitrofurantoin and Imipenem with varying locations. This shows that Ofloxacin might be the drug of choice in the treatment of infection caused by the *Salmonella* spp. isolated in this work. Gentamicin, Nitrofurantoin and Imipenem can be used as second line drugs as far as treatment of infections caused by the *Salmonella* spp. isolates in this work is concerned.

Resistance of *Salmonella* to various antimicrobials and specifically to beta-lactam antibiotics and the Cephalosporins, the Phenicol and Sulphonamides is high although it varied according to location. This finding can be as a result of high selection pressure for resistant strains in the abattoir facilities which might be a consequence of excessive use and indiscriminate dumping of antimicrobials. Similar high rate of resistance has been reported in previous studies by Igbinoza et al. (2016) who recorded 100% resistance to Ampicillin and Chloramphenicol, 92.3% to Augmentin and 85.2% to Sulfamethoxazole. The *Salmonella* isolates in their study were found to be sensitive to Gentamicin (89.5%), and Ofloxacin (89.3%). However, sensitivity to Ciprofloxacin (95.8%) contrasted that recorded in this work with most of the isolates in this study recording intermediate sensitivity to Ciprofloxacin in all the locations. This suggests that these isolates are either in their intermediate phase of acquiring resistance or a higher dosage of the drug is required to achieve sensitivity.

Wright (2010) defined antibiotic resistance as the evolutionary response by bacteria to the strong selective pressure that results from exposure to antibiotics. The work of Ayogu et al., (2018) showed some similarity to the susceptibility pattern of this work with their *Salmonella* isolates showing high sensitivity to Gentamicin (90%), Ofloxacin (60%), Ciprofloxacin and Cefuroxime (20%) and Cefazidime (0%). High resistance was recorded against Cefazidime (100%), Cefuroxime and Ciprofloxacin (80%) respectively.

Generally, *Salmonella* isolates from Ubakala abattoir were only sensitive to Ofloxacin (89.8%). Their sensitivity to Imipenem (42.6%) and Gentamicin(41.7%) were below average. This could be linked to either their water source (fetch water in gallons from unidentified water sources) or poor personal and environmental hygiene which makes for a high bacterial load with antibiotic resistance genes transmissible through human, animal and the environment.

Multiple antibiotic resistance (MAR) index is a measure of extent of resistance to antibacterial agent. It is an indirect suggestion of the source of the organism with MAR values >0.2 indicating isolates recovered from high risk sources where strict rules concerning antibiotic prescription and usage are compromised (Gufe et al., 2019). Most of the *Salmonella* spp. (99.5%) isolates had MAR index greater than (>) 0.2.

Most *Salmonella* spp. from this study were found to be multidrug resistant (resistance to three or more antimicrobials (Magiorakos et al., 2012)). According to Eng et al. (2015), *Salmonella* species resistant to antimicrobials like Ampicillin, Chloramphenicol and Cotrimoxazole which are used as traditional first line drugs for *Salmonella* infections are referred to as multidrug resistant (MDR). The resistance of the *Salmonella* spp. isolates from this study to most of the clinically important antimicrobials (CIAs) such as fluoroquinolones and third generation cephalosporins used in the treatment of life threatening disease conditions in man (Sibhat et al., 2011) has significant public health importance especially with no new antimicrobial in sight that will successfully withstand the resistance mechanisms of the microorganisms. The emergence of multidrug resistance in *Salmonella* spp is mainly promoted by the use of antibiotics in animal feed for promotion of growth and for treatment of infections in veterinary medicine (Hycon et al., 2011).

CONCLUSION

This study revealed a high occurrence of multidrug resistant *Salmonella* species in abattoir wastewater from Abia State, Nigeria. The presence of antibiotic resistant organisms in abattoir wastewater is of great public health concern as *Salmonella* species harboring antimicrobial resistant genes could be widely distributed in not just the abattoir environment but to other environment. This may create a pool of transferable resistant genes which can be transferred to a wide range of related and unrelated commensal and pathogenic organisms. This study suggests that these antimicrobial resistant or multidrug resistant *Salmonella* species can pose a serious threat to the treatment of infections in man in the studied area being not only a source of infection, but also reservoirs of antimicrobial resistant genes. Hence, effective implementation of good hygiene practices (GHP) in slaughter houses, provision of potable water in abattoirs and regulation of the use of antibiotics in animal and animal feed is recommended.

Author contributions: This work was carried out in collaboration between all authors. Authors EKC and IVI conceptualized the work. The design of the experiment, and the experimental procedures were done by EKC, IVI, AES and USI. The planning of the studies and supervising of this work was done by IVI, AES and USI. EKC acted for all correspondences and drafted the manuscript. All authors participated in the manuscript revision and approved the final manuscript.

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