

Antimicrobial Resistance Profile of *Salmonella* Isolates from Livestock

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Abstract

Food animals are important reservoirs of infectious pathogens. The use of antimicrobial drugs in food animals is a major source of selection of drug resistant pathogens. This study investigated a total of 1000 faecal samples of livestock and poultry between January 2012 and March 2013 to determine the prevalence rate of *Salmonella* species and their antimicrobial resistance profiles. Faecal samples of chicken, pig, cattle, goat and sheep (200 samples of each) were pre-enriched in Tetrathionate broth and Rappaport Vassiliadis R10 broth. The broth culture was subcultured on XLD agar and incubated. The isolates were identified by standard biochemical tests and confirmed by AP1 20E test kit. Antimicrobial susceptibility was performed by disk diffusion method. The result showed that the prevalence of *Salmonella* spp. in all the samples was 21.8%. Chicken faeces had the highest prevalence rate of 52.5% followed by pig faeces (40%), cattle (10%), goat (4.5%) and sheep (2%). The isolates have resistance profile ranging from 1 to 9 antimicrobial drugs. Tetracycline had the highest resistance (81%) of all the isolates followed by Streptomycin (68%). Gentamycin had the lowest resistance profile of 14%. We conclude that *Salmonella* species have high prevalence rate in chicken and pig, but less in cattle, goat and sheep, and that most of the isolates are resistant to most commonly used antibiotics. Effort is needed to adopt measures to control the spread of multidrug resistant pathogens to humans. Care must be taken in the use of antibiotics in farm animals to reduce the selection of multidrug resistant strains.

Keywords

Antimicrobials, Faeces, Livestock, Multidrug Resistance, *Salmonella*

1. Introduction

The genus *Salmonella*, a gram negative oxidase-negative and lactose-negative motile *bacillus* is a member of the

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family Enterobacteriaceae. *Salmonella* is a ubiquitous and hardy bacterium that can survive several weeks in a dry environment and several months in water. It has many pathogenic species and strains. Daniel Salmon, an American veterinarian first isolated *Salmonella choleraesuis* from pigs with hog cholera in 1884 [1] hence the name “*Salmonella*”. *Salmonella* is an enteric pathogen and is the major cause of bacterial foodborne infections (*Salmonella typhimurium* and *Salmonella enteritidis*), enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteremia, endovascular infections, focal infection and enterocolitis (typically *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella heidelberg*).

The nomenclature and classification of *Salmonella* species have undergone several changes and rearrangements over the years. Traditionally, *Salmonella* species were named according to the Kaufmann-White typing system. This comprises different combinations of somatic, surface, and flagellar antigens (labelled as O, Vi and H antigens respectively) [2] [3].

Recently, *Salmonella* species have serologically defined names appended as serovars or serotypes. Hence, *Salmonella enterica* was officially approved as the type species of the genus *Salmonella*. For instance, the current nomenclature of *S. typhi* is *S. enterica* serovar Typhi. To date, more than 2500 serovars of *S. enterica* have been described. Some serovars are host-specific and can reside in only one or a few animal species, for example, *Salmonella* Dublin in cattle; and *Salmonella choleraesuis* in pigs. When these particular serotypes cause disease in humans, it is often invasive and can be life-threatening. Others have a broad host range [4]. Typically, such strains cause gastroenteritis, which is often uncomplicated and does not need treatment, but can be severe in the young, the elderly and patients with weakened immunity.

According to Nouichi and Hamdi [5] food borne diseases often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* spp., and *Escherichia coli* O157: H7. *Salmonella* bacteria are widely distributed in domestic and wild animals. They are prevalent in food animals such as poultry, pigs and cattle, and in pets including cats, dogs, birds and reptiles such as turtles. *Salmonella* can pass through the entire food chain from animal feed, primary production, and all the way to households or food-service establishments and institutions.

Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin such as eggs, meat, poultry and milk and green vegetables contaminated by faecal manure. Human cases occur where individuals have contact with infected animals, including pets. These infected animals often do not show signs of disease [6].

Since the beginning of the 1990s, *Salmonella* strains which are resistant to a range of antimicrobials have emerged and are now a serious public health concern. *Salmonella* has a widespread distribution in the environment and certain host factors make humans particularly susceptible to infection. Its increasing antimicrobial resistance, prevalence, virulence, and adaptability are a challenge worldwide.

Livestock and poultry producers rely on antibiotics to treat a host of diseases and infections as well as use low doses of antibiotics as growth promoters. Such treatments help promote the health and wellbeing of the animal. However, there are concerns about the potential influence of antibiotics on the development of bacterial resistant factors that limit antimicrobial efficacy [7].

In this study the prevalence rate of *Salmonella* species and antimicrobial resistance profile of *Salmonella* species isolated from livestock faeces in selected farms in Owerri municipality was described and evaluated.

2. Methodology

2.1. Sample Collection

A total of 1000 fresh faecal samples comprising 200 each of cattle, pig, sheep, goat and chicken droppings were collected from different livestock farms in Owerri, Nigeria, using sterile containers.

2.2. Culture and Isolation

One gram of faecal sample each from cattle, sheep, goat, pig, and chicken were pre-enriched in Tetrathionate broth (TTB) containing 2% iodine-iodide solution for 24 hours at 37°C [8] [9]. This was followed by selective enrichment of 0.1 ml of TTB culture in 10 ml Rappaport Vassiliadis R10 broth (Difco) This was incubated for 24 hours at 41°C ± 0.5°C [10] [11]. After incubation, a loopful of the enriched culture was sub-cultured onto

XLD agar (made up of Yeast extract 3.0 g, L-lysine Hydrochloride 5.0 g, Xylose 3.75 g, Lactose 7.5 g, Sucrose 7.5 g, Sodium desoxycholate 1.0 g, Sodium chloride 5.0 g, Sodium thiosulphate 6.8 g, Ferric ammonium citrate 0.8 g, Phenol red 0.08 g and Agar 12.5 g in one litre of deionized water; pH = 7.4 ± 0.2) (Oxoid, UK) and incubated at 35°C for 18 - 24 hours.

Red colonies with or without black centres growing on the XLD agar were picked, purified and gram-stained. Biochemical tests for *Salmonella* were performed. The isolates were further confirmed as *Salmonella* spp. using AP1 20E test kit (Biomerieux, France).

2.3. Antimicrobial Susceptibility Testing

All isolates were subjected to antimicrobial susceptibility testing using disk diffusion method.

A bacterial lawn was prepared by transferring bacterial colonies to a glass tube containing 5 ml sterile peptone water with a sterile inoculating loop.

The suspension was vortexed and visually matched with 0.5 MacFarland standard for turbidity [12].

Sterile cotton tipped swab was immersed in the suspension, excess fluid removed by rolling the swab on the upper part of the tube, and spread onto Mueller Hinton agar (Oxoid, UK) to obtain a semi-confluent growth.

Disks impregnated with predetermined amounts of antibiotics were dispensed onto the bacterial lawn and the plates were incubated for 18 - 24 hours at 35°C.

After the incubation, the diameter of the inhibition zones were measured and interpreted as sensitive or resistant using the criteria described by the Clinical and Laboratory Standards Institute [13].

The antibiotics used include:

Ampicillin 25 µg, Amoxicillin 25 µg, Augmentin 30 µg, Chloramphenicol 30 µg, Streptomycin 25 µg, Tetracycline 25 µg, Cephalothin 30 µg, Gentamycin 10 µg, Ciprofloxacin 5 µg, Sulfamethoxazole 25 µg, Nalidixic acid 30 µg.

3. Results

Out of the total of 1000 samples examined, 218 (21.8%) yielded positive cultures for *Salmonella* species. Chicken faeces yielded the highest number of *Salmonella* isolates of 105 (52.5% of the chicken faecal samples inoculated) while sheep faeces yielded the lowest (2%, ieonly 4 isolates out of 200 samples: **Figure 1**).

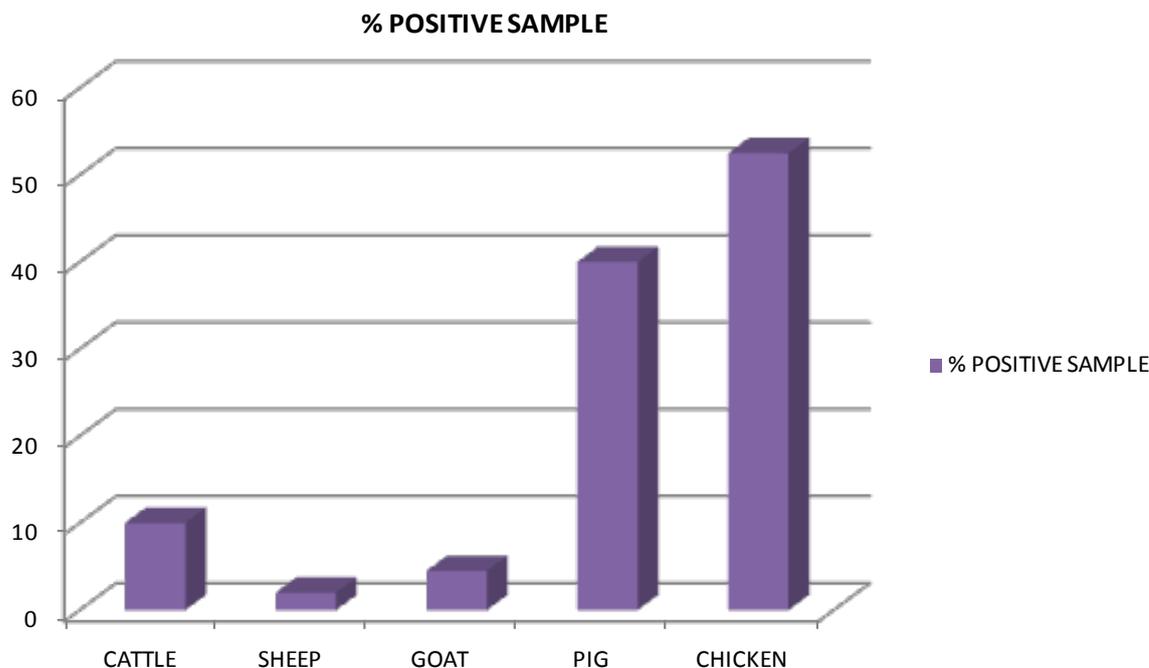


Figure 1. Percentage positive samples from each faecal sample. X-axis = source of livestock faeces; Y-axis = % positive sample.

Isolates from chicken, cattle and pig show highest resistance to most antimicrobial drugs (**Table 1**). Isolates from chicken show the highest resistance to Tetracycline followed by resistance to Nalidixic acid, Sulphamethoxazole, Streptomycin, Ampicillin and Cephalothin in that order. Isolates from pig follow similar pattern (**Figure 2**).

Tetracycline has the highest number of total resistant isolates from all the specimens put together. This was followed by Streptomycin and Nalidixic acid respectively. Gentamycin and ciprofloxacin have the least resistant isolates (**Figure 3**).

4. Discussion

This study has revealed that farm animals harbour a good number of *Salmonella* species (21.8% of all the samples

Table 1. Multidrug resistance pattern of *Salmonella* isolates from different specimens.

	Cattle (n = 20)	Sheep (n = 4)	Goat (n = 9)	Chicken (n = 105)	Pig (n = 80)	Total (n = 218)
Ampicillin	15 (75%)	1 (25%)	2 (22%)	70 (67%)	40 (50)	128 (59%)
Amoxicillin	3 (15%)	0 (0%)	1 (11%)	20 (19%)	15 (19%)	39 (18%)
Augmentin	7 (35%)	0 (0%)	1 (11%)	40 (38%)	20 (25%)	68 (31%)
Chloramphenicol	15 (75%)	1 (25%)	2 (22%)	75 (71%)	45 (56%)	138 (63%)
Streptomycin	15 (75%)	1 (25%)	2 (22%)	80 (76%)	50 (62%)	148 (68%)
Tetracycline	17 (85%)	1 (25%)	4 (44%)	95 (90%)	60 (75%)	177 (81%)
Cephalothin	10 (50%)	0 (0%)	2 (22%)	70 (67%)	40 (50%)	122 (56%)
Gentamycin	3 (15%)	0 (0%)	1 (11%)	14 (13%)	13 (16%)	31 (14%)
Ciprofloxacin	15 (75%)	0 (0%)	1 (11%)	10 (9.5%)	10 (13%)	36 (17%)
Sulfamethoxazole	10 (50%)	0 (0%)	2 (22%)	75 (71%)	45 (56%)	132 (61%)
Nalidixic acid	15 (75%)	1 (25%)	2 (22%)	80 (76%)	50 (62%)	143 (66%)

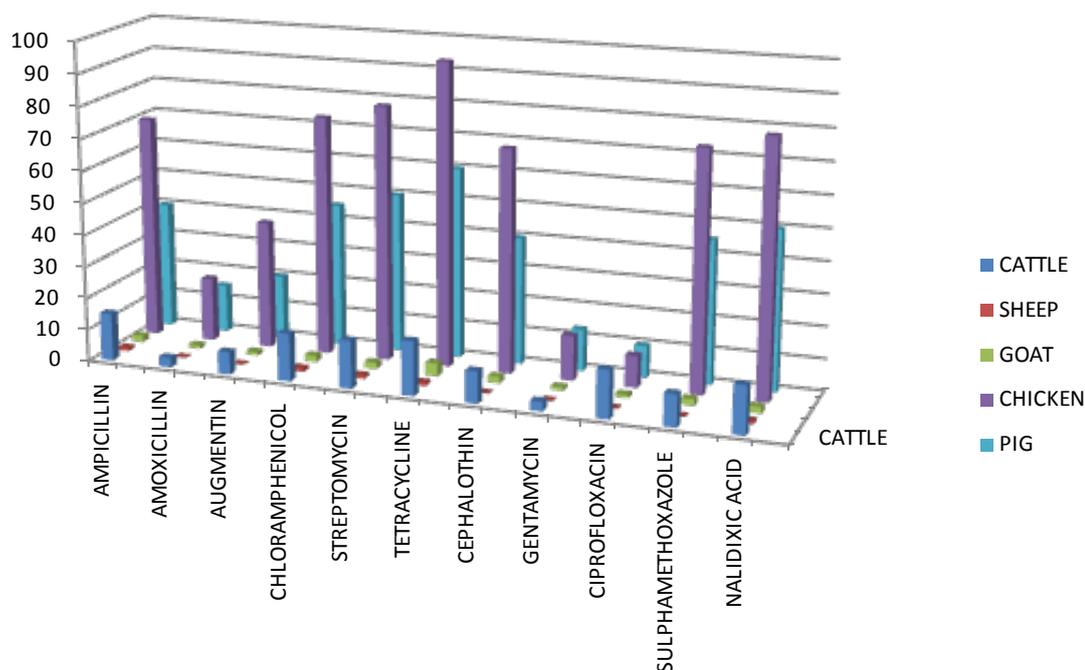


Figure 2. Comparison of number of resistant isolates from each live stock faeces. X-axis = antibiotics; Y-axis = Number of resistant isolates.

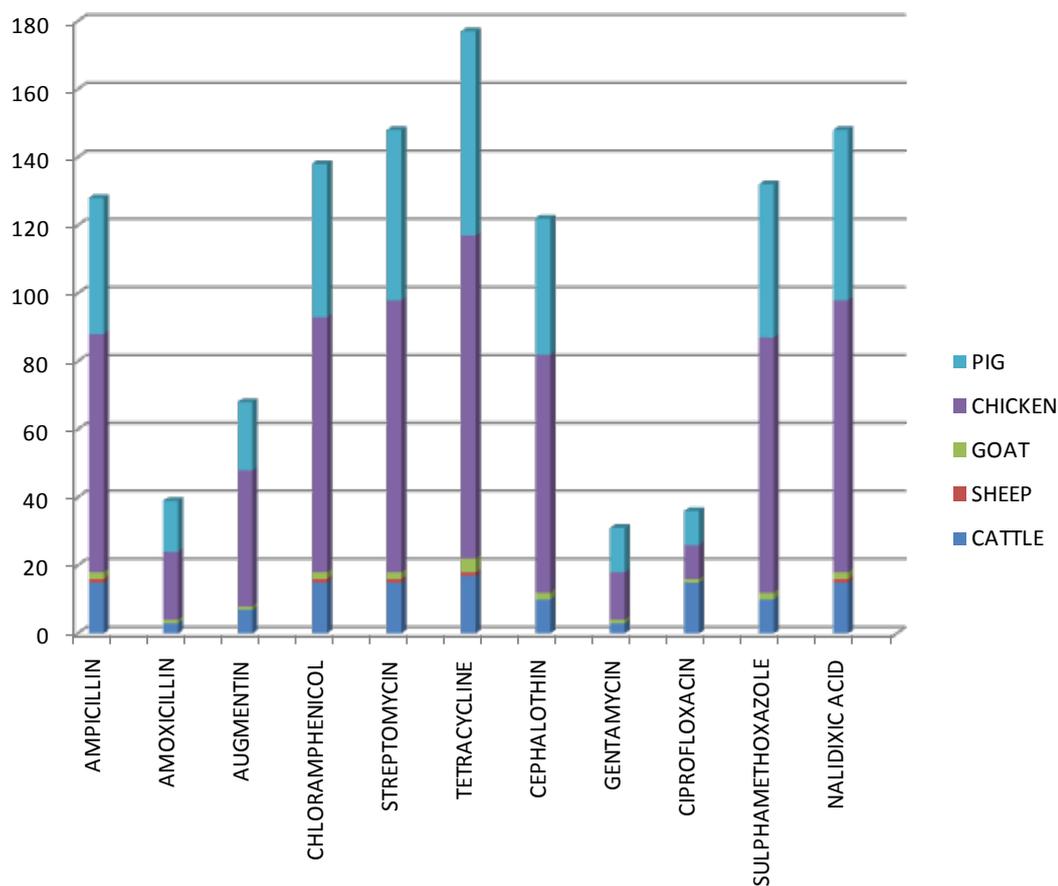


Figure 3. Contribution of each group of isolates to the total number resistant to each antimicrobial drug. X-axis = antibiotics; Y-axis = cumulative resistant isolates

yielded *Salmonella* spp.). The largest number of *Salmonella* was isolated from chicken faeces followed by pig faeces, while sheep and goat faeces yielded the least. A number of studies have reported similar high *Salmonella* prevalence in farm animals usually slaughtered for food. Kishima *et al.* [14] reported the isolation of 172 strains of *Salmonella* from 218 pig farms in Japan. In Australia, Duffy *et al.* [15] reported 46.3% *Salmonella* species isolation from the faeces of goat. Asseta Kagambega *et al.* [16] in Bukina Faso reported isolation of salmonella from 52% cattle faeces, 55% chicken faeces and 16% swine faeces. In Belgium, Botteldoorn *et al.* [17] reported 37% *Salmonella* prevalence in pig faeces. However, a few low prevalence rates of *Salmonella* have been reported by some researchers. In Ethiopia, Anbessa Dabassa *et al.* [18] isolated *Salmonella* from as low as 4.4% cattle and sheep faeces but none from goat faeces. Alao *et al.* [19] reported only 14% *Salmonella* isolation from goat and cattle faeces in Sango-Otta, Nigeria. Most of these studies reveal higher prevalence of *Salmonella* in chicken than in other farm animals which corresponds to our findings in this study. According to Asseta Kagambega *et al.* [16], the rate of *Salmonella* carriage in chicken is higher than the rate in cattle. But he points out that the rate of *Salmonella* isolation from these animal faeces may depend on the isolation methodology and the type of animal husbandry practiced in that country.

The administration of antimicrobial agents in animal production creates selection pressure that favours the survival of antibiotic resistant pathogens [20]. Resistance of *Salmonella* to commonly used antimicrobials is increasing both in veterinary and public health sectors and has emerged as a global problem. These animals are important reservoirs of antimicrobial resistant organisms. If resistant organisms are common in animals, the chances that they will be transmitted to humans are more likely since they are sources of food. Duffy *et al.* [15] has pointed out that goat meat is a potential source of *Salmonella* serovars associated with human disease. In this study, most *Salmonella* isolates show marked resistance to 1 - 9 of the 12 antimicrobial drugs used. Majority were resistant to Tetracycline, Ampicillin, Streptomycin, Chloramphenicol etc. These are the drugs widely used

in the treatment of human systemic salmonellosis [21]. A similar study in Alberta, Canada indicates high resistance of *Salmonella* from food and food animals to Ampicillin, Streptomycin, Sulphamethoxazole and Tetracycline [22]. In Ethiopia, resistance pattern of *Salmonella* isolates from chicken indicates large proportions of strains resistant to a variety of drugs [23]. Also in a study in Alberta swine farms, highest frequencies of resistance were observed for chloramphenicol, Ampicillin, Kanamycin, Sulphamethoxazole, Streptomycin and Tetracycline [24]. Dabassa *et al.* [18] further reports that *Salmonella* isolates from cattle and sheep faeces show multidrug resistance to Ampicillin, Nalidixic acid, Streptomycin, Tetracycline and Chloramphenicol from which he concludes that foodstuffs particularly beef, chevon and mutton parts could be a potential vehicle for food borne infections. Furthermore, our study reports low resistance of the isolates to Ciprofloxacin and Gentamycin. Dabassa *et al.* [18] had earlier reported zero resistance of *Salmonella* isolates to the two drugs in Ethiopia.

Antimicrobial drug over use in animal agriculture is contributing to human public health problems [20] [24]. Microbial studies show that antimicrobial drug resistance from farm animals not only infect humans but may transfer the resistance to other bacteria that colonize the animal and human gut by mechanisms of gene (plasmid) transfer, thus serving as a prelude to severe risk of health crisis. This study only investigated “within farm” carriage of *Salmonella*. The major point to note here is that of selection of resistant strains of pathogens by the use of antibiotics for treatment of infected animals and for growth enhancement. Another important point is that of lateral spread of the drug resistant pathogens from an infected animal to the others within the farms. Thirdly the transfer of the pathogens from the infected animals to humans (farm workers) by direct contact with infected animal faeces and other body fluid, and from infected animals to other meat and meat products at slaughter houses and distribution points [15] [16] [25] [26]. Furthermore, the use of untreated animal dung for manure provides another route of transfer of multidrug resistant pathogens to other food products such as vegetables and fruits that may be consumed raw or with insufficient cooking. The environment also gets contaminated with faecal pathogens in areas where farm animals are allowed to roam about and graze in open fields [16].

5. Conclusion and Recommendations

Our study has revealed high prevalence of *Salmonella* species in chicken and pig, and less prevalence in cattle, goat and sheep. We have further discovered that most of the isolates were resistant to most commonly used antibiotics for treatment of the infections. Effort is needed to adopt measures that will prevent the spread of these multidrug resistant pathogens to humans. Serious environmental hygiene and strict infection control measures must be adopted both in the treatment and disposal of animal dungs and at abattoirs to prevent contamination of the food chain. Use of untreated animal dung for manure should be discouraged. Care must be taken in the use of antibiotics in asymptomatic animals to reduce the selection of multidrug resistant strains. Authorities in Agriculture and Veterinary Medicine are advised to formulate code of conducts in this regard and ensure certain restrictions as it is done in the developed countries [25]. The public should be properly enlightened on the dangers of eating raw or insufficiently cooked food to forestall acquisition of these pathogens. Further studies are required to identify the particular strains of *Salmonella* harboured by the farm animals and the level of risks they pose to the public.

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