Comparison Of Prevalence And Antimicrobial Sensitivity Of Salmonella typhimurium In Apparently Healthy Cattle And Goat In Sango-Ota, Nigeria

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Citation

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Abstract

Salmonella
typhimurium has been extensively incriminated worldwide as common causes of Salmonellosis in humans, with food-animals serving as important reservoirs. This study was aimed at determining and comparing the prevalence of Salmonella typhimurium isolated from faeces of goats and cattle in Sango-Ota and also at determining the antimicrobial sensitivity of the isolated organisms. A total of 157 fresh faecal samples (50 from goat and 107 from cattle) were examined for the presence of Salmonella typhimurium using standard microbiological and biochemical methods. 22 (14%) of the total samples were positive for Salmonella
typhimurium and 13 (8.28%) were positive for Salmonella spp. 19 (86.4%) of Salmonella typhimurium were isolated from cattle while 3 (13.6%) from goats while 12 (92.31%) of Salmonella spp were isolated from cattle while 1 (7.69%) from goats. Other organisms isolated were Citrobacter spp, Providencia spp,
Pseudomonas spp,
Proteus spp, Klebsiella spp, Yersinia spp, Morganella spp, Enterobacter spp, Escherichia coli and Serratia spp. Antibiotic susceptibility test revealed that majority of the isolates were susceptible to ofloxacin, ciprofloxacin, pefloxacin, gentamicin, and nalidixic acid. A majority of Salmonella typhimurium, Salmonella spp, and other bacterial isolates were susceptible to all fluoroquinolones used in this study. A majority of the isolates were resistant to amoxicillin, ampicillin, augmentin, cotrimoxazole, streptomycin, tetracycline, and all cephalosporins used in this study. Salmonella typhimurium was present in both cattle and goat faeces in Sango-Ota and therefore if food from these sources are not properly handled it could lead to spread of the organism and occurrence of food borne salmonellosis.

INTRODUCTION

Salmonella is a Gram-negative, non-spore forming, rod shaped, facultative intracellular anaerobic bacterium in the family Enterobacteriaceae trivially known as enteric bacteria (Todar, 2005). They are cytophilic, non-capsulated bacilli that are motile with peritrichous flagella ranging in size from 2 µm to 3 µm by 0.6 µm (Paul and Colin, 1990). They metabolize glucose to acids; catalase-positive, oxidase-negative. However, they can also live under aerobic conditions. Salmonella lives in the intestinal tracts of warm and cold blooded animals i.e. humans and animals (Todar, 2005).

Salmonella typhimurium is the most widely distributed of the bacteria that causes enteric fever or gastroenteritis, which is designated as food poisoning and it is transmitted generally through contaminated food and water (Schlegel, 2002; Srivastava and Srivastava, 2003). Salmonellosis is considered as one of the widespread food-borne zoonoses in industrialised as well as developing countries (Molla et al., 2003). Due to the ability of Salmonella typhimurium to survive in meat and animal products that are not thoroughly cooked or not properly handled, animal products are the main sources of food borne salmonellosis (Akoachere et al., 2009). Therefore, cattle may carry this organism undetected into an abattoir at the time of slaughter.

Salmonella typhimurium is ubiquitous as it has been reported in diverse environment including water, grass, silages, decomposing organic matter, soil and faeces (Hassan

et al., 2000). Many animal species harbour Salmonella and can act as potential reservoirs for human infections. Salmonella may enter the food chain through carcass contamination with animal faeces at slaughter and during processing, or through food or food handlers (Hussein et al., 2010). Contact with farm animals, pets, reptiles and natural pet treats have also been associated with infection (Kariuki et al., 2006). Salmonella typhimurium can spread from farm to farm through exchange of livestock, by wildlife, or in runoff from fields (Todar, 2008).

Direct cattle-to-cattle contact from other herds can also result in the introduction of Salmonella typhimurium into dairy farms. (Van Schaik et al., 2002). Another potential source of Salmonella transmission includes livestock feed (Anderson et al., 2001; Davis et al., 2003).

Salmonellae are common inhabitants of the gastrointestinal tracts of all animals, including cattle and goat, and as a consequence, beef and dairy products can also serve as vehicles for human exposure to this organism (Kunze et al., 2008). However, human infection may also occur through contaminated water, pets and exotic animals (Hussein et al., 2010).The worldwide incidence of human non-typhoidal salmonellosis is estimated at 1.3 billion cases, with three million deaths annually (Hussein et al., 2010) and the non-typhoidal salmonellosis are caused mainly by Salmonella typhimurium and Salmonella enteritidis (Hussein et al., 2010; Kariuki et al., 2006).

Many S. typhimurium isolates (although not exclusively isolates of this serotype) have developed resistance to multiple antibiotics. Particularly S. typhimurium definitive type (DT) 104 are resistant to Ampicillin, chloraphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT), with an increasing number of isolates showing resistance to Trimethoprim and fluoroquinolones (Threlfall et al., 1997; Piddock, 2002).

Effective Antimicrobial therapy reduces morbidity and mortality from salmonella food poisoning. Without Therapy, illness may last 3-4 weeks and case-fatality rates may exceed 10% (WHO, 2003).

Therefore, this study was carried out to determine the role play by cattle and goat in this environment as reservoirs of S. typhimurium and also to determine antibiotic susceptibility of this organism and other isolates.

MATERIALS AND METHODS

SAMPLE COLLECTION/STUDY DESIGN

A total of 157 samples of fresh faeces of cattle and goats were collected and processed. The samples were collected from six sites in Ota: 29 samples from the Cattle Ranch at Toll Gate Abattoir (Study Site 1), 33 samples from Temperance (Study Site 2), 35 samples from Sifor (Study Site 3), 10 samples from Benja (Study Site 4), while 25 samples from Goat Markets at Oju-Ore (Study Site 5) and 25 samples from Sona (Study Site 6). All these were collected in a sterile Universal bottle, stored in ice packs and transported immediately to the Microbiology Laboratory in Bells University of Technology, Ota within two hours for microbiological analysis. The samples were collected from different sites based on the frequency of defecation by the animals. The name was assigned to each of the study sites based on the common name given to their locations.

MICROBIOLOGICAL ANALYSIS

A loopful of the faecal samples was inoculated aseptically into a McCartney bottle containing 9ml of selenite F broth (Oxoid). The sample was incubated for 24 hours at 37□C. After incubation, a loopful of selenite F broth culture of the faeces was aseptically streaked on Salmonella-Shigella agar (Oxoid). Plates were incubated at 37□C for 24 hours after which they were examined for typical colonies of Salmonella.

Colonies from all samples were then streaked on Nutrient agar in order to obtain pure culture. These colonies were then transferred onto a Kliger Iron agar slant (Oxoid), after which they were subjected to Gram-staining, citrate utilisation testing, sulphide-indole-motility testing, and sugar utilisation testing. Gram negative short motile rods with characteristic red slope and yellow butt reaction with production of gas and H_2S on KIA were reported as Salmonella typhimurium.

ANTIBIOTIC SENSITIVITY TESTING

The Clinical and Laboratory Standards Institute (CLSI), disc-diffusion method was used for antibiotic sensitivity testing (CLSI, 2005).

Turbidity of the inoculums of various isolates of enterobacteria is compared with 0. 5 McFarland standard and each of the isolates were inoculated onto the surface of a sterile nutrient agar plates using a sterile swab in order to ensure even distribution of the inoculums, the plates were

allowed to dry and Antibiotic discs with concentrations were placed on the surface of the agar plates. The antimicrobial discs include the following. Amoxicillin (30µg), Augmentin (30µg), Pefloxacin (10µg), Ofloxacin (5µg), Gentamicin (10µg), Ciprofloxacin (10µg), Cefotaxime (30µg), ceftazidime (30µg), Cefuroxime (30µg), Streptomycin (30µg), Erythromycin (10µg), Ampicillin (30µg), Cotrimoxazole (25µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Tetracycline (15µg). After 30 mins of applying the disc, the plates were inverted and incubated for 24 hours at 37 ^{o} C. The clear zone that developed around each disc were measured as the zones of inhibition from underneath each plate with the aid of a ruler in centimeter and converted to millimeter (mm) and on the basis of CLSI guidelines.

STATISTICAL ANALYSIS

The chi-square test was employed to compare the prevalence of Salmonella typhimurium in goats and cattle. The differences were considered significant at $p<0.05$.

RESULTS

A total number of one hundred and fifty-seven fresh faecal samples were analysed from herds of cattle and goat in different locations in Ota, Ogun State. These bacterial isolates were identified on the basis of their morphological, cultural and biochemical characteristics on various media and their ability to ferment various Carbohydrates.

Of the 157 samples analysed, 22 (14%) were positive for Salmonella typhimurium; out of which 19 (86.4%) were from cattle and 3 (13.6%) from goat. In this study, 13 samples were positive for other Salmonella species, 12 (92.3%) were from cattle and 1 (7.7%) from goat.

The distribution of the isolates recovered from the faecal samples obtained from the six study sites is as follows: from the study site 1, a total of 29 isolates were obtained representing-3 (10.3%) S. typhimurium, 3 (10.3%) Salmonella spp, 2 (6.9%) Escherichia coli, 3 (10.3%) Proteus spp, 2 (6.9%) Enterobacter spp, 12 (41.4%) Citrobacter spp, 1 (3.4%) Yersinia spp, 1 (3.4%) Morganella spp, 1 (3.4%) Providencia spp, and 1(3.4%) Klebsiella spp.

4 (12.1%) S. typhimurium, 4 (12.1%) Salmonella spp, 1 (3.0%) Pseudomonas spp, 1 (3.0%) Escherichia coli, 6 (18.2%) Proteus spp, 1 (3.0%) Enterobacter spp, 8 (24.2%) Citrobacter spp, 1 (3.0%) Yersinia spp, 4 (12.1%) Serratia spp, and 3 (9.1%) samples with no growth were recorded from study site 2 with a total isolates of 33.

12 (34.3%) S. typhimurium, 2 (5.7%) Salmonella spp, 3 (8.6%) Pseudomonas spp, 3 (8.6%) Proteus spp, 1 (2.9%) Enterobacter spp, 11 (31.4%) Citrobacter spp, 1 (2.9%) Yersinia spp, and 2 (5.7%) Providencia spp, were recorded from study site 3 with a total isolates of 35.

3 (30%) Salmonella spp, 2 (20%) Pseudomonas spp, 1 (10%) Enterobacter spp, 2 (20%) Citrobacter spp, 1 (10%) Morganella spp, and 1 (10%) Serratia spp, were recorded from study site 4 with a total isolates of 10.

2 (8%) S. typhimurium, 1 (4%) Salmonella spp, 1 (4%) Pseudomonas spp, 1 (4%) Escherichia coli, 4 (16%) Proteus spp, 1 (4%) Enterobacter spp, 13 (52%) Citrobacter spp, and 2 (8%) Providencia spp, were recorded from study site 5 with a total isolates of 25.

1 (4%) S. typhimurium, 13 (52%) Pseudomonas spp, 1 (4%) Escherichia coli, 1 (4%) Proteus spp, 8 (32%) Citrobacter spp, and 1 (4%) Providencia spp, were recorded from study site 6 with a total isolates of 25.

Therefore, samples from various study sites recorded a prevalence of 13.6% S. typhimurium from the cattle faecal samples in study site 1 (Toll Gate Abbatoir), 18.2% of S. typhimurium from study site 2 (Temperance Ranch), 54.5% of S. typhimurium from study site 3 (Sifor Ranch), 9.1% of S. typhimurium from study site 5 (Oju-Ore Goat Market) and 4.6% of S. typhimurium from study site 6 (Sona Goat Market). Study site three recorded the highest prevalence of S. typhimurium while study site six had the lowest percentage.

The antibiotic sensitivity test showed that S. typhimurium and other isolates were 100% sensitive to ciprofloxacin and pefloxacin but Pseudomonas aeruginosa and Proteus spp showed 85% and 58.8% respectively to ciprofloxacin and pefloxacin, while Citrobacter showed 75.9% sensitivity to pefloxacin and 79.6% to ciprofloxacin. Majority of the isolates showed low sensitivity rate to gentamicin, nalidixic acid, and nitrofurantoin; while majority of the isolates were resistant to ampicillin, streptomycin, amoxicillin, augmentin, cotrimoxazole, ceftazidime, cefuroxime, cefotaxime, tetracycline, and erythromycin.

Figure 1

TABLE 1- Prevalence of and other bacterial isolates in various study sites

Organisms	TG	TC	SC	BC	OG	SG	Total	Prevalence rate (%)
Salmonella typhimurium	3	4	12	0	2	1	22	14
Salmonella spp	3	4	2	3	1	0	13	8.3
Pseudomonas spp	0	1	3	2	1	13	20	12.7
Escherichia coli	2	1	0	0	1	1	5	3.2
Proteus spp	3	6	3	0	4	1	17	10.8
Enterobacter spp	2	1	1	1	1	0	6	3.8
Citrobacter spp	12	8	11	2	13	8	54	34.4
Yersinia spp	1	1	1	0	0	0	3	1.9
Morganella spp	1	0	0	1	0	0	2	1.3
Providencia spp	1	0	2	0	2	1	6	3.8
Serratia spp	0	4	0	1	0	0	5	3.2
Klebsiella spp	1	0	0	0	0	0	1	0.6
No growth	0	3	0	0	0	0	3	1.9
TOTAL	29	33	35	10	25	25	157	100

Key: TG-Toll Gate Abattoir, TC-Temperance Cattle Ranch, SC-Sifax Cattle Ranch, BC-Benja Cattle Ranch, OG-Oja Ore Goat Market, SG-Sona Goat Market

Figure 2

Table 2: Antiobiotic sensitivity pattern of and other bacterial isolates

Isolates	No of Isolates	Antibiotics								
		Cot (25µg)	Cef (30µg)	Gen (10µg)	Nal (30µg)	Nit (200µg)	Tet (15µg)	Cpr (10µg)	Pef (10µg)	Ofl (5µg)
Salmonella typhimurium	22	1 (4.5%)	R	8 (36.4%)	8 (36.4%)	5 (22.7%)	1 (4.5%)	22 (100%)	22 (100%)	15 (22.7%)
Salmonella Spp	13	R	R	10 (76.9%)	11 (84.6%)	2 (15.4%)	R	13 (100%)	13 (100%)	10 (76.9%)
Serratia Spp	5	1 (20%)	R	3 (60%)	5 (100%)	2 (40%)	1 (20%)	5 (100%)	5 (100%)	5 (100%)
Klebsiella Spp	1	R	R	1 (100%)	1 (100%)	R	R	1 (100%)	1 (100%)	1 (100%)
Pseudomonas aeruginosa	20	R	1 (5%)	16 (80%)	15 (75%)	2 (10%)	R	17 (85%)	17 (85%)	17 (85%)
Enterobacter Spp	6	R	R	5 (83.3%)	6 (100%)	3 (50%)	R	6 (100%)	6 (100%)	6 (100%)
Yersinia Spp	3	R	R	2 (66.7%)	2 (66.7%)	R	R	3 (100%)	3 (100%)	2 (66.7%)
Escherichia coli	5	R	R	4 (80%)	5 (100%)	R	R	5 (100%)	5 (100%)	5 (100%)
Citrobacter Spp	54	6 (11.1%)	R	33 (61.1%)	31 (57.4%)	8 (14.8%)	3 (5.6%)	43 (79.6%)	41 (75.9%)	43 (79.6%)
Providencia Spp	6	R	R	4 (66.7%)	4 (66.7%)	3 (50%)	R	6 (100%)	6 (100%)	3 (50%)
Morganella Spp	2	R	R	1 (50%)	1 (50%)	R	R	2 (100%)	2 (100%)	1 (50%)
Proteus Spp	17	R	R	4 (23.5%)	12 (70.6%)	R	R	10 (58.8%)	10 (58.8%)	3 (17.6%)
Total	157									

Key: Cot-Cotrimoxazole, Cef-Ceftazidime, Gen-Gentamicin, Nal-Nalidixic acid, Nit-Nitrofurantoin, Tet-Tetracycline, Cpr-Ciprofloxacin, Pef-Pefloxacin, Ofl-Ofloxacin, R – Resistant, Spp-Species

DISCUSSION

In this study, 22 (14%) Salmonella typhimurium and 13 (8.3%) Salmonella spp were isolated from animals (goats and cattle) found in various sites in Sango-Ota. The other organisms isolated from these sites were Citrobacter spp, Serratia spp, Pseudomonas spp, Enterobacter spp, Escherichia coli, Klebsiella spp, Proteus spp, Yersinia spp, Providencia spp and Morganella spp.

Salmonella enteric is an important cause of food borne diseases with an estimated 1.4 million illnesses and 500 deaths attributed to salmonellosis in the United States annually (Kunze et al., 2008). Poultry, pork, fresh produce and consumption of beef products are all vehicles of human salmonellosis which is attributable to faecal contamination (Kunze et al., 2008). Sheep and goats are potential carriers of Salmonella (Zhang et al., 2003).

This study was therefore conducted to determine and compare the prevalence and the antimicrobial susceptibility of Salmonella typhimurium and other organisms isolated from the faeces of cattle and goat in Sango- Ota, Ogun-State, where no such data exist.

This study detected that the prevalence of Salmonella typhimurium in cattle (86.4%) and goats (13.6%) are statistically significant. The prevalence rate of S. typhimurium in this study is 14%, however, previous studies have shown S. typhimurium with a higher prevalence but some others have shown S. typhimurium with a low prevalence (Akoachere et al., 2009; Molla et al., 2003).

The low occurrence of S. typhimurium (13.6%) and Salmonella spp (23.1%) in site one (abattoir) maybe attributed to the fact that the cattle come from different ranches and stay temporarily, that is, until they are slaughtered which probably reduced the contamination. During slaughtering, the organisms (if present) are washed from cattle and discharged on the grasses. Since the effluent is not properly disposed other cattle brought in can be infected. However, the presence of this organism in these animals is of health concern because if not properly handled and processed, it could result in the contamination of the meat (beef) which can be of health risk to the consumers.

The moderately low occurrence of S. typhimurium (18.2%) and Salmonella spp (30.8%) in site two can be related to the grazing site of these animals. The grazing sites of these animals are not changed and this has probably led to recontamination of the animals through their faeces.

The high incidence of S. typhimurium (54.5%) and Salmonella spp (15.4%) in the study site three is very significant to the fact that the animals graze on the same farmlands daily as confirmed by the herdsman. Therefore, the high prevalence of these organisms in these animals could result from continuous consumption of contaminated grasses on the farmland. This study confirms the report made by Kidd and co-workers (2002).

The low occurrence of S. typhimurium (0%) and Salmonella spp (23.1%) in site four can be related to the grazing sites being changed frequently. According to the herdsman in this site, the animals do not graze on these farmlands every day. Therefore, it reduces the incidence of re-contamination of the grasses (if at all, the grasses had been previously contaminated with the dung of the animals during grazing on the farmland) and consumption of this organism by the animals.

The 22% incidence of S. typhimurium in this study is an

appreciable importance. This is because, once there is an infective dose of this organism in the body with low immunity, there is high tendency of occurrence of Salmonellosis. Due to the ability of S. typhimurium to survive in meat and animal products that are not thoroughly cooked or not properly handled, animal products are the main sources of food borne salmonellosis (Akoachere et al., 2009). Infection of humans by the enteric pathogen S. typhimurium generally results in gastroenteritis, severe abdominal cramping, fever, weakness and severe diarrhoea (Srivastava and Srivastava, 2003). Therefore, adequate control measures should be adopted to eradicate the proliferation of this organism among cows. The usage of animal dung, as a form of manure in the farmlands should be controlled. Consumers of beef and other animal products should ensure that these products are properly cooked and preserved.

Sites five and six also had a low prevalence of S. typhimurium (9.1% and 4.6% respectively) and this probably occurred due to the fact that goat stalls had a special cubicle for the feed to prevent its faecal contamination. The goats eat once in a while from the ground which allows for recontamination through their faeces. The movement of these animals is restricted to the owner's stall where they are fed with straw.

Epidemiological surveillance of antimicrobial-resistant of S. typhimurium has become necessary for effective treatment and prediction of occurrence of resistant populations. Antibiogram of the isolates revealed marked susceptibility of isolates to fluoroquinolones-ciprofloxacin (100%), and pefloxacin (100%); except Citrobacter with 79.6% to ciprofloxacin and 75.9% to pefloxacin, Pseudomonas aeruginosa with 85% to both antibiotics, and Proteus spp with 58.8% to both antibiotics (Table 2). The result of this study corroborates the earlier findings of Esaki and co-workers, 2004 and that of Kawagoe and co-workers, 2007 that reported a marked susceptibility of S. typhimurium to fluoroquinolones that could be used in the treatment of infections caused by this organism. However, susceptibility of the isolates to another fluoroquinolones-ofloxacin is low in some of the organisms. However, the concentration of ofloxacin (5µg) used in this study is low compared with 10µg of ciprofloxacin and pefloxacin. Therefore, effort should be made to discourage indiscriminate use of these antibiotics in the society. Other active agents observed was Nalidixic acid (84.6%) to Salmonella spp. (100%), to other isolates namely Klebsiella spp, Enterobacter spp, Serratia spp, and Escherichia coli, with 70.6%, 57.4%, 75%, and 66.7% to Proteus Citrobacter, Pseudomonas aeruginosa, and Yersinia spp, respectively.

All the isolates were resistant to ampicillin, streptomycin, erythromycin, amoxicillin, augmentin, cotrimoxazole, tetracycline, ceftazidime, cefuroxime, and cefotaxime. This high level of resistance to these treatment drugs, corroborate the submission of Smith and co-workers, (2006) that, it is because of the across-the-counter purchase and street hawking of these drugs that still exist in this environment, thereby allowing easy access to these drugs and possibility of resistant strains. Therefore, the use of these antibiotics would lead to treatment failure and probably lead to development of resistant strains of Salmonella typhimurium and that of other isolates.

In conclusion, the findings of this study have established that cows and goats are the true reservoirs of S. typhimurium which has been known to be ingested by the animals through their continuous grazing on contaminated grasses. Health care officials should therefore educate the people on the need to thoroughly cook beef and other animal products in order to destroy the pathogens therein. Vegetables and other food products should also be cooked thoroughly and not steamed to prevent the consumption of the pathogens that have been introduced into them either through the use of the animal dung as manure or other faecal means of contamination.

All the isolates were sensitive to quinolones proving that these antibiotics will still remain the drug of choice for the management of salmonellosis in this environment. Therefore, adequate health education should be put in place to guide against indiscriminate use of quinolones antibiotics in order to prevent drug resistance by these organisms.

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