Salmonellosis and *Shigellosis* Associated with Cattle Dung Contaminant from Indigenous Abattoirs, Osun State, Nigeria

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Citation: Omololu-Aso J, Omololu-Aso OO, Atiene MT, et al. *Salmonellosis* and *Shigellosis* Associated with Cattle Dung Contaminant from Indigenous Abattoirs, Osun State, Nigeria. Br J Res 2017, 4: 1.

Abstract

Faecal shedding of cattle as well as poultry and poultry products have been implicated as a major source of *Salmonella* infections in humans. We sourced to isolate and identify *Salmonella* and *Shigella* species from cattle dung in different abattoir of Ile-Ife, Nigeria and to detect the route of cross-contamination of *Salmonella* and *Shigella* from animals to humans with antibiotic susceptibility of the isolates recovered. All 40 samples taken were collected from the dung of freshly killed cows which were placed within sterile plastic containers and transported to the Microbiology Laboratory. Each Sample was placed aseptically in sterile nutrient broth and incubated. Microbiological analysis was conducted with samples serially diluted and analyzed on *Salmonella-Shigella* agar by the direct plating method. Biochemical tests were employed to confirm the isolates including Gram staining, catalase, oxidase, urease, glucose fermentation and motility tests.

The results showed that out of the ten (10) samples collected in Odo-Eran area, Ile-Ife town, 4 (40%) samples showed *Escherichia coli* growth, 4 (40%) *Salmonella* were isolated and 2 (20%) *Shigella*-like organisms were isolated. At God's Love Tabernacle (GLT) area in Ile Ife town, From the 20 samples taken, 7 (35%) showed *Salmonella* isolates, 7 (35%) confirmed *Shigella*-like characteristics isolates. Six (30%) isolates left were observed to show *Escherichia coli* colonies. The *Salmonella/Shigella* isolates obtained from study areas were 100% resistant to chloramphenicol, 75% to septrin and 10% to pefloxacin and tarivid respectively while the isolates were 80% susceptible to gentamicin and 65% to ciprofloxacin and streptomycin used. Gentamicin streptomycin and ciprofloxacin antibiotic therapeutic agents will be effective in the treatment of cattle infections caused by *Salmonella* and *Shigella* species in the study area.

Epidemiological burden of *Salmonellosis* is expected to be much greater in the developing parts of the world.

Introduction

Salmonella are the leading cause of bacteremia, 28.5% with *S. typhi* accounting for 20.9% and non-typhi *salmonella* 7.6% [1]. Infections due to *salmonella* serotypes continue to be a major health problem [2]. Worldwide incidence of *shigellosis* is estimated to be 164.7 million cases per year of which 163.2 million were in developing countries, where 1.1 million deaths occurred. About 60% of all episodes and 61% of all deaths attributable to *shigellosis* involved cattle and children below five years. The incidence in developing countries may be 20 times greater than that in developed countries [3]. An estimated 30% incidence of these infections are caused by *S. dysenteriae* with case fatality rates reaching 30% [3].

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries [4]. Most *Salmonella* infections in cattle are subclinical, and shedding of the organism can occur for extended periods. *Salmonella* can also persist in the farm environment [5].

Shigella species are highly infective, particularly *S. dysenteriae* considered the most virulent, and can produce a potent cytotoxin known as "Shiga toxin". *Shigella dysenteriae* type 1 causes severe and sometimes fatal disease. *Shigella* species have been recently identified to be the most frequently identified agent of laboratory-acquired infections because of their high virulence and low infectious dose [6].

In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and in some cases resulting in deaths [7]. It is common in developing countries where affected populations are immunologically compromised due to poor nutrition and background infections leading to high morbidity attributed to shigellosis [8-10]. *Salmonellosis*, a major cause of bacteria enteric illness in both humans and animals cause diseases in humans and is referred to as a zoonotic infection/disease. It is a disease caused by the bacteria *Samonella* [4].

The aim of this research work was to isolate, identify and characterize *Salmonella* and *Shigella* from fecal contaminants on products and the antimicrobial susceptibility of the isolates obtained from the abattoirs.

Materials and Methods

Study area

The study area was Ife Central Local Government, Ile-Ife, Nigeria. Samples tested include the fresh cattle dung contaminant from separate meat products at various butcheries which were collected between February to June, 2016. Ife Central is a local government area in Osun State, Nigeria. Its headquarters are in the city of Ile-Ife to the south of the area. It has an area of 111km^2 and a population of 167,254 at 2006 census.

Sample collection and preparation

Each sample consisted of cattle dung contaminant collected with sterile applicator swabs. The various samples collected were from Odo-Eran area along Ede Road, as well as from an abattoir adjacent to God's Love Terbanacle, along Ibadan Road. All of the samples collected were from the dung of freshly killed cows.

Sample transportation

The samples were placed within sterile plastic containers and transported to the Microbiology Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria within an hour of collection. The samples were examined immediately upon arrival at the laboratory. Each Sample was placed aseptically in sterile nutrient broth and incubated.

Microbiological analysis of samples

The samples collected were serially diluted and analyzed on *salmonella-shigella* agar by the direct plating method. A measured amount of the moderately warm molten agar was poured into the sterile petri dishes and allowed to set after which the organisms were streaked on the *Salmonella-Shigella* agar plates evenly to allow for the spread of the organism. The plates were then incubated for 24-48 hours at 37°C in an inverted manner. Biochemical tests were employed to confirm the isolates including Gram staining, catalase, oxidase, urease, glucose fermentation, motility tests. For all the biochemical tests carried out 18-24 hours young cultures of the isolates were used.

Antibiotic susceptibility test

In vitro susceptibility of the isolates against antimicrobial agents was determined by the standard disc diffusion procedure (Bauer et al 1996). The following antibiotic discs were used: augmentin (30 µg), gentamycin (30 µg), streptomycin (30 µg), pefloxacin (10 µg), chloramphenicol (23.2 µg), amoxacillin (25 µg), ciprofloxacin (5 µg), streptomycin (30 µg), tarivid (5µg) spafloxacin (25 µg) and septrin (10 µg). Isolates were inoculated in nutrient broth and incubated at 37°C for 24hours. The inoculum was poured into the Mueller-Hinton agar plates, antibiotic discs were placed on the plates. Plates were incubated at 37°C for 18 to 24 hours. The zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard Institute provided (CLSI 2014).

Results

Biochemical characterization of isolates of *Shigella* and *Salmonella* strains

The isolates were Gram negative rods. They were catalase positive; motility positive for *Salmonella* but motility negative for *Shigella*. They were urease positive and glucose positive.

Table 1: Morphological reaction of isolate to the selected media.

Morphology	Results
Growth in Nutrient agar	Positive for all isolates
Growth in MacConkey agar	Positive for both *Salmonella* and *Shigella* species
Growth in *Salmonella-Shigella* agar	Positive for *Salmonella* and *Shigella* species
Motility testing	Positive for *Salmonella* species but negative for *Shigella* species
Gram staining	Negative for both *Salmonella* and *Shigella* species

Table 2: Antibiotic susceptibility pattern of *Salmonella/shigella* species isolated from cattle dung collected from abattoirs in Ile-Ife town, Osun State, Nigeria.

Antibiotics	Total Isolates	R (%)	I (%)	S (%)
Septrin	30	75	25	25
Chloramphenicol	30	100	0	0
Sparfloxacin	30	15	30	30
Ciprofloxacin	30	25	10	65
Amoxicillin	30	40	30	30
Augmentin	30	20	0	80
Gentamicin	30	35	10	55
Pefloxacin	30	10	60	30
Tarivid	30	10	60	30
Streptomycin	30	35	0	65
Key: S - Susceptible, I - Intermediate, R - Resistance				

However, *Salmonella* tested positive in the oxidase test. *Shigella* tested negative for oxidase (Tables 1 and 2) (Figures 1-4).
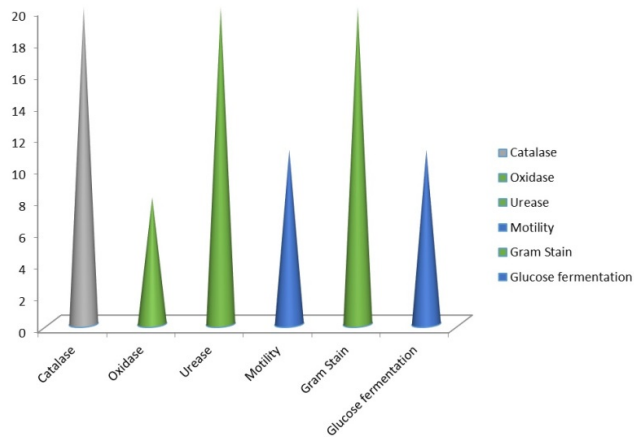
Figure 1: Biochemical test of Salmonella isolates on faecal contaminant of meat products at abattoirs in Ife town, Osun State, Nigeria.
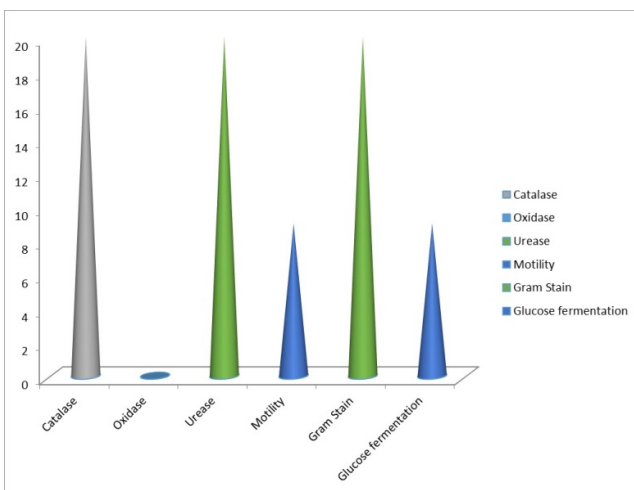

Figure 2: Biochemical test of Shigella isolates on faecal contaminant of meat products at abattoirs in Ife town, Osun State, Nigeria.

Discussion

In this study, out of the ten (10) samples collected in Odo-Eran area in Ife town, 4 (40%) samples showed *Escherichia coli* growth as seen in Figure 3. 4 (40%) *Salmonella* were isolated and 2 (20%) *Shigella*-like organisms were isolated. Thus, the probability of the occurrence of bacterial infection among those that consume meat or work in the butcheries in this community is high and which is in agreement with the reports of Joseph et al. (2012) on *Salmonellosis* and associated organisms as dangerous food borne pathogen.

From the 20 samples collected in God's Love Tabernacle (GLT) area in Ife town, 7 (35%) showed *Salmonella* growth as seen in Figure 1. 7 (35%) confirmed *Shigella*-like characteristics (Figure

2). Six (30%) isolates left were observed to show *Escherichia coli* growth as in Figure 3.
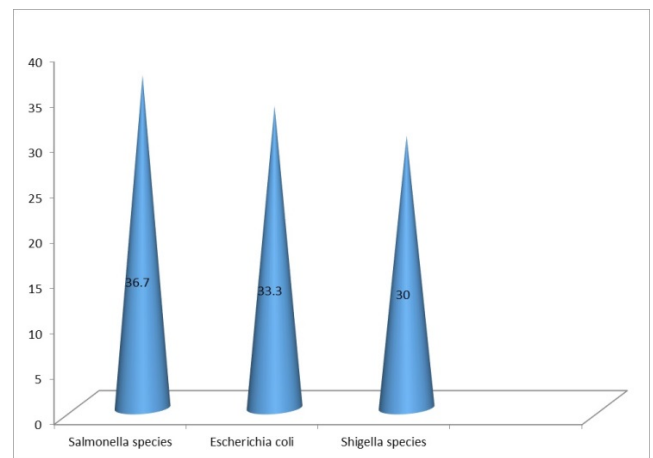

Figure 3: Frequency of occurrence of isolated organisms from faecal contaminant at abattoirs situated in Ife-Central, Ile- Ife town, Osun State, Nigeria.
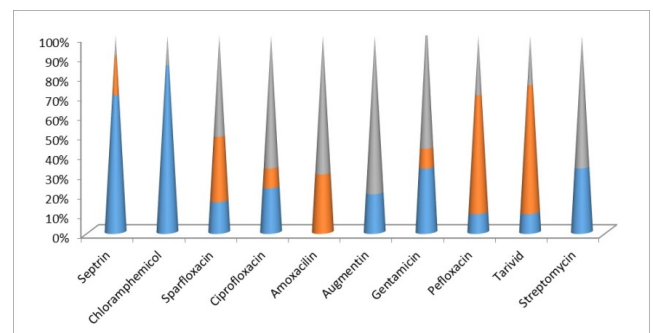

Figure 4: Antibiogram – *Salmonella/Shigella* species isolates obtained from cattle dung contaminant at abattoirs, Ile-Ife, Osun State, Nigeria. KEY: % S – Susceptible – Green, % I – Intermediate – Red, %R – Resistance – Blue.

All of the results suggest that livestock and in this case cow dung is a reservoir for bacteria for environmental contamination which is in concordance with the findings of Sayah [11].

Table 1 showing the reactions of isolates of *Salmonella* and *Shigella* to the various biochemical tests carried out, each indicating the distinctive properties of the isolates such as motility, with *Salmonella* being motile and *Shigella* being non-motile. These tests are effective in determining a basic level and type of bacteria present in the cow dung and hence relating to the type of infection suffered by the cattle.

From our findings, it can be established that, although the level of *Salmonella* may not be high in these understudy area, there is still a possibility for infection in cases of poor hygienic practices. Also, it can be inferred that the chances of infection remain high among the butchers and meat handlers which corroborated the reports of Hawkin [12] in the clinical management of public health risks from *Salmonella*.

From Table 2 showing the antibiotic susceptibility testing, the *Salmonella*/*Shigella* isolates were 100% resistance to chloramphenicol, 75% to septrin and 10% to pefloxacin and tarivid respectively while the isolates were 80% susceptible to gentamicin and 65% to ciprofloxacin and streptomycin used as illustrated in Figure 4. In our findings, it can be inferred that both gentamicin streptomycin and ciprofloxacin antibiotic therapeutic agents will be effective in the treatment of cattle infections caused by *Salmonella* and *Shigella* species and which is in variance with the work of Nyogi [13] on changing pattern of serotypes on antimicrobial susceptibility of *Shigella sp.* Isolated from children in Calcutta. Multiple antibiotic resistances shown by the isolates may suggest that the cattle are reared in areas where antibiotics are often abused [14].

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