

Antimicrobial Resistance Profile of *Escherichia coli* and *Salmonella* Isolated from Meat Sold in Sokoto Central Market, Sokoto State

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ABSTRACT

This study was designed to isolate and identify *Escherichia coli* and *Salmonella* spp. from raw-meat samples, on retail in the Sokoto central market and test their susceptibility to commonly prescribed antibiotics. Meat samples (n=40) were randomly collected from selected retail spots within the Sokoto metropolis, pre-enriched in buffered peptone water and incubated at 37°C for 12 hours. Pre-enriched samples were streaked on MacConkey agar and incubated at 37°C for 24 hours. The isolates were subjected to a series of biochemical tests for characterization and subsequent antimicrobial sensitivity testing using the disc diffusion method. Of the 40 samples collected, 7 were contaminated with *E. coli* and *Salmonella* spp. Antibiotic sensitivity testing showed that *E. coli* and *Salmonella* spp isolates were both resistant to chloramphenicol (10µg), ampicillin (30µg), amoxicillin (30µg), cefuroxime (30µg) and norfloxacin (10µg) In conclusion, this study showed that *E. coli* and *salmonella* spp. isolates from raw beef sold in Sokoto open market were multiple drug resistant strains which underscores a public health concern.

Keywords: *Escherichia coli*, Antimicrobial resistance, *Salmonella*.

INTRODUCTION

Escherichia coli and *Salmonella* are major food-borne pathogens belonging to the large family of bacteria; Enterobacteriaceae (Bintsis, 2017). *Escherichia coli* causes acute enteritis in humans and young animals including lambs, calves, piglets and poultry species. The *E. coli* strain 0157:07 was found to be associated with Haemorrhagic colitis and haemorrhagic uremic syndrome in humans (Erickson, 2019; Ameer, 2021).

Worldwide, Salmonellosis is one of the most common infectious diseases in animals and humans (Wiedemann, 2015; Pedersen *et al.*, 2007), and an important zoonotic problem as the pathogens can be transmitted through meat, milk and eggs (Radwowski, 2001 and Chabra *et al.*, 2003). Food-borne infections and illnesses due to *E. coli* strains and *Salmonella* spp are major international health problems (Zhao *et al.*, 200; CDC 2005). In addition, antimicrobial abuse is considered to be the most vital selecting force to resistance in these species of bacteria (Gutema *et al.*, 2021; Mora *et al.*, 2005). These resistance microbes may be potential sources in the transmission of antimicrobial resistance to humans when humans ingested foods contaminated by these resistance organisms (Mora *et al.*, 2005).

Beef is a major source of meat used in daily food preparations in the study area owing to its relatively lower cost and availability across seasons of the year. Over 60% of the indigenes of Sokoto state are traditionally engaged in rearing of at least one ruminant species by a free-range system. These animals are the major source of meat and milk and other livestock products and by-products. There is, however, an increasing public health concern over the unhygienic state of Abattoirs where meat is processed after slaughter, transportation, handling and storage as well as retail in markets. This may not be unrelated to the increasing prevalence of enteric fever (Salmonellosis) among human population in the study area. There is not much information on the prevalence of enteropathogens such as *E. coli* and

Salmonellae isolated from raw beef on sale in Sokoto metropolis that may have become resistant to commonly used antibiotics. Therefore, the present study was designed to isolate *E. coli* and *Salmonella* from raw beef samples on retail within the Sokoto metropolis and screen the isolates for resistance to common antimicrobial agents used in the area of study.

MATERIALS AND METHODS

Study Area

The study was carried out in Sokoto State, Northwest Nigeria. Sokoto Township housed a municipal abattoir where approximately 80 cattle and 62 sheep originating from throughout the state are slaughtered daily. The abattoir receives animals from several herds and flocks in groups of 12 and 25 animals, which are transported and penned together before slaughter.

Sample Collection

Between March to May 2018, a total of 40 beef samples were randomly collected from selected retail spots within the Sokoto meat and fish market (*Kasuwan Dan-kure*) and processed. Samples were collected by surface swabbing technique using a sterile swab. The swabs were then placed in tubes containing 5ml of buffered peptone water. The collected samples were then transported under ice to the Veterinary Microbiology Laboratory of Usmanu Danfodiyo University Sokoto for further analysis.

Culture and Identification of Isolates

Meat surface samples pre-enriched in buffered peptone water were incubated at 37°C for 12 hours. After enrichment, a loop-full of peptone water was streaked on MacConkey (Mac) and Eosin Methylene Blue (EMB) agar followed by incubation at 37 °C overnight. Those colonies that appear smooth and pinkish on Mac with a corresponding greenish metallic sheen appearance on the EMB agar surface were presumed to be *E. coli*. Colonies that appear small and colourless on Mac agar (most of the time without a corresponding greenish

metallic sheen appearance on the EMB agar) were subcultured on Xylose Lysine deoxycholate (XLD) agar and incubated at 37 °C for another 24 hours. Colonies with characteristic pinkish colour with or without blackish colouration (hydrogen sulphite) were presumed to be *Salmonella*. The presumed *E. coli* and *Salmonella* spp. were sub-cultured non-selective nutrient agar slants for antimicrobial susceptibility testing and further biochemical characterization.

Biochemical Screening

The following biochemical screening as described by Quinn *et al.* (2002) were conducted to characterize isolates: Indole, Methyl red, Voges Proskor and Citrate tests (IMViC), production of Hydrogen sulphide (H₂S) and Urease tests.

Antimicrobial Susceptibility Testing

The disc diffusion method by Kirby Bauer as described in CLSI (2018) was used to examine the bacterial susceptibility to ten (10) antimicrobial agents. Antibiotic disks (Oxoid®) containing Ciproflaxacin (5µg), Gentamycin (10µg), Tetracycline (50µg), Chloramphenicol (10µg), Ampicillin (30µg), Amoxicillin (30µg), Cefuroxime (30µg), Ofloxacin (10µg), Norfloxacin (10µg) and Nitrofurantoin (100µg) were used on Mueller-Hinton Agar. The diameters of the zones of inhibition were measured in millimetres. The measured zone of inhibition for each antimicrobial agent was interpreted either as susceptible or resistant following

Clinical and Laboratory Standards Institute guidelines CLSI (2017).

Data Analyses

Data generated were analyzed using Microsoft office excel 2007 (Microsoft Corp. USA) and results presented as charts and tables. The frequency of occurrence of *Escherichia coli* and *Salmonella* spp were calculated using percentages. Similarly, the antibiotic susceptibility profile of characterized isolates was presented as percentages.

RESULTS AND DISCUSSION

Cultural, colonial and phenotypic features on solid culture media were used for presumptive identification of the bacteria isolated. Biochemical characterizations were used to confirm the identification of *E. coli* and *Salmonella* spp as presented in Table 1. The results of occurrence of *Escherichia coli* and *Salmonella* spp. in meat sold in Sokoto metropolis indicated 7.5% of samples were contaminated with *E. coli* while 10.0% with *Salmonella* spp as shown in Figure 1. This result was in line with the results reported by Okonko *et al.* (2010) who isolated *E. coli* and *Salmonella* spp. from more than 11% of samples retrieved from meat. In another study, Zhao *et al.* (2001) isolated of *E. coli* and *Salmonella* from 11.9% and 11.1% meat samples. There are reports of high occurrence of *Salmonella* in meat samples as demonstrated by Chengappa *et al.* (1993) and Nawal (2008) that isolated *Salmonella* from 44.64% and 41.93% of swabs from raw meat samples.

Table 1: Biochemical characterization of isolates from meat sold in Sokoto metropolis

Biochemical tests						Identification
H ₂ S	Urease	Indole	Methyl red	Voges Proskor	Citrate	
-	+	+	+	-	-	<i>E. coli</i>
+	-	-	+	-	+	<i>Salmonella sp</i>

Key: H₂S = Hydrogen sulphide; + = Positive result; - = Negative result

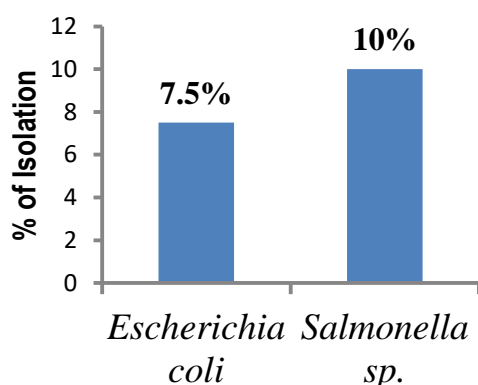


Figure 1: Occurrence of *Escherichia coli* and *Salmonella* spp. in meat sold in Sokoto metropolis

The results presented in Table 2 indicate that *E. coli* isolates showed resistance to all antimicrobial agents tested. *E. coli* Isolates from one sample were susceptible to gentamicin while 2 samples were susceptible to ciproflaxacin, ofloxacin and nitrofurantoin. Except for ciproflaxacin, ofloxacin, gentamicin and tetracycline, salmonella isolates proved to be completely resistant to all the other antibiotics tested. The contamination of the meat product might be while processing, transportation, handling by retailers or during storage of the product. Hence, there is need for employment of risk reduction strategies such as hazard analysis critical control point (HACCP) throughout the meat production processes from slaughter to retailed points to identify potential sources of contaminations with the aim of designing and execution of appropriate prevention and control strategies.

Table 2: Antibiotic Susceptibility Profile of *E. coli* and *Salmonella* spp. Isolated from raw Beef Retailed at Sokoto Market

Antimicrobial agents	Resistant strain	
	<i>Escherichia coli</i> (%)	<i>Salmonella</i> spp. (%)
Ciprofloxacin (5µg)	2 (66.6%)	1 (25.0%)
Gentamicin (10µg)	1 (33.3%)	3 (75.0%)
Tetracycline (50µg)	3 (100.0%)	3 (75.0%)
Chloramphenicol (10µg)	3 (100.0%)	4 (100.0%)
Ampicillin (30µg)	3 (100.0%)	4 (100.0%)
Amoxicillin (30µg)	3 (100.0%)	4 (100.0%)
Cefuroxime (30µg)	3 (100.0%)	4 (100.0%)
Ofloxacin (10µg)	2 (66.6%)	1 (25.0%)
Norfloxacin (10µg)	3 (100.0%)	4 (100.0%)
Nitrofurantoin (100µg)	2 (66.6%)	4 (100.0%)

Escherichia coli (n=3); *Salmonella* spp. (n=4), % = percentages, µg = Microgram,

Antimicrobial resistant bacteria are commonly recovered from livestock (Cattle, sheep, goats, camels, pigs and poultry) and have been shown to contaminate food products from animal origin (Meat, dairy, and egg) (FMARD *et al.*, 2017). Drug residues are frequently detected chemicals in food of animal origin, majority of which are commonly used antimicrobial agents in veterinary practice in Nigeria (Aliu, 2004). The indiscriminate use of antimicrobials for the prevention and treatment of bacterial infection in animals is a common practice in developing countries (Dino and Arowolo, 1991; Kabir *et al.*, 2004). A greater percentage of cattle in Nigeria are reared by nomadic herdsmen who administer antimicrobial agents without veterinary prescription, often with incorrect dosage and the drug withdrawal periods are usually not observed (Alhaji, 1976). Besides the effects of such practices on meat quality, drug residues in animal products constitute public health risks and economic concerns as it relates to the spread of antimicrobial resistance.

CONCLUSION

The findings from this study observe *E. coli* and *Salmonella* spp contamination of raw beef sold in the Sokoto market for public consumption. The isolated bacteria were strains with multidrug-resistant characteristics. Relevant regulatory agencies should intensify monitoring and advocacy on standard hygienic on-farm as well as abattoir practices that reduce incidence of contamination in the meat processing value chain.

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