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Research Article

Prevalence of Food-Borne Pathogens from Livestock and Their By-products in Gwagwalada Area Council, Abuja-Nigeria

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Abstract

Foodborne pathogens are widely distributed in nature and are responsible for illnesses also referred to as food poisoning. This study aimed to isolate and characterize pathogenic and toxigenic bacteria genera such as *Staphylococcus, Escherichia* and *Salmonella* species from food-animals and food products sold within Gwagwalada Area Council. A total of 240 samples were sourced from different animal food products and by products including raw meat (beef, liver, offals), suya meat, grilled/roasted chickens, dried and roasted fish, package milk amongst vendors, as well as swabs from live chickens and cattle in market and abattoir over a period of four months. Cultural morphology on Mannitol salt agar, Xylose Lysine deoxycholates agar, Eosin Methylene Blue agar, microscopy and biochemical characterization were employed in phenotypic detection of *Staphylococcus, Salmonella and E. coli* species respectively. Antibiotic sensitivity testing was carried out on confirmed isolates using the disk diffusion method. Overall prevalence showed, 75% *Staphylococci*, *Salmonella* 20% and *E. coli* 75%. Of the Staphylococci isolates, 20 (100%) were obtained from suya meat, 20 (100%) from grilled/roasted chicken meats and 20 (100%) from dried fish. *Salmonella* isolates obtained from chicken viscera and fecal samples were 8 (26.7%) and 8 (16%) respectively. *E. coli* isolates according to sample type showed cattle rectal swab had the highest isolation rate of 95% while fish samples had the lowest isolation rate of 55%. The antibiotic sensitivity testing results showed varying degree of susceptibility of the bacteria isolates. *Staphylococci* isolates were completely (100%) resistant to ampicillin, cefuroxime and amoxicillin. All (100%) the *Salmonella* isolates were resistant to sparfloxacin, perfloxacin and amoxicillin, while the *E. coli* isolates were also completely resistant to amoxicillin and augumentin. Amoxicillin resistance was common for all bacteria isolates in this study. In conclusion, this study documents the occurrence of antibiotic-resistant zoonotic bacteria in food-animals and their by-products and its of public health significance.

Keywords: Foodborne Pathogens; *Staphylococcus; E. Coli, Salmonella*; Meat Products, Antibiotics.

Introduction

Foodborne diseases accounts for one in ten illnesses with about 7.6% (600 million) individuals of the world populations being affected annually and over 420,000 deaths worldwide [1,2]. Pathogenic bacteria such *as Salmonella, E. coli, Campylobacter, Listeria, Staphylococcus aureus* and *Yersinia species* are the most common cause of bacteria food-borne illnesses owing to their ability to produce a wide range of toxins [3]. These pathogenic agents and their metabolites can get into the body through ingestion of contaminated foods such as meat, fish, milk and its products, including eggs and other food products [4,5]. This often result in

human food poisoning conditions, characterized by nausea, sweating, dizziness, vomiting, hypothermia, stomach cramps, weakness, lethargy and diarrhea occurring for few to several hours after consumption of contaminated foods. Unhygienic conditions and poor handling of animal-derived foods contaminated with pathogenic microbes has impacted the public health burden of Foodborne diseases (FBDs) [6].

Foodborne diseases are widespread and a growing public health problem in all countries of the world. But most prevalent in developing nations. Currently, Africa and Asia have the highest cases and mortality rates from foodborne diseases, even though the incidences of FBDs in the two regions are analogous, the estimated mortality rate from Africa is almost four times higher than that of Asia with 180, 000 FBD-related deaths per year (Jaffee et al., 2020). Most of the involved pathogens have a zoonotic origin and according to European Food Safety Authority's report on Zoonoses in Europe, *Salmonellosis* is ranked second after norovirus among zoonotic diseases followed by Shiga Toxin-producing *Escherichia coli* (STEC) infection in third position, Listeria infections in fourth position and in the category of microbiological contaminants subject to food safety criteria, the enterotoxins of *Staphylococcus aureus* are mentioned in second position after Histamine (European Food Safety Authority [EFSA].

Members of the genus *Staphylococcus* are opportunistic bacteria that colonizes mucous membranes and skin of both humans and animals [7]. Though coagulase negative *staphylococci* (CoNS) are believed to be less pathogenic than *S. aureus* but have been reported to produce enterotoxins, a heat stable toxin associated with staphylococcal food poisoning. *Salmonellae* are widely distributed worldwide, but more prevalent in developing countries of the world and in areas where little is done to prevent contamination of water and food supplies. The organism is a leading cause of enteric fever, gastroenteritis and septicemia in man and animals. *E. coli*, a member of the family *Enterobacteriaceae* is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals but some strains of *E. coli* are pathogenic, incriminated in most cases of human gastrointestinal disorders and foodborne illness [8]. This rod-shaped bacterium is an indicator organism used to determine the bacteriological quality of water and milk samples [9,10].

Studies by Oduori, et al., found *Staphylococcus, Salmonella, Klebsiella* and *E. coli* species as the most frequently incriminated bacteria in most FBDs in Nigeria [11]. These organisms are ubiquitous and possess potential to contaminate a wide variety of food products during processing, preparation as well as packaging and storage. Reports further incriminated food handlers as probable carrier and contamination source of pathogenic bacteria species on edible food. These pathogenic bacteria and their toxins often cause sporadic, unrecognized and unreported but high incidence of FBDs, associated chronic complications and challenging outbreaks over many states and nations annually. Hence, this research seeks to investigate the occurrence of *Staphylococcus, Salmonella* and *E. coli* in raw meat (beef, liver, offals), suya meat, grilled/roasted chickens, roasted fish and pasteurized milk and their antimicrobial profile in order to proffer better human safety awareness in the study area.

Methodology

The Study Area

The study was conducted in Gwagwalada Area Council of the Federal Capital Territory (FCT) including the University of Abuja and its environs, situated along the Abuja city Airport Road, Nigeria. Gwagwalada Area Council is located between latitude 8° 25" and 9° 25" North of the equator and longitude 6° 45" and 7° 45" East of the Greenwich. Lying between latitudes 8.25° and 9.20° north of the Equator and longitudes 6.45° and 7.39° East of Greenwich Meridian. It shares a boundary with Kuje Area council to the East, Abuja Municipal Area Council to the North, Abaji Area Council to the South and Zuba to the West, as shown in Fig. 1 [12].

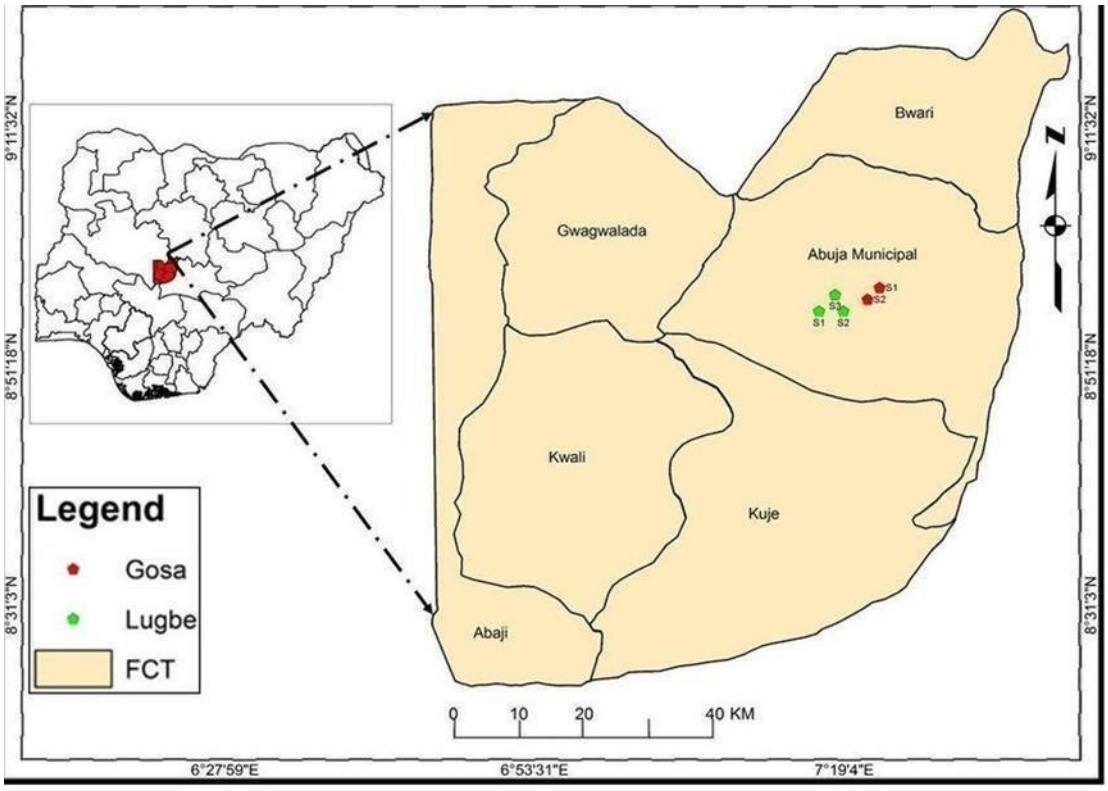

Figure 1: Map of Abuja showing the study area.

Source: Abuja Geographical Information System indicate locations on Gwagwalada.

Sample Collection and processing

A total of 240 samples comprising of these sample types: suya, grilled chicken, roasted fish, package milk, raw beef, fresh fish, offals, chicken liver, rectal swab of cattle and cloacal swabs of chickens were collected using purposive sampling technique from vendors in University of Abuja and Gwagwalada communities. The study was designed to collect Eighty (80) samples each for isolation of *Staphylococcus, Salmonella* and *E. coli*. For each sample type twenty (20) samples were collected into sterile sample tubes containing 5 ml of peptone water and placed in a cool thermos on ice packs for immediate transportation to the Department of Veterinary Microbiology laboratory, University of Abuja for processing and microbiological analysis.

Laboratory Analysis

All media used were prepared according to manufacturer's instructions. Bacteria organisms in this study were isolated according to standard methods described by International Organization for Standardization (ISO 6579) (2002) and Office International des Epizooties (OIE) (2011). In brief, each sample was analyzed individually by first inoculating in 5 ml of peptone water (Hi Media, Mubai India) and incubated at 37°C for 18 to 2 hours. After which 1 ml of the inoculum was inoculated into 9 ml of selective enrichment broth: MacConkey broth and Rappaport vassiliadis broth for *E. coli* and *Salmonella*, incubated at 37°C for 18 to 24 hours. After which a loopful from the selective broth was streaked onto prepared plates of MacConkey and XLD agar and then incubated at 37°C for 18 to 24 hrs. Pinkish colonies (lactose fermenters) were further sub cultured on prepared plates of EMB agar, incubated at 37°C for 18 to 24 hr and observed for the appearance of greenish metallic sheen colonies suggestive of *E. coli*. While colorless colonies on MacConkey were subculture on XLD and observed for the presence of typical colonies of *Salmonella* which are red colonies with black centers on XLD agar. For Staphylococci isolation, a loopful from inoculated peptone water was directly streaked onto prepared plates of MSA and incubated at 37°C for 18 to 24hrs. After incubation, all plates were examined for suspected Staphylococci growth, which were seen as small, round, convex, pink or yellow colonies on MSA. The yellow colonies were considered as presumptive or suspected *Staphylococcus aureus* due to fermentation of Mannitol and were sub-cultured on prepared plates of nutrient agar, incubated at 37℃ for 18- 24 hours to obtain a pure culture [13]. All presumptive bacteria isolates were inoculated on nutrient agar slants and incubated at 37℃ for 18- 24 hours, then stored at 4℃ for microscopic and biochemical characterization. Isolates were characterized based on their Gram's reaction and specific biochemical test as described by Cheesbrough [14].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of isolates was examined using the disc diffusion technique of Kirby-Bauer and in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). The antibiotic discs (Abtek Biologicals Ltd) used comprised of pefloxacin (10µg), gentamycin (10µg), ampicillin (30µg), cefuroxime (20µg), amoxacillin (30µg), ceftriazone (25µg), ciprofloxacin (10µg), azithromycin (20µg), levofloxacin (20µg) and erythromycin (10µg). The results were interpreted according to the guidelines provided by the Clinical Laboratory Standards Institute (CLSI, 2018).

Data Analysis

Data generated in this study were analyzed using descriptive statistics and presented using frequency, percentages and tables.

Results

Out of the 80 samples collected for Staphylococci study, 60 typical isolates were obtained from all the sample types: suya meat (20), grilled/roasted chicken meats (20) and roasted dried fish (20). While the package milk samples yielded no isolates with an overall prevalence of 75% Staphylococci contamination recorded in this study as shown in Table 1. A total of 16 (20%) isolates were confirmed positive as *Salmonella* species, across sample types with 8 (26.7%) of the isolates been obtained from liver/intestine of slaughtered chickens and 8 (16%) from chicken fecal samples with the overall prevalence of 20% for *Salmonella* species in this study (Table 1). 60 samples out of 80 were positive for *E. coli* with a distribution according to sample type that showed highest number of isolates from cattle rectal swab 19 (95%), fish 11 (55%), beef and offal had 16 (80%) and 14 (70%) respectively and an overall prevalence of 75% (Table 1).

The antimicrobial susceptibility testing result showed that 91.66% of Staphylococci isolates were sensitive to levofloxacin, 83.3% to ciprofloxacin, 66.6% to Azithromycin. All the Staphylococci isolates (100 %) were completely resistant to ampicillin, cefuroxime and amoxicillin clavulanic acid as shown in Fig. 2. *Salmonella* isolates in this study were completely susceptible to ofloxacin (100 %) but completely resistant to pepfloxacillin (100 %), sparfloxacillin (100), 87% resistance to amoxicillin and 46% and 44% to chloramphenicol and gentamicin respectively. The *E. coli* isolates in this study showed varying degree of sensitivity and resistance, 70% of the isolates were sensitive to ciprofloxacin, 68.3% were susceptible to ofloxacin and gentamicin. While all the isolates (100 %) were resistant to augumentin and amoxicillin as seen in Fig. 3,4.

Table 1: Occurrence and distribution of bacteria species in livestock and food products in Gwagwalada Area Council.

S/NO	Sample Type	No of Sample	Bacteria Specie	No of Isolates	Percentage Positive (%)
1	Suya meat	20	*Staphylococcus*	20	100
2	Grilled chicken	20	*Staphylococcus*	20	100
3	Roasted fish	20	*Staphylococcus*	20	100
4	Package milk	20	-	-	-
5	Chicken visceral	30	*Salmonella*	8	26.6
6	Chicken feces	50	*Salmonella*	8	16
7	Beef	20	*E. coli*	16	80
8	Offals	20	*E. coli*	14	70
9	Fresh fish	20	*E. coli*	11	55
10	Rectal swab	20	*E. coli*	19	95
	Total	**240**			

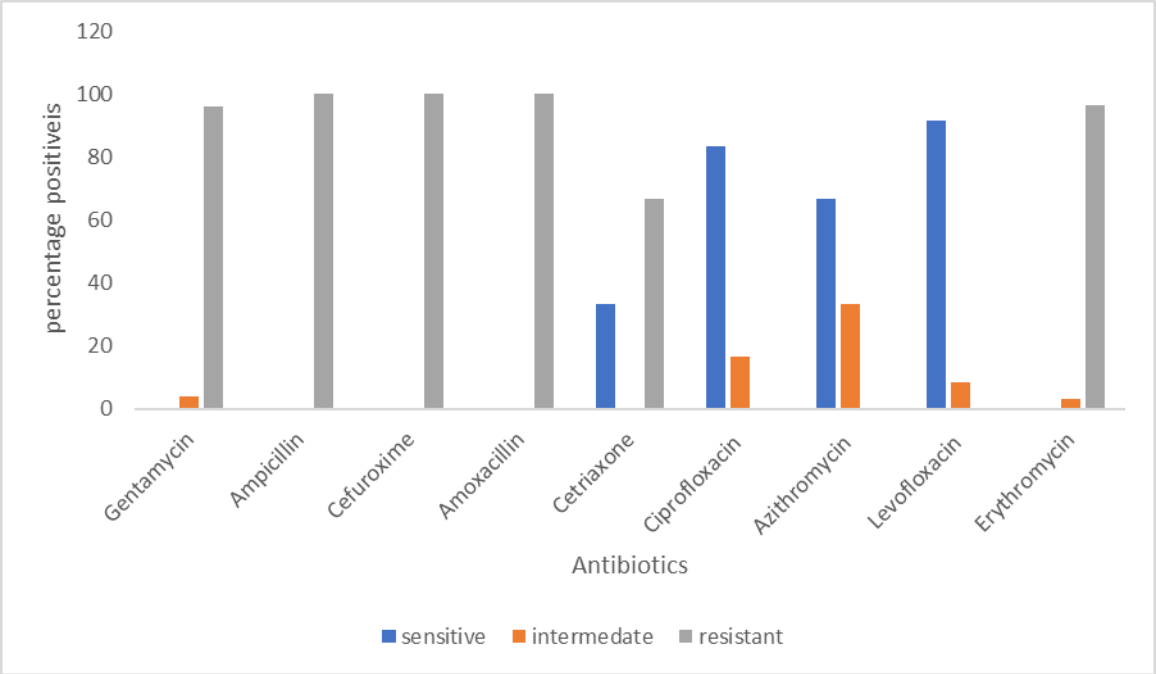

Figure 2: Dissipation of susceptibility studies of Staphylococci isolates to tested antibiotic agents

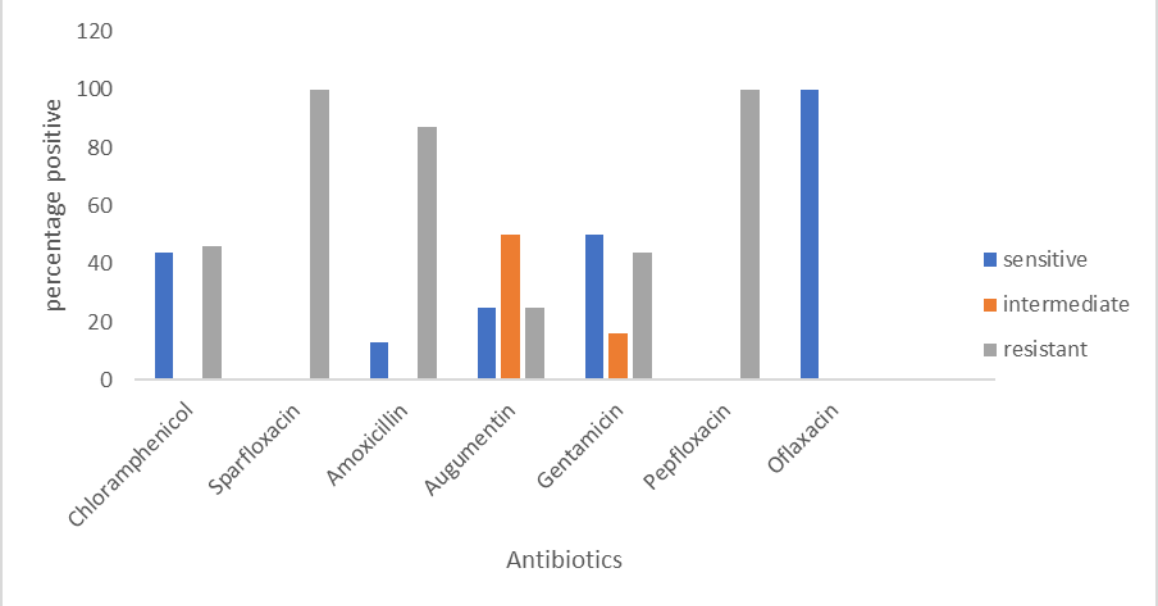

Figure 3: Dissipation of susceptibility studies of *Salmonella* isolates to antibiotic agents

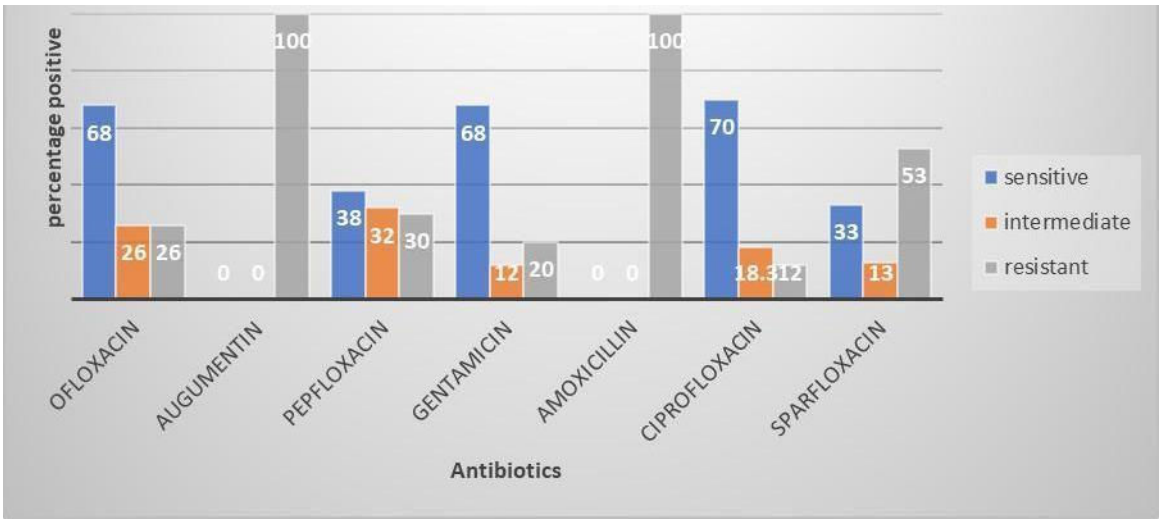

Figure 4: Dissipation of susceptibility studies of *E. coli* isolates to antibiotic agents.

Discussion

In this study, three predominant bacteria Foodborne pathogens associated with food contamination and foodborne illnesses were isolated using specific selective media and characterized biochemically according to standard methods. The overall prevalence of 75% Staphylococci contamination of ready-to-eat food products in this study is significantly higher than the 15.8% prevalence reported by Alabi, et al., and 28% reported by Mashak et al. [9,15]. This could be attributed to poor food handling and processing which poses public health threat to potential consumers of the various food products. *Staphylococcus* species are ubiquitous in nature, reported to have been isolated from a wide variety of food types such as meat milk and poultry and poultry products [16-20]. Several studies have also reported the occurrence of the organism from raw milk and milk products in Nigeria with the highest and lowest prevalence of 100 and 3.21% respectively [21]. However, the result of this study shows that the milk samples in this study were devoid of Staphylococci contamination. This could be attributed to pasteurization and other processing methods in which the packaged milk in this study were produced for commercial purposes under standard sanitary conditions, thus eliminating probable environmental bacteria contamination.

The occurrence of *Salmonella* in live bird and chicken meats in this study conforms to previous reports with the isolation rate of *salmonellae* being higher in liver/intestinal (26.7 %) samples than from fecal samples (16%) [10,22]. This is because chickens can become chronic carriers of *Salmonella* organism and thus excretes the organism in their faces intermittently in small quantum over a prolong period of time [23]. In addition, this organism is an important cause of avian Salmonellosis in poultry and a potential source of zoonotic foodborne transmission of *Salmonella* to humans through consumption of contaminated chicken meat originating from farm animals (Humphrey, 2006). Consequently, contaminated chicken meat remains an internationally important vehicle for human Salmonellosis [24].

The overall prevalence of *E. coli* in this study is higher than the 60.2% reported by Azabo, et al., but lower than the 83.1% reported by Mailafia, et al., suggesting endemic occurrence of this bacteria characterized by an unstable pattern but of serious public health significance because some *E. coli* strains are toxigenic and highly pathogenic [25]. In this study, the isolation rate of *E. coli* was higher from the rectal swabs of cattle than that obtained from fish, beef and offals. This could be attributed to the fact that the organism is a commensal bacterium of the gastrointestinal tract, hence could be easy isolated since they are extensively distributed in the gut of animals. However, the occurrence of the organism in beef samples is an indication of contamination which could be associated with poor hygienic practices along value chain process such as during and after slaughter, during transportation from the abattoir to the market with the source of contamination originating either from the animals, abattoir

environment, water used in washing the meats or even from the service providers including meat handlers such as the butchers, transporters and sellers.

All the bacteria isolates (*Staphylococcus, Salmonella* and *E. coli*), exhibited high level of antimicrobial resistance to commonly prescribed antibiotics in the study area suggesting the occurrence of an increasing antimicrobial resistance in these potential foodborne pathogens. This conforms to previous reports of Antimicrobial Resistance (AMR) being a growing public health concern because multi-drug-resistant bacteria can be transmitted to humans via the food chain, leading to potential infections that poses refractory challenges in the healthcare sector due to limited treatment options [26-29]. AMR has been established as one of the most important microbial threats of the twenty-first century. This could occur naturally as a survival strategy by microbes to a new environment but more importantly is due to indiscriminate use of antibiotics by man for therapeutic purpose or as growth promoters in food-animals [25].

Generally, poor sanitation during food processing and storage is responsible for most food contamination, often complicated by high environmental temperatures and relative humidity which favors most bacteria growth and multiplication [19]. Nonetheless, as part of a preventative strategy and microbiological safety checks based on Hazard Analysis and Critical Control Point (HACCP) principles, it is crucial for operators in the food industry that their products contain no pathogenic microbes and enterotoxins for the entirety of the food's shelf life.

Conclusion

This preliminary finding reveals an endemic occurrence of multidrug resistant *Staphylococcus, Salmonella* and *E. coli* incriminated as potential pathogenic foodborne pathogens associated with livestock meat products and their by-products sold in Gwagwalada Area Council including the University community in Abuja-Nigeria. Appropriate government action is therefore needed to curtail the menace of this disease in the study area.

Conflict of Interest

The authors have declared that they have no conflict of interest during the research and publication of this manuscript.

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