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Identification of bacteriological quality and antimicrobial resistance of microorganisms isolated from animal foods collected from the abattoir, butcher shops and local seafood market

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Abstract

The current study aimed to isolate bacteria that harbour various animal food products like meat, chicken and seafoods collected from the abattoir, butcher shops and local seafood market and to determine the antimicrobial resistance pattern of isolated pathogens which are responsible for various foodborne illnesses in human beings. A total of forty raw animal product samples were collected from the abattoir, butcher shops and local seafood market of Visakhapatnam. The samples selected for the study include raw chicken, meat, crab, prawns and different varieties of fish. A classic random sampling technique was employed to collect the study samples. All the samples were processed immediately using standard microbiological protocols. The bacteria isolation and characterization were done by studying morphological characteristics with staining methods, cultural characteristics by isolating and growing the pathogenic microorganisms in various selective and differential culture media. Antimicrobial susceptibility testing was performed by the Kirby -Bauer method by following Clinical and Laboratory Standards Institute (CLSI) guidelines. EDTA-Disc Potentiation Test and Imipenem-EDTA Double disc synergy test are used to detect the metallo beta-lactamase production of isolated pathogens. The highest number of isolates belong to *Salmonella* species (18), *Pseudomonas aeruginosa* (18) followed by *Vibrio* species (14) and few isolates belong to *Enterobacter* species (4). Majority of the microbial isolates obtained in the current study were multidrug resistant. The isolates from the abattoir environments, slaughterhouses, fish markets were found to exhibit variable resistance pattern to aminoglycosides, macrolides, β-lactams, cephalosporins, quinolone antibiotics used in the present study and at the same time most of them were sensitive to carbapenem antibiotic imipenem. Antimicrobial resistance (AMR) prevents the designing and assessment of effective interventions. If such a link can be established, then the tracking of antibiotic use and consumption data could be furthermore used as a surrogate indicator for the risk of potential antibiotic resistance (ABR) emergence.

1. Introduction

Animal products include any material obtained from an animal's body, this has the well-known flesh, blood, fat, flesh, eggs, bones, milk, skin and less familiar products, viz rennet and isinglass. Parts of animals that are not appropriate for human consumption are termed as animal by-products and some of the marketing value-added animal by-products allow the meat industry to economically compete with vegetable protein sources (Bengtsson and Holmqvist, 1984; Chatli *et al*., 2005). Meats of various animals including, fish play a significant role in the diet of a large group of people, and this is attributed to their appreciable palatability and nutritive value. Standards for fish and fish products and meat and meat products were set in Section 2.5 of Food Safety and Standards (Food Product Standards and Food Additives) Regulations (2011). These standards apply to both chemical requirements and microbiological requirements. A special manual has been designed for the microbiological examination of frozen meat and fish products (Rabia Shabir *et al*., 2018).

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Foodborne pathogens are a significant source of illness, sometimes with severe and fatal outcomes, thus consequently leading to a considerable expenditure of money on medical management. In general, children are more affected by food poisoning due to their underdeveloped immune systems. Chances of cross-contamination are more during the handling of raw meat than cooked meat, and this is due to high water activity in raw flesh (Hutt, 1984; CDC, 2012). Food handlers, equipment and the environment serve as the primary sources for meat contamination. Seafood associated infections are caused by a variety of viruses, bacteria and parasites; hence seafood consumption is at significant risk (Kromhout *et al*., 1985; Kris-Etherton *et al*., 2002). The recent data disclosed by the Union Health Ministry's Integrated Disease Surveillance Program (IDSP) reveals the fact that food poisoning is one of the most common outbreaks of India reported in 2017.

Bacterial infections are the major limiting factors for the significant production of animal-based food products as they are strongly involved in causing illness both in food-producing and their companion animals. Research reveals a piece of potent evidence that human ingestion of foods carrying antibiotic-resistant microbes has resulted, either directly or indirectly, in acquiring many antibiotic-resistant infections. All animals naturally carry bacteria in their intestinal tract. Giving antibiotics to animals as a part of the treatment will kill many bacteria. Nevertheless, resistant bacteria can survive and proliferate. Once the food animals are slaughtered and processed, these bacteria contaminate the meat and other animal products. These microbes can even infect the environment through animal stool and may reach the produce that is irrigated with contaminated water, thereby causing various water and foodborne illnesses (Jose Luis, 2017).

The judicious application of antimicrobial agents is the major challenge to minimize the risk of drug resistance. There is an urgent requirement to establish a sustainable antimicrobial surveillance system that can be piggybacked and strengthen the existing communicable disease surveillance system. The antimicrobial resistance surveillance system can help control the impending microbial resistance epidemic. An increase in antimicrobial resistance (AMR) in animal bacteria is attributed to their shorter generation time; plasmid-encoded resistance mechanism, exchange of genetic material etc. The drug-resistant animal microbes form a potential threat to humans by causing diseases when they are transferred through food or if they transfer their genetic material coding for resistance to pathogenic human bacteria. Investigation of AMR in bacteria that are threatening public health is routinely done using standardized phenotypic methods. However, Molecular methods are often replacing phenotypic methods in many laboratories due to their speed and accuracy they provide in detecting the underlying genetic mechanisms for AMR (Julian and Dorothy, 2010; Muna *et al*., 2017).

Our current study aimed to isolate bacteria that harbour various animal food products like meat, chicken and seafood collected from the abattoir, butcher shops and local seafood market and to determine the antimicrobial resistance pattern of isolated pathogens which are responsible for various foodborne illnesses in human beings.

2. Materials and methods

2.1 Study design, study area and sampling

The present study was carried from January 2020 to June 2020. Forty raw animal product samples were collected from the abattoir, butcher shops and local seafood market of Visakhapatnam. The samples selected for the study include raw chicken, meat, crab, prawns and different varieties of fish. A classic random sampling technique was employed to collect the study samples. The randomly collected samples of weight 25 g each were packed in sterile plastic bags and kept in an ice-cold box and were labelled appropriately with sample type sources, and date of collection. The sampling areas were clearly delineated with sterile aluminium foil templates (10×10 cm). A sterile cotton swab (2×3 cm) fitted with a wooden shaft was soaked in 10 mL of sterile buffered peptone water (BPW) (Himedia Pvt. Ltd) and swabbed gently over the delineated area horizontally and vertically several times. After completion of the swabbing, the used swab was placed in the test tube containing BPW, and the upper tip of the wooden shaft was broken down and disposed of leaving the cotton swab. All the samples were arranged in an icebox containing ice packs and without delay transported to the Microbiology Laboratory and were immediately processed upon arrival.

2.2 Bacterial isolation and characterization

Approximately 1 g of each raw animal product sample was homogenized in a blender for 2 mins with 10 mL of 1% buffered peptone water. An aliquot (0.1 mL) of the homogenized sample filtrate was carefully transferred onto each of the Thiosulphate citrate bile salt agar medium, blood agar, Mac-Conkey agar, Chocolate agar, Nutrient agar, Salmonella-Shigella agar and Eosin-methylene blue agar medium plates and incubated for 24 hrs at 37°C. The developed colonies were identified by their morphological characteristics, biochemical

properties, staining characteristics, by using standard microbiological protocols. The colony characteristics on the solid culture medium were studied in terms of qualitative measures of size, surface, shape, edge, opacity, colour and elevation, and the haemolysis pattern on blood agar. The characteristics observed in the liquid medium are surface pellicle formation, uniform turbidity, and formation of deposit at the bottom of the flask. Gram characters were revealed by performing Gram staining. Motility was identified by performing the hanging drop method. The presence of the capsule was recognized with the Indian-ink method. Various biochemical tests are performed for the confirmation of the culture, and these include catalase, oxidase, IMViC tests, carbohydrate fermentation tests, coagulase test, urease test, phenyl-alanine deaminase test, nitrate reduction test etc.

2.3 Antimicrobial susceptibility test

The antimicrobial susceptibility of isolated microorganisms was performed by using Kirby- Bauer disc diffusion method according to CLSI guidelines using Mueller-Hinton Agar (Hi-Media, India). The antibiotic susceptibility pattern was examined by using commercially available antibiotic discs of ceftazidime (30 µg), amoxicillin (10 µg), Gentamicin (10 µg), tetracycline (30 µg), Azithromycin (15 µg), Cotrimoxazole (1.25 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg) and Imipenem (10 µg). The *Escherichia coli* isolate ATCC 25922 and *Staphylococcus aureus* isolate ATCC 25923 were used as reference organisms for quality control for antimicrobial susceptibility testing. The interpretation usually categorizes each result as susceptible (S), Intermediate sensitive (I) and Resistant (R).

2.4 EDTA-disc potentiation test

Test organisms are inoculated onto plates with Mueller Hinton agar as described for the disc diffusion test. A blank filter paper (Whatman No. 2) was placed, and the discs of ceftazidime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), Ceftriaxone (30 µg) were placed 25 mm centre to centre from blank disc. 10 µL of 0.5 M EDTA solutions were added to the blank disc, and the plates were incubated overnight at 37°C. Development of the inhibition zone in the area that lies between the EDTA disc and anyone of the four cephalosporin discs, in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.

2.5 Imipenem-EDTA double disc synergy test (DDST)

Double disc synergy test was conducted as described by Lee *et al.* (2006). Test organisms were inoculated onto the plates of Mueller Hinton agar as recommended by CLSI guidelines. A 10 µg imipenem disc was placed 20 mm centre to centre from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). The formation of the inhibition zone in the region between imipenem and EDTA disc in comparison with the inhibition zone on the far side of the drug was considered as a positive result.

3. Results

In Table 1 it was reported that both Gram-positive and Gram-negative microorganisms were isolated from forty different types of tested animal products like raw meat, raw chicken, prawns, crabs, and varieties of fishes from a local abattoir, butcher shops and local seafood markets of Yendada and Madhurawada, Visakhapatnam. Table 2 represented the total number of microbial isolates screened from different animal food products used in the current study. The highest number of isolates belong to *Salmonella* species (18), *Pseudomonas aeruginosa* (18) followed by *Vibrio* species (14) and few isolates belong to *Enterobacter* species (4) (Figure 1).

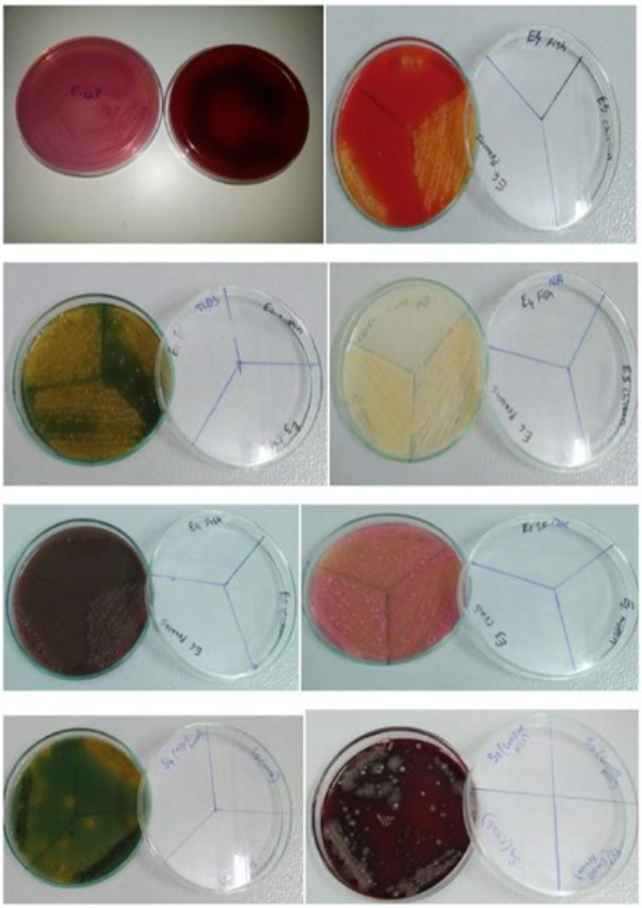

Figure 1. Screening of microorganisms from animal food products on various selective media

Table 3 displays the antibiotic sensitivity pattern of isolated strains, *E. coli* showed maximum susceptibility (66%) to imipenem followed by tetracycline and cefoxitin (50%) and showed the least sensitivity to other

Table 1. Isolation of microorganisms from various animal-derived food products

S. NO	Type of food sample	Number of samples collected from the areas (Yendada and Madhurawada, Visakhapatnam)	Microorganisms isolated
1.	Raw meat (abattoir)	02	*Staphylococcus aureus*, *Proteus* species, *Salmonella* species, *Klebsiella* species
2.	Raw meat (butcher shop)	02	*Pseudomonas aeruginosa, Vibrio* species, *Proteus* species, *Klebsiella* species
3.	Raw chicken (abattoir)	02	*Klebsiella* species, *Enterococcus faecalis, Vibrio* species
4.	Processed meat	02	CONS, *Shigella* species
5.	Processed chicken	02	*Pseudomonas aeruginosa, Proteus* species
6.	Turkey meat	02	*Staphylococcus aureus*, *Enterococcus faecalis, Salmonella* species, *Klebsiella* species
7.	Raw chicken (butcher shop)	02	*Escherichia coli, Proteus* species, *Enterobacter* species, *Salmonella* species
8.	*Penaeus monodon* (Prawns)	02	*Klebsiella* species, *Pseudomonas aeruginosa, Enterococcus faecalis, E.coli, Vibrio* species
9.	*Cancer pagurus* (Crab)	02	*Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* *Vibrio* species
10.	Sardina pilchardus fish	02	CONS, *Pseudomonas aeruginosa, Salmonella* species
11.	Rastrelliger kanagurta fish	02	*Pseudomonas aeruginosa, Proteus* species, *Salmonella* species
12.	Carassius auratus fish	02	*E. coli, Salmonella* species
13.	Parupeneus indicus fish	02	*Vibrio species, Enterobacter* species
14.	Salmosalar fish	02	*Enterococcus faecalis, Proteus* species, *Vibrio species*
15.	Scombero morus guttatus fish	02	*Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* species
16.	Thunnini fish	02	CONS, *Vibrio species, Klebsiella* species
17.	Pangasius bocourti fish	02	*Pseudomonas aeruginosa, E. coli, Salmonella* species
18.	Labeocatla fish	02	*Shigella* species, *Enterococci* species
19.	*Engraulidae (Anchovy)*	02	CONS, *Shigella* species, *E. coli*
20.	*Penaeus indicus*	02	*Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* species

Table 2. Total number of microbial isolates from different animal derived food products

S. NO	Microorganisms	Total number of isolates
1.	*E. coli*	12
2.	*Staphylococcus aureus*	10
3.	*Proteus* species	12
4.	*Vibrio* species	14
5.	*Salmonella* species	18
6.	*Pseudomonas aeruginosa*	18
7.	Coagulase-negative *Staphylococci* (CONS)	08
8.	*Enterobacter* species	04
9.	*Enterococcus faecalis*	10
10.	*Shigella* species	06
11.	*Klebsiella* species	12

tested antibiotics. *S. aureus* shows an equal range of susceptibility (60%) to imipenem, azithromycin, amoxycillin, tetracycline, ceftazidime and cefoxitin. *Proteus* species showed maximum susceptibility (66%) to imipenem and least sensitivity towards ciprofloxacin, gentamycin, amoxicillin and cotrimoxazole (33%). *Vibrio* species showed the highest susceptibility (86%) to imipenem and least sensitivity (14%) to ciprofloxacin, amoxycillin and cotrimoxazole. *Salmonella* species showed the highest sensitivity (78%) to imipenem followed by tetracycline (55%) and the least susceptibility to ciprofloxacin, amoxicillin and cotrimoxazole (22%). *Pseudomonas aeruginosa* showed the highest sensitivity (55%) to imipenem and least sensitivity to ciprofloxacin, gentamycin and cotrimoxazole (22%). The isolated 4 coagulase-negative *Staphylococci* strains showed 100% sensitivity to

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Table 3. Antibiotic sensitivity pattern of isolates

Microorganisms and Total number of isolates	Imipenem (10µg)	Ciprofloxacin (5 µg)	Azithromycin (15 µg)	Gentamicin (10µg)	Amoxycillin (10 µg)	Tetracycline (30 µg)	Cotrimoxazole (1.25µg)	Ceftazidime (30 µg)	Cefoxitin (30 µg)
	Number and percentage of sensitive strains to the tested antibiotics								
E. coli (12)	8 (66%)	4 (33%)	4(33%)	4 (33%)	4 (33%)	6 (50%)	4(33%)	4(33%)	6 (50%)
Staphylococcus aureus (10)	6 (60%)	4(40%)	6 (60%)	4 (40%)	6(60%)	6(60%)	2(20%)	6(60%)	6(60%)
Proteus species (12)	8 (66%)	4 (33%)	6 (50%)	4(33%)	4(33%)	6(50%)	4(33%)	6(50%)	6(50%)
Vibrio species (14)	12 (86%)	2 (14%)	8 (57%)	6 (43%)	2 (14%)	6(43%)	2(14%)	6(43%)	6(43%)
Salmonella species (18)	14 (78%)	4 (22%)	8 (44%)	6 (33%)	4 (22%)	10(55%)	4(22%)	6(33%)	6(33%)
Pseudomonas aeruginosa (18)	10 (55%)	4 (22%)	8 (44%)	4(22%)	6 (33%)	8(44%)	4(22%)	8(44%)	8(44%)
Coagulase-negative *Staphylococci* (CONS) (08)	8 (100%)	2 (25%)	4 (50%)	2(25%)	4 (50%)	4(50%)	4(50%)	4(50%)	4(50%)
Enterobacter species (04)	4 (100%)	2 (50%)	2 (50%)	2(50%)	2(50%)	2(50%)	2(50%)	2(50%)	2(50%)
Enterococcus faecalis (10)	8 (80%)	4 (40%)	4 (40%)	4(40%)	4(40%)	6(60%)	4(40%)	4(40%)	4(40%)
Shigella species (06)	4 (67%)	2 (33%)	2 (33%)	2(33%)	2(33%)	2(33%)	2(33%)	2(33%)	2(33%)
Klebsiella species (12)	6 (50%)	2 (17%)	4 (33%)	6(50%)	2(17%)	6(50%)	2(17%)	6(50%)	6(50%)

Table 4. Detection of carbapenemase production in isolated microorganisms

Organisms	Total isolates	Imipenem Resistance	Percentage (%)	Imipenem Sensitivity	Percentage (%)
E. coli	12	4	34	8	66
Staphylococcus aureus	10	4	40	6	60
Proteus species	12	4	34	8	66
Vibrio species	14	2	14	12	86
Salmonella species	18	4	22	14	78
Pseudomonas aeruginosa	18	8	45	10	55
Coagulase-negative *Staphylococci* (CONS)	8	0	0	8	100
Enterobacter species	4	0	0	4	100
Enterococcus faecalis	10	2	20	8	80
Shigella species	6	2	33	4	67
Klebsiella species	12	6	50	6	50

Table 5. Detection of Metallo Beta-Lactamase by EDTA disc potentiation test

Total number of isolates	Positive	Percentage (%)	Negative	Percentage (%)
18	16	88	2	12

Table 6. Detection of Metallo Beta-Lactamase by double disc synergy test

Total number of isolates	Positive	Percentage (%)	Negative	Percentage (%)
18	17	94	1	6

imipenem and least sensitivity (25%) to ciprofloxacin and gentamycin. The two isolated *Enterobacter* strains showed the highest sensitivity (100%) to imipenem and showed 50% sensitivity to remaining all tested antibiotics. *Enterococcus faecalis* showed the highest susceptibility (80%) to imipenem followed by tetracycline (60%). Out of three isolated Shigella strains, two strains were susceptible (67%) to imipenem and showed the least susceptibility (40%) to all other tested antibiotics. *Klebsiella* species showed the highest sensitivity (50%) to imipenem, gentamycin, tetracycline ceftazidime and cefoxitin. All the isolated microorganisms from various animal products showed maximum sensitivity to carbapenem and imipenem and the least sensitivity to antibiotics, specifically ciprofloxacin, amoxicillin and cotrimoxazole. Figure 2 showed the antibiotic susceptibility pattern of isolated strains by measuring their zone of inhibitions to the tested antibiotics. Among the different isolated strains, some of the strains were capable of producing carbapenemases and acquire resistance to carbapenem antibiotics like imipenem. Highest number of *Pseudomonas aeruginosa* strains showed carbapenemase production and no production was detected in the *Enterobacter* species and Coagulase-negative *Staphylococci* (CONS) (Table 4). EDTA disc potentiation test was performed with four different cephalosporins. By detecting the Metallo beta-lactamase production in 18 bacterial strains, 16 strains showed positive (88%) by EDTA disc potentiation test and 17 strains by double-disc synergy test (94%) (Tables 5 and 6).

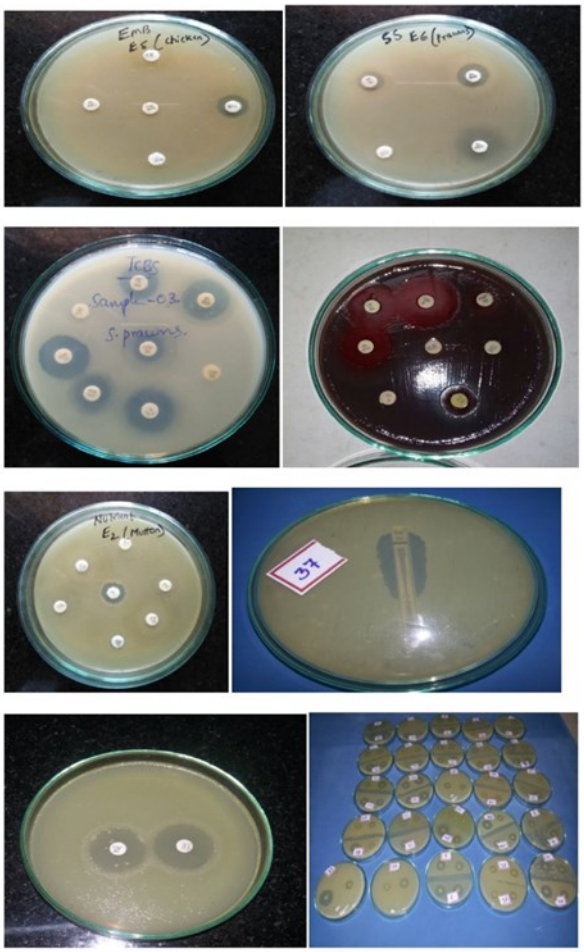

Figure 2. Antibiotic susceptibility of microorganisms isolated from animal food products

4. Discussion

Most of the veterinary antibiotic trade was unregulated in these nations, and the animal feed was less nourishing compared to developed nations, thus leading to an exponential increase in developing antibiotic resistance among animals. Over usage of antibiotics in animals raised for animal food products is associated with the rise in antibiotic-resistant infections in animals and humans. The researchers developed a geospatial model utilizing data from 901 point-prevalence surveys between 2000 and 2018 and reported rates of antibiotic resistance in animals and food products. Their analysis concentrated particularly on regular foodborne disease-causing microbes like *Campylobacter* species, *Escherichia coli*, non-typhoidal *Staphylococcus aureus* and *Salmonella* species. The development of resistance is not merely limited to the classical and frequently used drugs but also observed with novel drugs.

In the present study, all the tested forty samples yielded 124 bacterial strains on different selective and differential culture medium. All the forty samples showed a mixed culture of microorganisms, and most of the isolates were observed to belong to *Salmonella* species and *Pseudomonas aeruginosa* these strains showed maximum susceptibility to carbapenem antibiotics like imipenem and least or no susceptibility to ciprofloxacin, gentamycin, amoxicillin and cotrimoxazole etc. Since most of the veterinary drugs were designed exclusively for veterinary use. However, the compounds in the formulations are identical or nearly similar to those employed in human medicines for the reason that they belong to the same antimicrobial classes such as aminoglycosides, macrolides, β-lactams, cephalosporins, sulfonamides, fluoroquinolones and tetracycline. Most of the resistance towards different classes of antibiotics has appeared in humans, animals, and animal products. The findings of the present study were in harmony with previous studies (Akiyama and Khan, 2012).

Salmonella species are the most significant pathogens and the third leading cause of human death. *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi do not present animals as reservoirs, except for higher primates; their presence suggests contamination during food and water handling

by insufficient hygiene management. Motsoela *et al*. (2002) and Bello *et al*. (2011) reported that salmonella was present in all sampled abattoir environments in Botswana and western Nigeria. The majority of the microbial isolates obtained in the current study were multidrug-resistant. The isolates from the abattoir environments, slaughterhouses, fish markets were found to exhibit variable resistance pattern, and the antibiotic susceptibility pattern was in accordance with the previous studies (*Sallam et al*., 2014; Nseabasi-Maina *et al*., 2017). The presence of multidrug-resistant (MDR) *Salmonella* spp., could create major public health risks to consumers. Firstly, ingestion of various products contaminated with the MDR Salmonella species could cause salmonellosis which demands antibiotic therapy and the treatment can then be compromised due to the resistant strain (Omololu-Aso *et al*. 2017). However, it is noteworthy that even the cooking process will not destroy genes accountable for the resistance. Secondarily, non-pathogenic resistant bacteria can move into a human through the consumption of contaminated foods. Thus, resistance genes are subsequently transferred to other bacteria residing in the gut via mobile genetic element *viz* transposon, integrons, plasmid, gene cassette, and insertion sequence (Mboto *et al*., 2012; Bagudo *et al*., 2014). Finally, antibiotics may remain as residues in animal products such as meat, blood and liver and lead to the selective propagation of resistant clones in the consumer. The rate of isolating salmonellae from the slaughter environment is growing with multidrug resistance and with an increased tendency of their resistance. Consequently, it is essential to develop antibiogram research and bacterial resistance monitoring to direct rational drug therapy (Bello *et al*., 2011).

5. Conclusion

In conclusion, the results of the present study provide preliminary data on the prevalence of antimicrobial-resistant bacteria in raw animal products and make clear that biologically they are unsafe for human consumption. Many governments and industry policies, including standard operating procedures for sanitation, and the Hazard Analysis Critical control point (HACCP) system are framed to trim down the flow of foodborne pathogens from animals to humans. HACCP particularly focus on product protection and this has been widely adopted, particularly at slaughter or processing. These programs could help to decrease the flow of antimicrobial-resistant pathogens related to foods into humans.

Conflict of interest

The authors declare no conflict of interest.

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