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Antibiotic resistance pattern of bacteria isolated from abattoir table at Amansea Market, Awka

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Abstract

Food borne 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. Food handlers, equipment and the environment serve as the primary sources for meat contamination. The current study aimed to isolate bacteria from the abattoir tables at Amansea market and to determine the antimicrobial resistance pattern of isolated pathogens which are responsible for various foodborne illnesses in human beings. A total of five raw animal product samples were collected from the abattoir and butcher shops. The samples selected for the study include raw chicken and meat. 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. From the result, the highest number of isolates belong to *Staphylococcus aureus* (9), *Escherichia coli* (8) followed by *Proteus mirabilis* (6) and *Klebsiella pneumonia* (5) with a few isolates belonging 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. From the research, it was inferred that these tables contain large amounts of multidrug resistant bacteria that could impact on public health of humans, especially the abattoir workers and residents around the abattoir. There is need for sensitization of these food and meat handlers on the risks associated with their unhygienic practices.

Keywords: Antibiotic resistance; Abattoir; Food-borne illness; Pathogens

1. Introduction

Any material derived from an animal's body is considered an animal product; this includes less well-known items like flesh, blood, fat, eggs, bones, milk, and skin. Animal by-products are portions of animals that are unfit for human consumption. Because of their notable palatability and nutritional worth, meats from a variety of animals, including fish, are a staple in the diets of many people. Section 2.5 of the Food Safety and Standards (Food Product Standards and Food Additives) Regulations (2011) established standards for meat and meat products as well as fish and fish products. Both chemical and microbiological requirements are covered by these standards. A special manual has been designed for the microbiological examination of frozen meat and fish products (Rabia *et al*., 2018).

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A large amount of money is spent on medical management since food-borne viruses are a major cause of illness, often with serious and fatal consequences. Generally speaking, food poisoning is more common in youngsters because of their immature immune systems. Because raw flesh has a higher water activity than cooked meat, handling raw meat increases the risk of cross-contamination (CDC, 2012). The main causes of meat contamination include food handlers, equipment, and the environment. According to recent data released by the Integrated Disease Surveillance Program (IDSP) of the Union Health Ministry, food poisoning was one of the most prevalent outbreaks in India in 2017.

Due to their important role in generating illness in both food-producing and companion animals, bacterial infections are the main issues limiting the large production of animal-based food items. There is strong evidence from research that eating foods contaminated with antibiotic-resistant bacteria has caused many antibiotic-resistant infections in humans, either directly or indirectly. The intestinal tracts of all mammals are naturally home to bacteria. Many germs will be killed if animals are treated with antibiotics. However, resistant bacteria are able to endure and grow. These germs infect the meat and other animal products after the food animals are killed and processed. Through animal feces, these microorganisms can even spread throughout the ecosystem and get to products that are irrigated with contaminated water, thereby causing various water and foodborne illnesses (Jose, 2017).

The main obstacle to reducing the possibility of drug resistance is the prudent use of antimicrobial drugs. Establishing a sustainable antimicrobial surveillance system that can support and enhance the current communicable disease surveillance system is imperative. The upcoming microbial resistance epidemic can be managed with the use of the antimicrobial resistance surveillance system. Because animal bacteria have a shorter generation period, plasmid-encoded resistance mechanisms, genetic material interchange, etc., they are more likely to exhibit antimicrobial resistance (AMR). When drug-resistant animal microorganisms spread through food or pass on their genetic material that codes for resistance to harmful human bacteria, they pose a risk of sickness and infection to people. Standardized phenotypic techniques are frequently used to investigate AMR in bacteria that pose a concern to public health. However, because of their speed and precision in identifying the underlying genetic processes for AMR, molecular methods are frequently replacing phenotypic methods in many laboratories (Muna *et al*., 2017).

This current study aims to isolate bacterial species that harbour various animal food products like meat, chicken and seafood collected from the abattoir table at Amansea Market, Awka Anambra State, and to determine the antibiotic resistance pattern of isolated pathogenic bacteria which are responsible for various foodborne illnesses in human beings.

2. Material and methods

2.1. Study Area and Collection of Samples

The samples were collected from Amansea Abattoir Market, Awka, Anambra State. Five raw animal product samples were collected from the abattoir's table inside Amansea Abattoir Market Awka, Anambra State. A classic random sampling technique was employed to collect the study samples. A sterile cotton swab fitted with a wooden shaft was soaked in 10 mL of sterile buffered peptone water (BPW) and swabbed gently over the abattoir tables horizontally and vertically several times. After completion of the swabbing, the used swab was placed in the test tube containing Buffered Peptone Water. All the samples were arranged in a sterile plastic bag and without delay transported to the Microbiology Laboratory and were immediately processed upon arrival.

2.2. Serial Dilution of samples

The sample was diluted through a series of standard volumes of distilled water. The serial dilution was done in five fold. After the first tube, each tube was the dilution of the previous dilution tube.

2.3. Isolation and Characterization of Organisms

One ml of the 10^{-5} dilution was dispensed into sterile Petri dishes that were labelled and agar (prepared according to manufacturer's procedures) poured into the petri dishes after it had cooled to about 50°C. After solidifying, petri dishes containing agar and sample were incubated at 37°C for 24 hours. After 24 hours incubation, plates were observed and colonies of organisms observed were sub cultured using sterilized inoculating loops on freshly prepared agar plates. Plates containing sub cultured organisms were incubated for another 24 hours at 37°C. After incubation, plates were also observed for microbial growth. Following observation of organisms, Gram staining was done and nutrient slants were made using bijou bottles and left to stand overnight. The following day, slants were checked for contaminants. The sub cultured organisms were stored in bijou bottles for identification.

2.4. Biochemical tests

2.4.1. Gram Staining

Under sterile conditions, a smear was made from 24 hours old culture by placing a drop of sterile saline on a clean grease free slide and a colony was picked from the culture plate with the aid of a sterile cooled loop and emulsified. The smear was air dried and heat fixed by passing the slides over a flame. Slides were flooded with Crystal Violet and the stain was allowed to act for 1 minute. It was then rinsed under slow running tap water and flooded with Lugol's iodine for another 1 minute, followed by rinsing with water and decolorizing with acetone for 30 seconds (care was taken to avoid over decolonization). The slide was then flushed with water and allowed to drain. Slides were later counter stained with safranin for 1 minute, rinsed with water and viewed under a microscope using oil immersion objective (x100).

2.4.2. Catalase test

Using a sterile dropping pipette, a drop of 3% hydrogen peroxide solution was placed on a slide and a colony of the test organism was added to the drop of hydrogen peroxide solution. Formation of oxygen bubbles which is indicative of the presence of catalase was looked out for. This is done to differentiate between staphylococci and streptococci. Effervescence of gas indicates the presence of gram positive organisms.

2.4.3. Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interpret “drift” or “Brownian motion” as motility. Results were recorded as motile or non-motile.

2.4.4. Urease test

Urease test is a biochemical test that detects the alkaline fermentation of urine (urea) with the resultant production of ammonia by microorganisms. It is performed by growing the test organisms on the urea broth or agar medium containing the pH indicator phenol red. Positive results show deep pink colour while no colour change is a negative result.

2.4.5. Citrate utilization test

The test differentiates among bacteria by determining their ability to utilize citrate as their only source of carbon. Test organisms were inoculated on Simmons citrate agar slants streaking gently and incubated at 37°C for 24 hrs. A colour change in the agar from green to blue indicates a positive reaction.

2.4.6. Methyl red test

Wire loops full of isolates under investigation were inoculated onto MRVP broth in the bijou bottles and incubated for 24 hours. Three drops of methyl red solution was added and colour change observed. A colour change from light yellow to pink indicates a methyl red positive reaction, meaning that acid is produced; while no change in colour (colour remains yellow) indicates a methyl red negative reaction.

2.4.7. Indole production test

The indole test is used to distinguish among members of the families of *Enterobacteriaceae* by testing their ability to degrade an essential alpha amino acid, tryptophan to produce indole. The isolates were inoculated in nutrient broth and incubated at 37°C for 16 - 24 hours. After incubation, few drops of Kovac's reagent were added and the tube shaken gently and allowed to stand. The pinkish and ring-like colour at the upper layer indicates indole production in the tubes, and if otherwise, no indole production.

2.4.8. 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. 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).

Bacteria isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by (CLSI, 2005). In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 h incubation. After the inoculums were dried, antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37 C for 24 h. Finally, the zone of inhibition was measured including the disk diameter. The susceptible and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer's leaflet attached to them. The standard antibiotic discs (Oxoid,England) and their concentrations used against the isolates were tetracycline (TET) – 10 g, cephalothin(CEF) – 30 g, norfloxacin (NOR) – 10 g, penicillin G(PEN) – 10 g, azithromycin (AZM) – 15 g, streptomycin(STR) – 10 g, cephotaxime (CTX) – 30 g, gentamycin(GEN) – 30 g, imipenem (IPM) – 10 g, cefpirome (CPO)– 30 g, compound sulphonamides (CS3) - 30 g, nalidixicacid (NAL) – 30 g, erythromycin (ERY) – 10 g,oxytetracycline (OXY) – 30 g, sulphamethoxazole (SMX)– 25 g and cefuroxime sodium (CXM) – 30 g.

3. Results

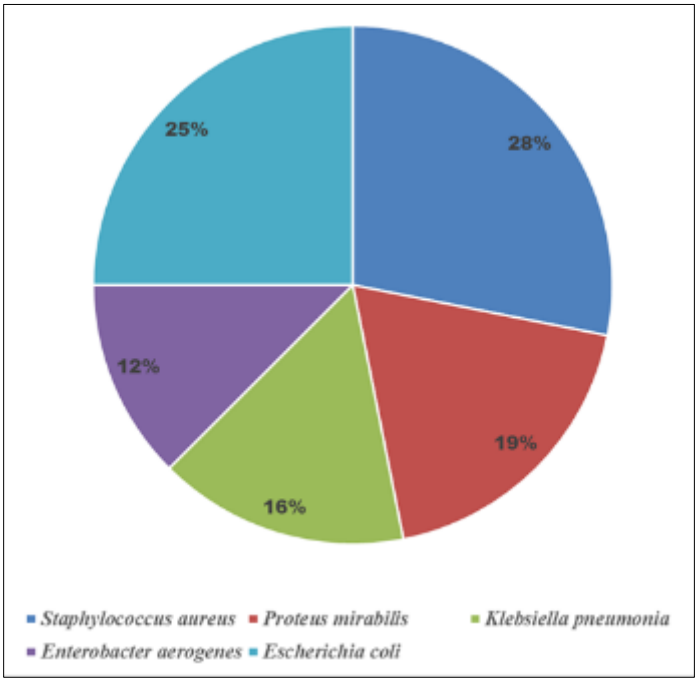

Figure 1 Frequency of occurrence of bacterial isolates

Table 1 Morphological and Biochemical Identifications of the Various Bacterial Isolates

Isolate	Form	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
A	Circular	Cream	Entire	Raised	Transparent	- Rod	+	+	-	+	+	+	+	+	+	-	+	-	+	*Proteus mirabilis*
B	Irregular	Cream	Entire	Raised	Opaque	-Rod	+	-	-	+	-	+	+	+	+	(-)	+	-	+	*Klebsiella pneumoniae*
C	Circular	Yellowish	Entire	Raised	Opaque	+ cocci	+	+	-	+	-	-	AG	AG	A	A	AG	-	+	*Staphylococcus aureus*
D	Circular	White	Entire	Convex	Moist	-Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	*Enterobacter aerogenes*
E	Circular	Whitish	Entire	Convex	Translucent	-Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*Escherichia coli*

Key: Gram: Gram reaction Cat: Catalase test Mot: Motility test Ind: Indole test MR: Methyl-red test VP: Voges-Proskauer test Cit: Citrate Utilization test Sugar Fermentation Tests: Lac: Lactose Fermentation Glu: Glucose Fermentation Suc: Sucrose Fermentation Fru: Fructose Fermentation Mal: Maltose Fermentation Oxi : Oxidase Ure: Urease

Table 2 shows the antimicrobial susceptibility of isolated microorganisms which was performed by using Kirby- Bauer disc diffusion method according to CLSI guidelines using Mueller-Hinton Agar (Hi-Media, India).

Table 2 Antibiotics sensitivity pattern of isolates

Bacterial isolates	Imipenem 10µg	Ciprofloxacin 5µg	Azithromycin 15µg	Gentamicin 10µg	Amoxicillin 10µg	Tetracycline 30µg	Clotrimoxazole 1.25µg	Ceftazidime 30µg	Cefoxitin 30µg
	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)	Percentage of sensitivity (%)
Proteus mirabilis	66%	33%	50%	33%	33%	50%	33%	50%	50%
Klebsiella pneumoniae	50%	17%	33%	50%	17%	50%	17%	50%	50%
Staphylococcus aureus	60%	40%	60%	40%	60%	60%	20%	60%	60%
Enterobacter aerogenes	100%	50%	50%	50%	50%	50%	50%	50%	50%
Escherichia coli	66%	33%	33%	33%	33%	50%	33%	33%	50%

4. Discussion

Due to the intestinal content that is released during defeathering and evisceration, these two procedures are thought to be the most significant sources of contamination and microorganism release into the slaughter environment (Pacholewicz *et al*., 2015). Previous research have shown that the bacterial load is largest during the initial step of the entire process, known as "hanging of poultry," and then lowers during the defeathering process, particularly for poultry slaughterhouses.

Several bacterial genera were recovered and identified in this study by swabbing Abattoir tables in Amansea Awka. Bacteria are classified using the Gram staining technique according to the structural features of their cell walls. The gram staining method was used to identify five distinct bacterial species based on the results. In addition to being more dangerous to people, gram-negative bacteria are also more resistant to antibiotics. Human guts typically include gram-positive bacteria, which are believed to help with digestion. Gram-negative cocci are known as harmful microorganisms and are typically linked to illnesses. The most varied category of human pathogens found in slaughterhouses are bacteria. Amansea market's abattoir tables were found to contain major human bacterial pathogens, including Escherichia coli and Staphylococcus species, followed by enterobacteria from the genera Proteus and Enterobacter (Table 1); Rousham *et al*. (2018) confirmed the presence of these bacteria on the abattoir table. The different activities could account for the presence of these microorganisms.

The following bacteria were proven to be present in the abattoir table by the results of the microbiological analysis: E. coli, Proteus species, Klebsiella species, Staphylococcus species, and Enterobacter species. Numerous human illnesses have been linked to and implicated by these bacteria (Irham *et al*., 2016). Certain bacteria isolated from the slaughterhouse table can cause wound infections, such as Escherichia coli. These bacteria have extra genes that encode virulence factors like adhesion factors and toxins, making them pathogenic and causing intestinal or extraintestinal diseases like meningitis, diarrhea, hemolytic uremic syndrome, and gastroenteritis (Anastasi *et al*., 2010). A class of bacteria known as Staphylococcus can also cause a variety of infectious disorders in different bodily tissues. Since Staphylococcus aureus is a common flora found on the skin of healthy mammals and appropriate hygiene procedures were not followed during the slaughter process, the presence of this bacteria in the samples may have been caused by contamination from animal hides as well as the hands and skin of abattoir employees.

Based on antibiotic susceptibility, Table 2 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 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%). The isolated Staphylococci strains showed 100% sensitivity to imipenem and least sensitivity (25%) to ciprofloxacin and gentamycin. The isolated Enterobacter strains showed the highest sensitivity (100%) to imipenem and showed 50% sensitivity to remaining all tested antibiotics. Klebsiella species showed the highest sensitivity (50%) to imipenem, gentamycin, tetracycline ceftazidime and cefoxitin. All the isolated microorganisms from abattoir's table showed maximum sensitivity to imipenem and the least sensitivity to antibiotics, specifically ciprofloxacin, amoxicillin and cotrimoxazole.

5. Conclusion

The bacterial profile and antimicrobial resistance pattern of bacteria isolated from the Amansea slaughterhouse table have been examined and presented in this research work. The hygienic circumstances in which carcasses are dressed are far from optimal, according to on-site observations of the slaughterhouse environment. At the abattoir, heaps of bones and animal feces are a common sight. The butchers and workers, the water quality, the utensils, and the state and condition of the slaughter slabs were all below required hygienic standards. Animal urine, blood, intestinal contents, lipids, undigested food, aborted fetuses, feces, hairs, and other untreated slaughterhouse wastes are also released into the environment. From the research, it was inferred that these tables contains large amounts of multidrug resistant bacteria that could impact on public health of humans, especially the abattoir workers and residents around the abattoir.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest

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