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RESEARCH PAPER

# ANTIBIOGRAM PROFILE OF PATHOGENS ISOLATED FROM PROCESSED COW MEAT FROM EKPOMA AND IRRUA METROPOLIS, EDO STATE, NIGERIA.

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## ABSTRACT

This study determined the extent of bacterial contamination of cow meat processed and sold in Ekpoma and Irrua abattoirs in Edo State, Nigeria, and their antibiotic susceptibility patterns. One hundred and twenty (120) samples comprising 40 samples each, from blood, table surfaces and processed row meat, were collected from the sampled abattoirs and analyzed using standard bacteriological procedures. The results showed that 60 samples, representing 50% prevalence, were positive for different bacteria species, but the table surface samples were the most contaminated. The comparative difference in rates of contamination, were observed to be statistically significant ($p<0.05$). Of note, is the fact that the bacteria specie - *Bacillus cereus* (31; 51.7%), accounted for most of the bacteria isolate, while the antibiotic resistance tests revealed varied, but interesting susceptibility patterns. Our findings does highlight the fact that there exist obvious vehicles for pathogenic bacteria proliferation within our abattoirs, and hence, the need for caution.

**Key words:** Abattoirs, *Bos taurus*, Pathogenic bacteria, Antibiotics, Resistance

## INTRODUCTION

Cow meat or beef is the culinary name for meat from bovines especially cattle. The generic name of cow meat is *Bos taurus* and the habitable weather of *Bos taurus* includes temperature of $101.5^0$F ($38.6^0$C) and ability to live in a harsh terrains (Li *et al.,* 2006). The processing of cow meat begins from buying of the cow and then taken to abattoirs for killing and processing as described by Komba *et al.* (2012). The contamination chain starts from the abattoir to branders and then retailers (Komba *et al.,* 2012). The contaminant could be gotten from the environment, the materials used for processing like knives, the water used and the hands of the processors (Ukut *et al.*, 2010).

Food safety is a matter of great concern and of public health importance, particularly when the environment in which the food is handled is heavily contaminated (Soyiri *et al.*, 2008). Most fresh foods especially that of animal origin like beef, is highly vulnerable to microbial invasion and food poisoning, since meat is an ideal medium for growth of a number of microorganisms due to its nutritive value (Soyiri *et al.*, 2008). The main constituents of *Bos taurus* (cow meat) are water and protein. In addition to water and protein, are fat, phosphorus, iron and vitamins.

The major primary unit of meat is carcass which represents the ideal meat after removal of head, hide, intestines and blood (Li *et al.*, 2006). The edible parts of a carcass include lean flesh, fat flesh and edible glands or organs which include the heart, kidney, liver, brain and tongue. Tissues from healthy animals are normally sterile, but can be contaminated by

microorganisms from the exterior of the animal and its intestinal tract during slaughter, dressing and cutting (Ukut *et al*., 2010).

The safety of raw and processed meat has indeed, become a great concern to public health officers' due to the degree of antibiotics resistant bacteria isolated from them. In spite of increased consumer demand for food safety standards for beef in Nigeria, there are still poor hygiene and sanitary practices along the food production chain, which contributes to unacceptable level of microbial load in meat. This poses a health risk to consumers (Mtenga *et al*., 2000).

This study therefore, was designed to determine the extent of bacterial contamination of cow meat processed and sold in Ekpoma and Irrua abattoirs within Edo State, Nigeria, as well as their antibiotic susceptibility patterns. It is expected that the information obtained from this study, would help educate the public on possible vehicles for meat contamination and its link to outbreaks of multi-drug resistance (MDR) bacteria.

## MATERIALS AND METHODS

**Study Area:** This study was carried out in the Abattoirs in Ekpoma and Irrua -the administrative Headquarters of Esan West Local Government Area and Esan Central Local Government Area of Edo State, Nigeria, respectively.

**Ethical Consideration:** Ethical approval was obtained from the Health Research Ethics Committee and informed consent was sought from the various abattoirs' supervisors

**Inclusion and Exclusion Criteria:** Table surfaces and other abattoir materials that have been disposed or had not been in use, were excluded from the study. Regularly used table surfaces, blood samples and processed meat for commercial purposes, were included in the study

**Sampling Technique:** Simple random sampling technique was used for sampling in this study.

**Sample Size/ Sample Collection:** A total of 120 samples comprising of 40 samples each of blood, table surfaces and processed meat, were collected from the abattoirs. Sterile swabs with sterile peptone water were used to collect samples from the table surfaces. Meat samples were collected into universal containers with 5ml of peptone water, while the blood samples were collected into sterile diphasic medium and were taken immediately to the microbiology laboratory of the department of Medical Laboratory Science of Ambrose Alli University for laboratory analysis.

**Laboratory Analysis:** The blood was introduced into the blood culture bottles by using sterile syringe and was inoculated directly and was then vented before incubation at 35˚– 37˚C.Venting was accomplished by inserting a sterile cotton-plugged needle into the diaphragm (i.e., rubber part) of the biphasic blood bottle, it was observed up to 7 days before disregarding it as negative result. It was been observed even 48 hours for growth. Sterile swabs with sterile peptone water were used to collect samples from the table surfaces and meat samples. Swabs were cultured on Nutrient agar and Blood agar and incubated at 37°C.

**Identification of Isolates:** The isolated bacteria were identified using Gram staining, microscopy and biochemical tests such as Catalase test, Oxidase test and Coagulase test.

**Gram Reaction:** The Gram reaction was used to classify the isolates into gram positive and gram negative bacteria after examining the agar plates. The gram stained slides were examined first with x40 objective lens to check for the staining and distribution of the gram stained bacteria, then with oil immersion objective lens (x100) to look for the bacteria. Gram positive bacteria appeared purple while gram negative appeared red or pink.

**Statistical Analysis:** Statistical analysis was done using the Statistical Package for Social Sciences (SPSS), version 17.0.

## RESULTS

Out of the 120 samples analyzed, 60 samples were positive for different bacteria, giving an overall prevalence of 50%. Table 1 shows bacterial contamination on the samples collected and examined, with the blood samples of cow meat analyzed having a contamination rate of 50%, while the processed meat had a contamination rate of 20% and samples from the table surfaces were the most contaminated with 75% contamination rates. Table 2 shows the prevalence of bacteria



species in relation to sample types. Out of the 20 blood samples that had bacterial isolates, *Bacillus cereus* were 10 (50.0%), and *Staphylococcus aureus* were 10 (50.0%). Out of the meat samples collected and examined 10 had positive bacteria isolates, *Bacillus cereus* were 5 (50.0%), and *Staphylococcus aureus* were 2 (20.0%), while *Pseudomonas aeruginosa* were 3(30.0%). And samples from the table surfaces were statistically significant with 30 samples having positive bacteria isolates, *Bacillus cereus* were 16 (53.3%), and *Staphylococcus aureus* were 6 (20.0%), while *Pseudomonas aeruginosa* with 8 (26.7%). Table 3 shows the biochemical characterization and identification of the isolated bacteria species. Table 4 and 5 shows the antibiogram of the isolated gram negative and gram positive bacteria.

The antibiotics used during this study were multiple disc (positive and negative antibiotics disc), which included; Ampliclox, Rifampicin, Norfloxacin, Gentamicin, Levofloxacin, Ciprofloxacin, Chloraphenicol, Erthromycin, Amoxil and Streptomycin while the Negative Discs Were Tarivid, Peflacine, Gentamycin, Nalidixic Acid, Augmentin, Ciprofloxacin, Septrin, Streptomycin, Ampicillin and Ceporex.

Antibiotic resistance tests showed that up to 100.0% of *Pseudomonas aeruginosa* were resistant to Streptomycin and ampicillin, and had resistance prevalence to Augmentin, Travid, Septrin, and Ceproflex of 90.0%,63.6%, 81.9% and 72.7% respectively, while Ciprofloxacin, Peflacine and Gentamycin had 90.9%, 81.9% and 72.7% respectively, were the most active antibiotics against *Pseudomonas aeruginosa* while *Bacillus cereus* isolated were resistant to Erythromycin, Streptomycin and Ampliclox., but were sensitive to Rifampicin, Norfloxacin, Gentamicin, Levofloxacin, ciprofloxacin, Chloraphenicol, Amoxil and Streptomycin, while *Staphylococcus aureus* isolated were resistant to Erythromycin and Streptomycin but were sensitive to Rifampicin, Norfloxacin, Gentamicin, Levofloxacin, Ciprofloxacin, Chloraphenicol, Amoxil and Streptomycin.

**TABLE 1: Bacterial Contamination on the Samples Collected and Examined**

| Samples | Number Examined | Number Contaminated | Percentage Contaminated | $X^2$Cal | P-Value |
|---|---|---|---|---|---|
| Blood | 40 | 20 | 50% | | |
| Meat | 40 | 10 | 25% | | |
| Table Surfaces | 40 | 30 | 75% | | |
| Total | 120 | 60 | 50% | 25.000 | 0.000004 |

Key: $X^2$cal=chi-square, degree of freedom (df) =2

**TABLE 2: Prevalence of Bacteria species in relation to Sample types**

| Samples | Total | *Bacillus Cereus* | *Staphylococcus Aureus* | *Pseudomonas aeruguinosa* | $X^2$Cal | P-Value |
|---|---|---|---|---|---|---|
| Blood | 20 | 10(50.0) | 10(50.0) | 0(0.0) | | |
| Meat | 10 | 5(50.0) | 2(20.0) | 3(30.0) | | |
| Table | 30 | 16(53.3) | 6(20.0) | 8(26.7) | | |
| Total | 60 | 31(51.7) | 18(30.0) | 11(18.3) | 48.78 | 0.0000 |

Key: $X^2$cal=chi-square, degree of freedom (df) =2

**Table 3 Biochemical Characterization of Gram Negative Isolates**

| Gram reaction | Catalase | Coagulase | Oxiddase | Citrate | Indole | Urea | Mot | Lactose | Man | Glucose | Sucrose | isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram PB | + | - | - | - | - | - | + | - | - | - | - | *Bacillus cereus* |
| GPC | + | + | - | - | - | - | - | - | - | - | - | *Staplylococcus aureus* |
| GNB | - | - | + | + | - | - | + | - | - | - | - | *Pseudomonas aeruginosa* |

Key: Positive = +; negative = - ; Gram postive cocci = GPC; Gram negative bacilli = GNB; Gram Positive bacilli = GPB

**Table 4: Distribution of the Overall Antibiotic Pattern with Various Antibiotics with the Isolated Gram Negative Bacteria**

| Antibiotics | *Pseudomonas aeruginosa* Number of isolates=11 | |
|---|---|---|
| | Resistant n (%) | Sensitive n (%) |
| Tarivid | 7(63.6) | 4(36.4) |
| Nalidix acid | 8(72.7) | 3(27.3) |
| Peflacine | 2(18.1) | 9(81.9) |
| Gentamycin | 3(27.3) | 8(72.7) |
| Augmentin | 10(90.9) | 1(9.1) |
| Ciprofloxacin | 1(9.1) | 10(90.9) |
| Septrin | 9(81.9) | 2(18.1) |
| Streptomycin | 11(100.0) | 0(0.0) |
| Ampicillin | 11(100.0) | 0(0.0) |
| Ceporex | 8(72.7) | 3(27.3) |

Key: n = number, % = percentage

**Table 5: Distribution of the Overall Antibiotic Pattern with Various Antibiotics with the Isolated Gram Positive Bacteria**

| Antibiotics | *Bacillus cereus* Number of isolates=31 | | *Staplylococcus aureus* Number of isolates=18 | |
|---|---|---|---|---|
| | Resistant n (%) | Sensitive n (%) | Resistant n (%) | Sensitive n (%) |
| Ampliclox | 21(67.7) | 10(32.3) | 1(5.6) | 17(94.4) |
| Rifampicin | 8(25.8) | 23(74.2) | 6(33.3) | 12(66.7) |
| Norfloxacin | 2(6.5) | 29(93.5) | 7(38.9) | 11(61.1) |
| Gentamycin | 0(0.0) | 31(100.0) | 2(11.1) | 16(88.9) |
| Levofloxacin | 4(12.9) | 27(87.9) | 2(11.1) | 16(88.9) |
| Ciproflox | 5(16.1) | 26(83.9) | 5(27.8) | 13(72.2) |
| Chloramphenicol | 3(9.7) | 28(90.3) | 211.1) | 16(88.9) |
| Erythromycin | 28(90.3) | 3(9.7) | 9(50.0) | 9(50.0) |
| Amoxil | 11(35.5) | 20(64.5) | 211.1) | 16(88.9) |
| Streptomycin | 16(51.6) | 1548.4 | 14(77.8) | 4(22.2) |

Key: n=number, %=percentage.

## DISCUSSION

The isolation of bacteria agents from blood and meat samples, as well as the table surfaces within the abattoirs under study, further explain the concerns raised by experts on the handling and processing of meat for public consumption. Specifically,



the results did show that all the samples had high bacteria contaminants, and most contaminated sample source was the table surfaces (75% contamination rate), followed by the blood samples of the cows being processed with contamination rate of 50% and then the processed meat with 20% contamination rate. This finding agrees totally with the findings by Eze *et al*., (2010), who reported similar observations in Imo state, Nigeria; suggesting that the blood, meat and tables in the abattoirs may be vectors in the transmission of overt or opportunistic pathogenic microorganisms, as well as the spread of multidrug resistant bacteria strains. It is a known fact that *Staphylococcus aureus, Bacillus cereus* and Pseudomonas *spp*. are of pathogenic and public health importance (Cheesbrough, 2000).

Furthermore, the results of this study show that these organisms are multidrug resistant, with strains of *Pseudomonas spp* isolates for example, exhibiting more than 50% resistance to seven antibacterial agents. In a similar vein, more than 60% of the *Bacillus cereus* isolates were resistant to four antibiotics. While the medical implication of these is obvious, the public health importance is more glaring in the light of the transferability of these traits among both pathogenic and potentially pathogenic bacteria (Eze *et al.,* 2010).

Considering the fact that bacteria isolated from cow blood, processed meat and table surfaces have shown similar attributes, raises serious concern over allergic reactions, chronic indigestion, constipation and inflammation of the appendix. *Staphylococcus aureus* is acknowledged as the very harmful bacteria in food poison or intoxication in humans. Their presence in this study samples is therefore undesirable. The risk this may portend is corroborated by the more than 26% presence of *Staphylococcus aureus* on table surfaces.

Of interest also, is the antibiotic resistance test results showing that up to 100.0% of *Pseudomonas aeruginosa* were resistant to Streptomycin and ampicillin, and had resistance prevalence to Augmentin, travid, septrin, and ceproflex with 90.0%,63.6%, 81.9% and 72.7% respectively, while Ciprofloxacin, Peflacine and gentamycin with 90.9%, 81.9% and 72.7% respectively, were the most active antibiotics against *Pseudomonas aeruginosa. Bacillus cereus* were resistant to erythromycin, streptomycin and ampliclox., but sensitive to Rifampicin, Norfloxacin, Gentamicin, Levofloxacin, ciprofloxacin, Chloraphenicol, Amoxil and Streptomycin, while *Staphylococcus aureus* isolated were resistant to erythromycin and streptomycin, but sensitive to Rifampicin, Norfloxacin, Gentamicin, Levofloxacin, ciprofloxacin, Chloraphenicol, Amoxil and Streptomycin. These observations are in line with the reports from an epidemiological survey conducted by Bahrndorff *et al.* (2013), on the possible route of microbial transmissions from abattoirs to markets in India. According to Ali *et al*. (2010), the observed high resistance of some of the isolates could be attributed to the use of antibiotics to treat cows or their addition in cow feed and water, which surely can precipitate resistance development by such isolated bacteria species against known antibiotics.

## CONCLUSION

These findings show that blood, processed meat and table surfaces in the abattoirs studied, were vehicles for pathogenic bacteria proliferation. The table surfaces however, proved to be most potent vehicle when compared to those of blood and processed meat samples and the comparative difference was observed to be statistically significant ($p<0.05$).

## RECOMMENDATIONS

We therefore recommend that:

1. Proper amenities must be provided in abattoirs, especially those located in rural areas.
2. Routine periodic microbial quality tests should also be conducted in the various abattoirs by public health officers while initiating and sustaining enlightenment campaigns on relevant advanced technique(s).
3. Regular disinfection of the abattoirs is advocated to reduce or eliminate possible pathogenic organisms that could cause food borne diseases or illnesses.
4. Abattoirs should be sited far away from residential areas to reduce cross transmission, while all categories of workers in the abattoirs should be reminded via seminars, workshops, posters and/or signboards, on the need for safe hygienic practices.

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