Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 10 October 2024

Review Article

# Characterization and Estimation of Public Health Relevance of Bacteria Isolated from Butcher Tables in Ekpoma Market

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**DOI:** 10.31080/ASMS.2024.08.1928

**Received:** July 24, 2024

**Published:** September 17, 2024



## Abstract

Microbiological methods are not commonly used to inspect the hygienic status of butcher tables. This study aimed to identify and assess the bacteriological quality of butchers' tables within Ekpoma, Edo State, Nigeria. A total of 30 swab samples were obtained from the butchers' processing tables. The swab samples were inoculated on different selective media to isolate pathogenic bacteria. The bacterial isolates were characterized and allowed to be screened through an antibiotic sensitivity test. The bacteria isolated from this study revealed the presence of *Klebsiella* spp., *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* spp., and *Escherichia coli. E. coli* was the predominant isolate (26.9%), followed by *Salmonella* ssp (23.1%). The least bacterial isolate was *Pseudomonas aeruginosa* (11.5%). The antibiotic susceptibility pattern of this study also showed that the isolates were not completely multidrug-resistant, though all antibiotics tested were effective against some of the isolated organisms, 90% of the isolates were resistant to amoxicillin and chloramphenicol. The findings from this study point to the fact that there was a poor level of personal hygiene and inadequate sanitation at the butcher shops. Thus, there is a need to educate butchers and meat handlers on the importance of practicing sound sanitation and meat handling techniques.

**Keywords:** *Escherichia coli*; *Staphylococcus aureus*; World Health Organization (WHO)

## Introduction

Foodborne infections and illness are major international health problems, resulting in severe consequences and economic stagnation [1]. It is a significant cause of some fatal illness and death worldwide. Due to this detrimental effect, the World Health Organization (WHO) developed its global food safety strategy. In developing countries, foodborne infections can be traced to the death of many children, and the resulting diarrheal disease can have a long-term debilitating effect on children's growth and their physical and cognitive development. In an industrialized society, foodborne infection causes severe illness, heavily affecting the health care system [1]. According to [3], foodborne diseases are diseases resulting from the ingestion of microorganisms and their waste products, such as toxins present in foods, and such a symptom may vary with the amount of these microbes and their toxins present in the contaminated food ingested and the susceptibility of the individuals to the toxin.

Meat is a highly perishable food and a vital substrate to support the growth of microorganisms [9]. Most meat has a high water

content, corresponding to a water activity of approximately 0.99, which is suitable for microbial growth. Meat is considered to be spoilt when it is unfit for human consumption due to the enzymatic actions of microbes present in the meat, which eventually alters the organoleptic characteristics of the meat, and its fats may be oxidized chemically. Microorganisms grow on meat, causing visual textual and organoleptic Changes when they release metabolites. In an actual sense, tissue from healthy animals is sterile. However, studies have shown that during slaughter, dressing and cutting, microorganisms gain access to this animal tissue from the exterior of the animals and the intestinal tract. Still, more are added from knives, clothes, air, carts, and equipment. External contamination of these meats is possible from bleeding until consumption. Bacterial contamination of meat products is an unavoidable consequence of meat processing [8].

Due to evaporation, airborne microorganisms may be carried on dust particles in large droplets suspended briefly in the surroundings. The ultimate fate of survival of these airborne microorganisms is governed by a complex of circumstances, which include atmospheric conditions such as humidity, sunlight, temperature, size of the particles bearing the organisms and the nature of microorganisms, the degree of susceptibility, the resistance of a particular species to the new physical environment or its ability to form resistant spores [5]. Foodborne diseases occur in developing countries such as Africa because of unstable and undefined food safety laws, weak regulatory systems, lack of financial investment in safe equipment and lack of proper education for food handlers [15]. Animal products like meats and fish are considered high-risk commodities concerning microbial contamination and pollutants [16].

Raw retail meats have been identified as potential drivers for spreading foodborne diseases. Hence, there is a need to increase the implementation of hazard analysis of critical control points (HACCP) and consumer food safety education efforts [16]. According to [13], about 90% of food poisoning cases caused by meat and meat products may be attributed to post-mortem operations and handling, and about 10% of the cases are due to diseases already present in living animals. The HACCP program is a preventive approach to consistent, safe food production, and this program is based on two essential concepts of safe food production: prevention and documentation [11].

The following principles can achieve the HACCP [3]: conducting a hazard analysis, identifying the potential risk involved in food production at all stages up to the point of consumption, assessing the likelihood of occurrence of the hazards and identifying the preventive measures necessary for their control. The determination of the critical control points (C.C.P.), and the identification procedures and operational steps that can be adopted to eliminate the hazards or minimize the likelihood of their occurrence and also establishment of critical limit(s), set target levels and adjustments which must be maintained to ensure that the C.C.P. is under control, using of monitor control of the C.C.P.s and The corrective actions to be taken when monitoring indicates that a particular C.C.P. is not under control.

Establishing procedures for verifying and confirming that the HACCP system is working effectively is concerned with all procedures and records appropriate to these principles and their application, even in sanitation, “the creation and maintenance of hygienic and healthful conditions”. It is the application of science to provide wholesome food that is processed, prepared, merchandised, and sold in a clean environment by healthy workers and to minimize the proliferation of food spoilage microorganisms [11]. It can be achieved by chlorine, iodine, peracetic acid, and quaternary ammonium compounds, which are applied on all food contact surfaces and for environmental control.

According to [6], official or officially recognized programme for specified zoonotic agents should include measures to:-

- Control and eradicate their presence in animal populations or subsets of populations.
- Prevent the introduction of new zoonotic agents.
- Provide monitoring and surveillance systems that establish a baseline data analysis and guide a risk-based approach to controlling such hazards in meat.
- Control movement of animals between primary production units and abattoirs where trade animal populations are under quarantine restrictions.

## Materials and Methods

### Study area

This study was conducted in the Ambrose Alli University, Ekpoma, Edo State microbiology laboratory. Geographically,

Ekpoma is located at latitudes 6°15S to 48°E. Its population is about 25,000 people, and its significant occupations include farming, teaching, civil service, and trading.

### Sample collection

Thirty swab samples were taken from five butchers' tables in Ekpoma, Edo State, Nigeria. The swabs were taken from the surface of the butcher's table. The contaminated swabs were transported in an icebox to the microbiology laboratory of Ambrose Alli University, Ekpoma. The time frame between the collection of samples and sample analysis was at most two hours.

### Bacteriological analysis

After the sample was collected and transported with swab sticks to the microbiology laboratory, the swabs were streaked onto Blood agar base, Nutrient agar, Mannitol salt agar, Salmonella-Shigella agar, and MacConkey agar before incubating at 37°C for 24 hours.

### Bacterial counts

The samples were placed on trypticase soya agar (T.S.A.) for the trophic bacterial count. The agar plates were incubated at 37°C for 24 hours. The media used were weighed correctly according to the manufacturer's instructions. The serial dilution method was used for total bacterial counts.

### Identification of bacteria

After incubation, morphology was examined and recorded based on the colonies' shape, colour, texture and general appearance. Thereafter, colonies were sub cultured onto newly prepared nutrient agar plates aseptically to obtain pure cultures of the isolates. The bacterial colony on each plate and a single representative of each colony were gram-stained, followed by biochemical tests to identify the bacteria. The isolates were then stored at 4 °C using a nutrient agar slant.

### Gram staining

Some colonies were taken and spread on microscopic slides using a sterile wire loop to make thin smears. They were fixed with heat and placed in a staining rack. They were covered with crystal violet for two minutes, washed off with tap water, decolourized with acetone for a few seconds, washed off with tap water, and then covered with carbol fuchsine for thirty seconds. Finally, the stained smears were washed and air-dried. Then, they were examined under an oil immersion lens. The shapes and arrangement of the gram-positive and negative organisms were identified according to [2].

- **Catalase test:** Using a sterile glass rod, a part of the isolated colony was emulsified in one drop of hydrogen peroxide on a clean slide. Gas bubbles indicated a positive reaction.
- **Coagulase test:** Distilled water was dropped on a glass slide. The test organism was emulsified on the drop to make a thick suspension; a loopful of plasma was added to the suspensions and mixed gently. Clumping of the test organism after 10 seconds indicated that the organism is coagulase positive.
- **Citrate test:** Simmon's citrate agar was prepared according to the manufacturer's instructions, sterilized, poured, and allowed to solidify at an angle of $45^0$C. The bacteria were streaked on the surface of the agar slant and then incubated for about 24-48 hrs at $37^0$C. A change in the colour of the agar medium from greenish to blue indicates a positive test, while the absence of colour change indicates a negative test.
- **Motility:** The isolates were studied for motility using the Craigie technique [3], in which the bacteria was inoculated into a central tube containing semisolid agar and placed in a test tube using a straight wire. After incubation at 37oc for 24 hours, the tubes were examined for bacteria migrating outside the tube.
- **Indole:** A 1% tryptophan broth in a test tube was inoculated with the bacterial colony. After incubation at $37^o$C for 48 hours, one litre (1ml) of chloroform was added to the broth. The test tube was gently agitated, and then a two in1 of Kovac's reagent was added and gently shaken before being allowed to stand for about twenty minutes. After that, the formation of red on the top layer indicated cheerful and yellow colouration indicated negative.
- **Urease:** Christensen's medium was prepared according to the manufacturer's instructions and autoclaved at $121^o$C for 30 minutes, pH 6.8 glucose and urea sterilized by steaming at $100^o$C for 15 minutes were added and gently mixed, which were then poured into tubes as deep slopes. The test

organism was then inoculated on the agar slopes, incubated at 37°C for 4 hours, and then overnight. Change of colour to purple pink is indicative of urease positive.

### Antibiogram

Antibiograms of all bacteria isolates were carried out using the Kirby - Bauer disk diffusion method. Briefly, isolates were suspended in normal saline to a turbidity equivalent to 0.5 McFarland turbidity standard. The suspensions obtained were then streaked on a Mueller-Hinton agar plate using a sterile swab stick. Multiple antibiotic paper discs were gently but firmly placed on Mueller Hinton agar plates and incubated at 37°C for 24 hours. The numerous paper discs comprised Gentamycin, Ofloxacin, Ciprofloxacin Cloxacillin, Augmentin and Ceftriazidime, Septrin, Chloramphenicol, and Streptomycin. After 24 hours of incubation, the inhibition zones were carefully measured, and interpretation was done according to the Clinical and Laboratory Standard Institute [4].

## Result and Discussion

### Results

In the incidence of characterization and estimation of public health relevance of bacteria isolated from butcher table in Ekpoma, Edo State, Nigeria, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp and *Klebsiella* spp were isolated following standard microbiological procedures. Table 1 depicts the cultural, morphological, and biochemical characteristics of the bacterial isolates based on the results obtained from this study. In contrast, table 2 illustrates the occurrence of the bacterial isolates from the butcher's tables in Ekpoma. Finally, table 3 shows the antibiogram of the bacterial isolates obtained from the butcher's table against some panel of antibiotics.

| Cultural Characteristics | Gram Reaction | Cat | Coag | Ind | Ur | OX | MANNOSE | GLUCOSE | CITRATE | Organism identified |
|---|---|---|---|---|---|---|---|---|---|---|
| Golden yellow | + Cocci | + | + | - | - | - | + | + | + | *Staphylococcus aureus* |
| Greyish white | - Rod | + | - | - | + | - | + | + | + | *Klebsiella spp* |
| Pink colonies | - Rod | + | - | + | - | - | + | + | - | *Escherichia* coli |
| Green colonies | - Rod | + | + | - | - | + | + | - | - | *Pseudomonas aeruginosa* |
| Colorless with | - Rod | + | + | - | - | - | - | - | + | *Salmonella spp* |

**Table 1:** Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates.

Keys: Cat= Catalase, Coag= Coagulase, Ind= Indole, Ur= Urease

+ = Positive, - = Negative, OX= Oxidase.

| Organisms | No. of isolates |
|---|---|
| *Staphylococcus aureus* | 5(19.2%) |
| *Salmonella* spp | 6(23.1%) |
| *Escherichia coli* | 7(26.9%) |
| *Klebsiella* spp | 5(19.2%) |
| *Pseudomonas aeruginosa* | 3(11.5%) |
| Total | 26(100%) |

**Table 2:** Occurrence of bacterial isolates in the Butchers tables.

| Isolates | Resistance Antibiotics | Susceptible Antibiotics |
|---|---|---|
| *Escherichia coli* | Septrin, Chloramphenicol, Erythromycin | Augmentin, Gentamicin, Pefloxacin, Ofloxacin, Amoxicillin, Ciprofloxacine. |
| *Salmonella* spp | Chloramphenicol, Amoxicillin, Cipro-floxacin | Septrin, Augmentin, Gentamicin, Pefloxacin, Ofloxacin Erythromycin |

| | | |
|---|---|---|
| *Staphylococcus aureus* | Chloramphenicol, Amoxicillin. | Augmentin, Erythromycin, Pefloxacin Ceftriaxone, Streptomycin, Cloxacillin, Septrin |
| *Klebsiella* spp | Amoxicillin, Ceftriaxone | Augmentin, Ciprofloxacin, Chloramphenicol, Cloxacillin Streptomycin, Septrin, Pefloxacin |

**Table 3:** Antibiogram of bacterial isolates from butcher tables in Ekpoma.

## Discussion

The contamination of butchers' tables may be connected to meat processing, transportation, and display in the market. It also indicates that the presence of meat on tables provides suitable nutrition and environmental conditions for the growth of the bacterial isolates. This study is in line with [16], who described animal products such as meat, fish and some other animal products as commodities with a high tendency to be entangled with pathogen contents, natural toxins and other possible contaminants and pollutants [10]. It also described meat as the most perishable of the most commonly encountered food since it contains sufficient nutrients to support the growth of microorganisms.

The study identified bacterial contaminants as *Escherichia coli, Staphylococcus aureus, Klebsiella* spp, *Pseudomonas aeruginosa,* and *Salmonella* spp. Other researchers have reported similar organisms in foods, water and environmental samples [14]. *Escherichia coli* was the predominant isolate (26.9%), followed by *Salmonella* spp (23.1%), *Staphylococcus aureus* (19.2%), *Klebsiella* spp (19.2%), and *Pseudomonas aeruginosa* had the minor total frequency of (11.5%). This issue is similar to previous reports by [12], where they isolated almost similar organisms from raw meat and seafood. The high contamination rate of meat processing tables by these organisms in this study indicates the deplorable state of the hygiene and sanitary practices employed by Ekpoma butchers regarding washing, cleaning and sterilizing meat contact surfaces. Hygiene problems are not limited to knives and tables but are also associated with hand contamination.

According to a study by [1], most butcher shop workers handle money while processing the meat. Since it is well known that money is full of microbes, it can contaminate the meat. Handling foods with bare hands may also result in cross-contamination. Hence, microbes are introduced into safe food. Because meat handlers are potential sources of microbial contamination, all possible measures must be taken to reduce or eliminate such contamination [7]. Reported that most personnel working in the butcher shops do not apply hygienic practices, which could be due to a lack of knowledge based on the bacteria isolated and bacterial load on different surfaces in the butcher shops; meat could be contaminated by contact with contaminated surfaces and equipment in the butcher shops which could pose public health hazards. Thus, to safeguard the public against the risks of foodborne bacterial infections, educating and advocating for practising sound sanitation and detailed meat handling techniques in the butcher shops is necessary.

This study also observed that all the bacterial isolates were resistant to some of the antibiotics used, especially Chloramphenicol and Amoxicillin. This high resistance profile may be due to the extensive use of these antibiotics in animal feeds and indiscriminate use by man [14]. This practice promotes and enhances the spread of bacterial drug resistance across other bacterial species. Thus, foodborne diseases associated with resistant bacteria strains are difficult to treat, resulting in more extended hospitalization, high morbidity and mortality, decreased productivity and increased cost.

## Conclusion and Recommendation

It has been observed in this study that most of the bacterial isolates were resistant to some of the antibiotics used. This antibiogram profile may be due to the extensive use of these antibiotics in animal feeds and indiscriminate use by man. Studies have shown that the use of overalls could assist in mitigating the spread of microbes within the butcher's table; overalls should be suitable to wear over other clothing. Also, butchers could employ someone responsible for collecting money or handling the meat only to reduce the problem of adding currencies to meat products. Periodic antibiotic susceptibility testing of foodborne pathogens should be encouraged to detect any emergence of resistance by previously sensitive microbes.

- The scientific community should join regulatory authorities to spread awareness about basic hygiene principles.
- It is essential to provide training to meat handlers regarding food safety.

## Bibliography

1. Adak GK., *et al*. "Disease risk from foods, England and Wales, 1996 – 2000". *Emerging Infectious Disease* 11 (2015): 365-372.
2. Barrow GI and Feltham R K A. "Cowan and steel's Manual for the identification of Medical Bacteria". London: Cambridge University Press (2020): 45.
3. Cheesbrough M. "District oratory practice in tropical countries, part 2". Cambridge University Press. Comparison of microbial contamination on sheep/goat (2019): 54-60.
4. CLSI. "Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters". 5th ed. CLSI guideline M23. Wayne, PA: Clinical and Laboratory Standards Institute (2018).
5. Editor F T. "Food microbiology and food safety". Springer Science + Business Media, L.L.C (2019): 14.
6. FAO/WHO. Report of the 9th session of the codex committee on meat and poultry hygiene, Wellington, New Zealand, 17-23 February 2003, Food and Agriculture Organization of the United Nations, (2013): 38-45.
7. Jepsen A. "Bacteriological examination of manufactured meat products". In meat hygiene. F.A.O. Agricultural Studies No. 34. Rome (2013).
8. John TN and Anthony J S. "Microbiology of food and food processing, New York". American Elsevier Publishing Co. Inc. (2018): 45-77.
9. Kramer J M and Gillbert R J. "Bacillus cereus and other Bacillus species". In: Doyle, M. P. (ed). Foodborne Bacterial pathogens. New York, Marcel Dekker (2017): 21-70.
10. Magnus P. "Meat Composition. Food Science and Technology". 4th edition. Gohumunary Pub., London (2017): 108- 215.
11. Magnus P. "Meat Composition. Food Science and Technology". 4th edition. Gohumunary Pub., London (2014): 108- 215.
12. Marriott NG and Gravani R B. "Principles of food sanitation". 5th ed. Springer science + business media, Inc. (2016): 24.
13. Oko JO., *et al*. "Bacteriological and Nutritional Analysis of Groundnut Cake Sold in an Open Market in Samaru, Zaria-Kaduna State". *International Journal of Scientific and Technology Research* 4.5 (2015): 224-228.
14. Ukut IOE., *et al*. "Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria". *EJEAF Chemistry* 9.1 (2010): 89-100.
15. Waters AE., *et al*. "Multi-drug Resistant *Staphylococcus aureus* in meat and poultry". *Clinical Infectious Dis*ease 52 (2021): 1227-1230.
16. Yousuf AHM., *et al*. "Prevalence of Microbial Load in Shrimp, *Penaeus monodon* and Prawn, *Macrobrachium rosenbergii* from Bangladesh". *World Journal of Agricultural Science* 4.S (2018): 852-855.