Antibiotic susceptibility pattern of gram-negative bacteria isolated from apron and tables of meat vendors in Elele Market, Rivers State

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Abstract

Because of poor sanitary practices and a lack of knowledge about proper hygiene, meat vendors’ aprons and tables could be potential sources of cross-infection of many public health bacteria.

The purpose of this study was to determine the prevalence and antibiotic susceptibility of gram-negative bacteria isolates obtained from meat vendors' aprons and tables.

100 fresh swabs from meat vendors’ aprons and tables were streaked on Eosin methylene blue, Cetrimide, and MacConkey agar and identified by the biochemical test. The antibiotic susceptibility pattern was evaluated on Mueller Hinton agar using the Kirby-Bauer disc diffusion method.

Results: Out of the 100 samples processed, 66 Gram-negative bacteria were isolated. Klebsiella pneumoniae (74.24%) was the dominant gram-negative isolates, followed by E. coli (18.18%), Pseudomonas aeruginosa (4.55%), and Proteus sp (3.03%) respectively. The isolates from the tables recorded 100% resistance to Erythromycin and Cloxacillin with the highest sensitivity to Ofloxacin (66.7%) followed by Gentamicin (50%) while the isolates from aprons were 100% resistant to Augmentin and Cloxacillin followed by cefuroxime (93.3%) and Erythromycin (90%) with the highest sensitivity to Ofloxacin (100%) followed by Gentamicin (33.3%).

This study confirmed the presence of multidrug-resistant gram-negative bacteria on the apron, and the levels of contamination detected on the aprons and tables used by meat vendors in Elele market presented an inadequate microbiological standard and quality.

It is critical to implement proper orientation and workshops to educate meat vendors on standard practices to be followed when handling meat.

Keywords: Apron; Susceptibility; Pseudomonas aeruginosa; Multidrug; Contamination

1. Introduction

An apron is a protective piece of clothing made from fabric or other materials that covers the front of the body. Meat vendors wear aprons for hygienic reasons as well as to prevent spills and accidents. These aprons which are often used...
can become agents for the transmission of infections due to poor or lack of proper hygienic or sanitary practices during and after daily usage [1]. Meat vendors who harbor bacteria may contaminate meat with their fingers during processing, leading to infection of consumers. 

Meat is regarded as a perfect culture medium for most microorganisms due to its high percentages of nitrogenous compounds of varying degrees of complexity, high moisture, abundant supply of minerals, accessory growth factors, and some fermentable carbohydrates (glycogen) of a suitable pH [2]. It is on record that cross-contamination is a crucial factor in the outbreaks of foodborne diseases/infections [3]. Contamination could be due to unhygienic slaughtering, handling as well as the processing of the meat products [4]. Table surfaces contaminated with microbes run the potential risk of transmission of the pathogens to food during processing [5].

The source of water for abattoir activities is very paramount to meat hygiene as water is needed in maintaining the cleanliness of the abattoir environment and for washing off blood from the meat [6]. Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitary conditions [7]. Furthermore, when compared to advanced countries where waste generation, analysis, and treatment are considered before constructing abattoirs [8], abattoirs in resource-limited countries like Nigeria are generally less developed. The use of contaminated water and tools for washing and cutting, as well as unsanitary practices such as poor handling and display of meat for sale on contaminated tables, are likely to result in a high level of contamination of livestock products for sale.

Microorganisms are primarily derived from the exterior of the animal and its intestinal tract during slaughter, dressing, and cutting, but more are derived from knives, cloths, air, workers, carts, boxes, and equipment in general [9]. Antibiotics are frequently used in the treatment of infected humans and animals, as well as in the prevention and promotion of growth in food-producing animals [10].

The prevalence of antimicrobial resistance to bacterial pathogens is a global concern and its treatment has become more complex due to increasing resistance and empirical therapy leading to therapy failures of most infectious diseases associated with gram-negative bacteria [11]. This study was designed to assess the incidence and antibiotic susceptibility pattern of gram-negative bacteria obtained from the aprons and tables of meat vendors in Elele market.

2. Material and methods

2.1. Sample Collection

One hundred (100) samples were collected from the aprons and tables of meat vendors from different retail meat shops at Elele, Market using sterile swabs sticks. The samples were labelled, properly and stored in an icebox transported aseptically to the laboratory section of the Department of Pharmaceutical Microbiology and Biotechnology, Madonna University, Elele, Nigeria for further processing and analysis.

2.2. Sample Culturing and Purification

The swab sticks containing the samples were aseptically swirled into a sterile nutrient broth and incubated at 37 °C for 24 hours. Following incubation, the bacterial isolates were purified by growing/inoculating them in various bacteriological media, including MacConkey agar, Eosin methylene blue agar, and Cetrimide agar. For proper identification, the characteristic bacteria observed on these media were aseptically isolated and subjected to microscopical and appropriate biochemical tests.

2.3. Identification and Confirmation of Isolates

The obtained isolates were characterized using morphological (colonial morphology) and biochemical tests [12]. The isolates were then Gram-stained, and the Indole and Oxidase biochemical tests were performed to further identify the organisms to species level [13].

2.4. Antibiogram Screening

The antimicrobial susceptibility pattern of the bacterial isolates was evaluated using the Kirby's Bauer (disc diffusion assay) method. ABTKE Antibiotic multidisc (Abtek biologicals UK) used consisted of Gentamicin (10µg), Cefuroxime (30µg), Cefazidime (30µg), Augmentin (30µg), Ofloxacin (5 µg), Cloxacillin (5µg), Erythromycin (10µg) and Ceftriaxone (30µg). The medium used was Mueller Hinton agar (MH). The MH agar was inoculated by streaking the organisms using a sterile wire loop. The antibiotic disc was applied using sterilized forceps. The agar plates were left on the bench for 30 minutes to allow diffusion of antibiotics and the plates were incubated at 37 °C for 24 hours. The clear zone of inhibition around each antibiotic disc was measured with a meter rule in millimeters and recorded based on the Clinical and Laboratory Standards Institute [14].
3. Results

A total of one hundred (100) samples were collected and screened from meat vendors in Elele market, Rivers State. Fifty (50) were obtained from aprons, while the remaining Fifty (50) were obtained from tables. The meat vendors’ aprons and tables were contaminated by a variety of bacteria, many of which had been identified as food-borne pathogens. As shown in Table 1, the total gram-negative bacteria recorded from the aprons are 30 bacterial isolates (45.5%), while the total gram-negative bacteria recorded from the tables are 36 bacterial isolates (54.5%). Table 2 below shows the number of each bacterium isolated with its percentage occurrence.

Table 3 shows the antibiogram of gram-negative bacteria isolated from the Table of meat vendors to some antibiotics. It is observed that all the isolates were resistant to Erythromycin and Cloxacillin, recording 100% resistance. The isolates recorded 50% and 66.7% sensitivity to Gentamicin and Ofloxacin, respectively.

Table 4 shows the antibiogram of gram-negative bacteria isolated from the Apron of meat vendors to some antibiotics. It is observed that all the isolates were resistant to Augmentin and Cloxacillin, recording 100% resistance followed by Erythromycin at 90%. The isolates recorded 100% sensitivity to Ofloxacin.

**Table 1** Frequency of Gram-negative bacteria isolated from Aprons and Tables of meat vendors

| Source | Number of Bacteria isolated | Percentage (%) occurrence |
|--------|-----------------------------|---------------------------|
| Apron  | 30                          | 45.5                      |
| Table  | 36                          | 54.5                      |
| Total  | 66                          | 100                       |

**Table 2** Number and percentage of occurrence of each bacterium isolated

| S/N | Gram-negative bacteria          | No. of occurrence | Percentage (%) |
|-----|---------------------------------|-------------------|----------------|
| 1   | *Klebsiella pneumoniae*         | 49                | 74.24          |
| 2   | *Escherichia coli*              | 12                | 18.18          |
| 3   | *Pseudomonas aeruginosa*        | 3                 | 4.54           |
| 4   | *Proteus sp.*                   | 2                 | 3.03           |
|     | **Total**                       | **66**            | **100**        |

**Table 3** Antibiotic susceptibility pattern of Gram-negative bacteria isolated from Tables of meat vendors

| Antibiotics    | Number susceptible | % | Number resistant | % |
|----------------|--------------------|---|------------------|---|
| Gentamicin     | 18                 | 50| 18               | 50|
| Ceftriaxone    | 12                 | 33.3| 24              | 66.7|
| Ofloxacin      | 24                 | 66.7| 12              | 33.3|
| Augmentin      | 11                 | 30.6| 25              | 69.4|
| Erythromycin   | 0                  | 0  | 36              | 100|
| Cloxacillin    | 0                  | 0  | 36              | 100|
| Ceftazidime    | 12                 | 33.3| 24              | 66.7|
| Cefuroxime     | 14                 | 38.9| 22              | 61.1|
Table 4 Antibiotic susceptibility pattern of Gram-negative bacteria isolated from Aprons of meat vendors

| Antibiotics     | Number susceptible | %    | Number resistant | %    |
|-----------------|--------------------|------|------------------|------|
| Gentamicin      | 10                 | 33.3 | 20               | 66.7 |
| Ceftriaxone     | 8                  | 26.7 | 22               | 73.3 |
| Ofloxacin       | 30                 | 100  | 0                | 0    |
| Augmentin       | 0                  | 0    | 30               | 100  |
| Erythromycin    | 3                  | 10   | 27               | 90   |
| Cloxacillin     | 0                  | 0    | 30               | 100  |
| Ceftazidime     | 6                  | 20   | 24               | 80   |
| Cefuroxime      | 2                  | 6.7  | 28               | 93.3 |

4. Discussion
Aprons are worn by the meat vendors to maintain hygiene and protect clothes from spills and wear and tear while the table is being used to cut and display meat [5, 15]. Because of poor personal hygiene and inadequate knowledge meat vendors can contaminate foods with their aprons and tables during food processing and finally lead to infection of consumers [4]. This study was carried out to determine the incidence and antibiotic susceptibility pattern of gram-negative isolated bacteria from aprons and tables of meat vendors at Elele, Rivers State. The results obtained showed important bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus* spp. This has a similar report to other studies conducted in Awka and Portharcourt City were *Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* were isolated [15, 16]. *Klebsiella pneumoniae* recorded the highest incidence as it accounted for 74.24%. *Klebsiella* sp is a colonizing opportunistic pathogen of humans and animals and a common contaminant of retail meat [17]. The least encountered bacterium in this study was *Proteus* spp and *Pseudomonas aeruginosa* which both accounted for 3.03 and 4.54% respectively. The presence of these microbes on the tables used for displaying meat is an indication that meat placed on such tables could have been contaminated in the process. Nevertheless, other factors such as unhygienic practices may also account for the contaminations of the tables and aprons samples which were observed during the time of sample collection where they were being worn and used. This recent finding showed that the presence of *Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumonia* occurring in the tables and aprons samples suggest contamination from the kind of water used during and after meat handling and also meat sold in open-air shops. Many of these illnesses are caused by *Escherichia coli*. The situation is worsened by the production of toxins by *Escherichia coli* [18]. The knives and cutlasses used in cutting meat are also important contaminants since they are rarely sterilized due to the lack of facilities for sterilization of tools at the abattoirs. Furthermore, the indiscriminate use of these tools in the cutting of the intestines constitutes another possible source of meat contamination as the gut contents can easily be spread to the table and the entire meat to be sold [19].

The antibiotic susceptibility pattern of the bacterial isolates shown in Tables 3 and 4 revealed that the isolates developed complete sensitivity to Ofloxacin. Thus, bacteria isolated from Tables and Apron demonstrated high resistance to beta-lactam antibiotics such as Cloxacillin, Ceftazidime, and Cefuroxime. This is similar to the work of Abuchi et al. (2016) [15], who discovered complete resistance to beta-lactams (ampicillin, ceftriaxone, and cefuroxime) [15]. Their resistance to cephalosporins could be due to the production of beta-lactamase enzymes, which are known to inactivate antibiotics, particularly beta-lactams [15].

5. Conclusion
The isolation of multi-drug resistant gram-negative bacteria from meat vendors' tables and aprons at Elele Meat Market in Rivers State raises serious health concerns for the final consumers. Meat vendors should be briefed on proper hygiene practices when handling meats, such as regular washing and decontamination of tables and aprons. Also, raising awareness about the misuse of antibiotics in the treatment of animals or their feed.
Compliance with ethical standards

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Disclosure of conflict of interest
The authors declare no conflict of interest.

References

[1] Akagha TN, Gugu TH, Enemor EC, Ejikeugwu PC, Ugwu BC, Ugwu MC. Prevalence and Antibiogram of Salmonella species and Staphylococcus aureus in Retail meats sold in Awka metropolis, Southeast Nigeria. International Journal of Biological Pharmaceutical Research. 2015; 6(12): 924-929.

[2] Holck A, Axelsson L, McLeod A, Rode TM, Heir E. Health and safety considerations of fermented sausages. Hindawi Journal of Food Quality. 2017; 1: 1- 25.

[3] Käferstein F, Abdussalam M. Food safety in the 21st century. Bull World Health Organization. 1999; 77: 347-351.

[4] Elhadi N. Prevalence and antimicrobial resistance of Salmonella spp. in raw retail frozen imported freshwater fish to eastern province of Saudi Arabia. Asian Pacific Journal on Tropical Biomedicine. 2014; 4(3): 234-238.

[5] Eruteya OC, Akpan SA, Ubogu M. Antibiotic sensitivity of bacteria isolated from aprons of beef vendors in Port Harcourt, Nigeria. African Journal of Food Science. 2012; 6(15): 401-406.

[6] WHO (2008) Foodborne Disease Outbreaks: guidelines for investigation and control. World Health Organisation, France, 2008.

[7] Younes M, Bartram J. Waterborne health risks and the WHO perspectives. International Journal on Hygiene and Environmental Health. 2001; 204(4): 255-263.

[8] Ogbonna C. Analysis of Groundwater pollution from Abattoir waste in Minna Nigeria. Res. Journal of Sciences. 2008; 2(4): 74-77.

[9] Bhandare SG, Sherikarv AT, Paturkar AM, Waskar VS, Zende R. A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control. 2007; 18: 854-868.

[10] Kolar M, Karel Urbanek K, Tomas’La’tal T. Antibiotic selective pressure and development of bacterial resistance. International Journal of Antimicrobial Agents. 2001; 17: 357-363.

[11] Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O’Brien TF, Pablos-Mendez A, Klugman KP. Antimicrobial resistance in developing countries. Part I: recent trends and current status. The Lancet infectious diseases. 2005; 5(8): 481-493.

[12] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Test: CLSI Supplement Twenty-ninth Edition. M100. 2019; 39: 1.

[13] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Test: Approved Standards-Eleventh Edition. M02-A11. 2012; 32: 1.

[14] Cheesbrough M. District laboratory practice in tropical countries part 2. United Kingdom. University press Cambridge. 2012; 267-317.

[15] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Test: Approved Standards-Eleventh Edition. M02-A11. 2012; 32: 1.

[16] Podschun R, Ullmann U. Klebsiella spp. As nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998; 11: 589–603.

[17] Sales I, Zouhairi O, Alwan N, Hawi A, Barbour E, Harakeh S. Antimicrobial Resistance and Pathogenicity of Escherichia coli isolated from common Dairy Products in Lebanon. Annuals of Tropical Medicine & Parasitology. 2009; 103(1):39-52.

[18] Fasanmi OG, Olukole SG, Kehinde OO. Microbial studies of table scrapings from meat stalls in Ibadan metropolis, Nigeria: Implications on meat hygiene. African Journal on Biotechnology. 2009; 9 (21): 3158-3162.