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Research Article

Incidence and antibiotic profile of gram-positive and gram-negative bacteria isolated from aprons and tables used in abattoir located in Abraka and Obiaruku, Delta State, Nigeria

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Abstract

This study was designed to investigate the presence of gram-positive and gram-negative bacteria in aprons and tables used in abattoirs in Abraka and Obiaruku.

A total of one hundred samples were obtained. Fifty samples were obtained from the aprons of butchers and meat vendors working in an abattoir located in Abraka and fifty samples from the sectioning tables in an abattoir located in Obiaruku using sterile swab sticks. Biochemical tests were carried out to characterize the bacterial isolates and susceptibility carried out using Kirby-Bauer disc diffusion method.

Out of the 50 aprons sampled, 47 were gram negative bacteria and 3 were gram positive bacteria. The bacteria were: *Proteus spp.* (16), *Citrobacter spp*. (15), *Salmonella spp*. (10), *Shigella spp*. (3), *Aeromonas spp*. (2), *Providentia spp*. (1), *Mycobacterium spp*. (1), *Enterococcus spp*. (1) and *Streptococcus spp*. (1). From the table samples, all 50 were gram negative bacteria. The gram-positive bacteria showed high susceptibility (100%) with high zones of inhibition to most antibiotics used. Gram-negative bacteria showed highest susceptibility to erythromycin (95.7%)

This study reveals that aprons and tables of butchers and meat vendors at the abattoirs are reservoir of various bacteria, some of which are food borne pathogens and are multidrug resistant. The high prevalence of some of these organisms in this study coupled with their high antibiotic resistance profile is reflective of the poor hygiene practices carried out at the abattoirs in Abraka and Obiaruku and thus, pose a serious public health concern to the consumers of meat from such abattoirs.

Keywords: Bacteria, isolates, abattoir, butchers, meat vendors

INTRODUCTION

An abattoir is a place where livestock such as cattle, goats and others are slaughtered, processed and distributed for human consumption as well as for other industrial purposes.[1] The abattoir industry is an extremely important component of livestock industry in Nigeria as it provides domestic meat supply to over 150 million people and employment opportunities for the teeming population.[2] Notwithstanding, the abattoir industries are less developed in developing countries like Nigeria.[2] Meat serves as a major source of protein and so its daily demand is high. In Nigeria, there has been a continuous increase in the development and growth of livestock production, as it serves as a means of providing steady supply of food animals for slaughter and processing for human consumption.[3] The poor state of abattoirs and meat processing plants, lack of meat inspection services with a resultant consumption of unhealthy meat by the public have become a major cause of concern to all stakeholders and public in general and despite the passage of time, there has not been reasonable improvement in abattoir practices in Nigeria.[3] There are various means by which contamination of meat occurs in an abattoir. The major sources of contamination of the meat are the animals being slaughtered (from the hides and intestinal tract), the workers, the abattoir environment and to a lower level from the air through aerosols and from the dressing water used for carcass.[4,5] Surfaces which are contaminated with microorganisms have the potential risk of transmitting the pathogens to food during processing; therefore, food borne diseases which occur as a result of contact with hands and surfaces depends largely on the level of contamination.[3,6] Antimicrobial resistance is a major challenge in the management of severe food borne illness, since antimicrobial use in animals selects for resistant food borne pathogens that may be transmitted to humans as food contaminants. This study presents a comprehensive review on gram-positive and gram-negative bacteria, assessing their susceptibility and resistance to antimicrobial agent, pointing out their significance in the health sector as main causes of infections resistant to common antimicrobial agents.

MATERIAL AND METHODS

Materials

Materials used are: Nutrient agar, Peptone water, Nutrient broth, Muller Hinton agar, Simmons Citrate agar, MIU broth media, Crystal violet, safranine stain, Lugol iodine, ethanol, hydrogen peroxide, Kovac's reagent, glass wares, wire loop, glass rods, lead acetate paper.

Collection of Samples (Apron and Table samples)

A total of hundred (100) samples of apron and table specimen were obtained of which fifty (50) samples were obtained from the aprons of butchers and vendors working in an abattoir located in Abraka and fifty (50) samples from tables used for sectioning the carcasses and displaying the raw meat from an abattoir located in Obiaruku using sterile swab sticks labelled. After collection, the swab sticks were sealed properly and stored at a temperature of 4 °C in the fridge prior to use within 2 hrs of collection.

Test for Microbial Growth and Isolation of Bacteria

The work bench was swabbed using a disinfectant and Nutrient agar previously prepared according to Manufacturers specification and dispensed aseptically on hundred (100) sterile petri- dishes and were inoculated with apron and table specimen with previously preserved swab stick containing collected specimen labelled (1 – 100). Inoculated Petri- dishes were inverted and placed in an incubator at a temperature of 37 ºC for 24 hrs, after which Petri-dishes were examined for microbial growth, observations were recorded.[7,8] Gram test was carried out to classify organisms on their ability to retain the colour of the dye. Catalase, fermentation, oxidase, indole, H^2S, MR - VP (Methyl Red and Voges - Proskauer test), urease, citrate utilization test, coagulase and MIU (Motility Indole Urease) test were carried out to identify the organisms isolated.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried out using Kirby-bauer disc diffusion method for an already prepared antibiotic multidisc. The antibiotic disc contained the following antibiotic and concentration; Ciprofloxacin 10 µg, Norfloxacin 10 µg, Gentamycin 10 µg, Amoxil 20 µg, Streptomycin 30 µg, Rifampicin 20 µg, Erythromycin 30 µg, Chloramphenicol 30 µg, Ampiclox 20 µg and Levofloxacin 20 µg.

The table was swabbed using disinfectant and the media used (Mueller-Hinton Agar) was prepared and sterilized according to Manufacturer's instructions on the label. It was then allowed to cool before aseptically dispensed into sterile Petri - dishes. The agar medium was allowed to solidify at room temperature on the working bench. The isolate was inoculated on the Mueller-Hinton agar using sterile swab stick by dipping it in the broth medium containing the isolate and rubbing it gently on the surface of the Mueller-Hinton agar uniformly. The antibiotic disc was gently and firmly placed on the agar plates using sterile forceps. The impregnated agar plates were then incubated at 37°C for 24hrs and the zone of inhibition was determined.

RESULTS

In this Study, a total of one hundred (100) swab samples comprising 50 samples from aprons of butchers and meat vendors and 50 samples from the tables of butchers and meat vendors were aseptically collected from an abattoir located at Abraka and Obiaruku respectively using sterile swab sticks and these were bacteriologically analyzed for the isolation of gram-positive and gram-negative bacteria. On inoculation, one hundred (100) samples yield growth and they were identified. A total number of 100 bacterial isolates were obtained from both locations.

Synopsis of Isolated Gram-negative and Gram-positive Bacteria

The synopsis of isolated gram-negative and gram-positive bacteria and their frequency of occurrence is given in Table 1. *Proteus spp.* was the most dominant gram-negative bacteria species isolated (Table 1) while *Mycobacterium spp, Enterococcus spp, Streptococcus spp* were the gram-positive isolated (Table 2).

Table 1: Synopsis of Gram-negative Bacteria Isolates

Isolated Gram-Negative Bacteria	Aprons (n= 50)	Table (n = 50)	Total
Citrobacter spp	15(30%)	12(24%)	27(54%)
Salmonella spp	10(20%)	10(20%)	20(40%)
Proteus spp	16(32%)	21(42%)	37(74%)
Shigella spp	3(6%)	4(8%0	7(14%)
Aeromonas spp	2(4%)	1(2%)	3(6%)
Providentia spp	1(2%)	1(2%)	2(4%)
Pseudomonas spp	-	1(2%)	1(2%)

Table 2: Synopsis of gram-positive bacteria isolates and their frequency of occurrence

Isolated Gram-Positive Bacteria	Aprons (n= 50)	Table (n = 50)	Total
Mycobacterium spp	1 (2%)	-	1 (2%)
Enterococcus spp	1 (2%)	-	1 (2%)
Streptococcus spp	1 (2%)	-	1 (2%)
Total	3(6%)	-	3(6%)

Key: n- sample size

Zone of Inhibition of Gram-Negative Organisms

The zones of inhibition for the different organisms revealed that levofloxacin (LEV) and ciprofloxacin (CPX) were the optimized antibiotics for the different gram-negative organisms (Figure 1).

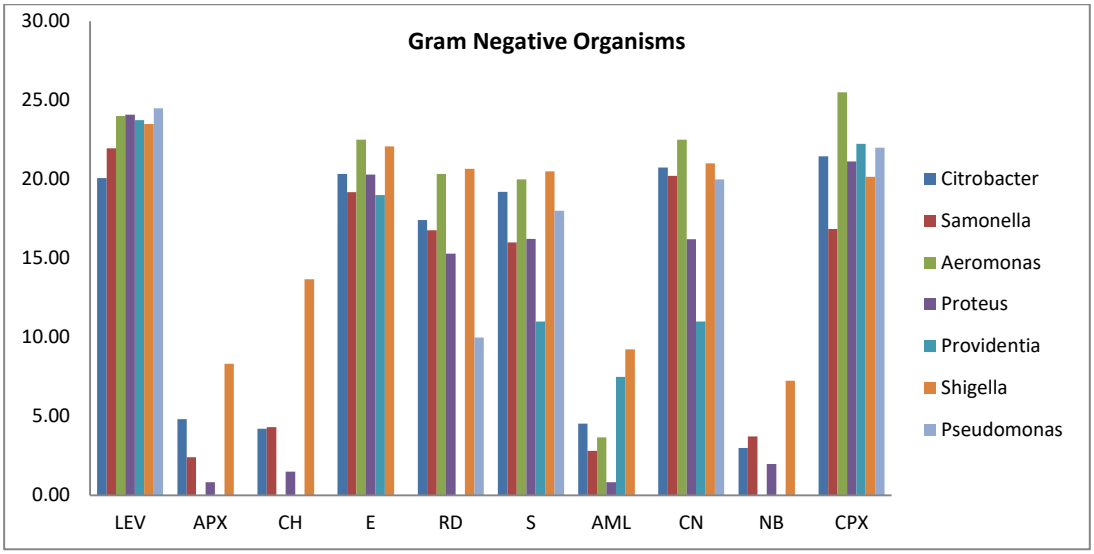

Figure 1: Shows the zone of inhibition of the gram-negative and gram positive bacteria against the various antibiotics

LEV: Levofloxacin; APX: Ampliclox, CH: Chloramphenicol, E: Erythromycin, RD: Rifampicin, S: Streptomycin, AML: Amoxicillin, CN: Gentamicin, NB: Norfloxacin, CPX: Ciprofloxacin

Zones of Inhibition for Gram-positive Organisms

The zones of inhibition for the different organisms revealed that levofloxacin (LEV) and ciprofloxacin (CPX) were the optimized antibiotics for the different gram-positive organisms (Figure 2)

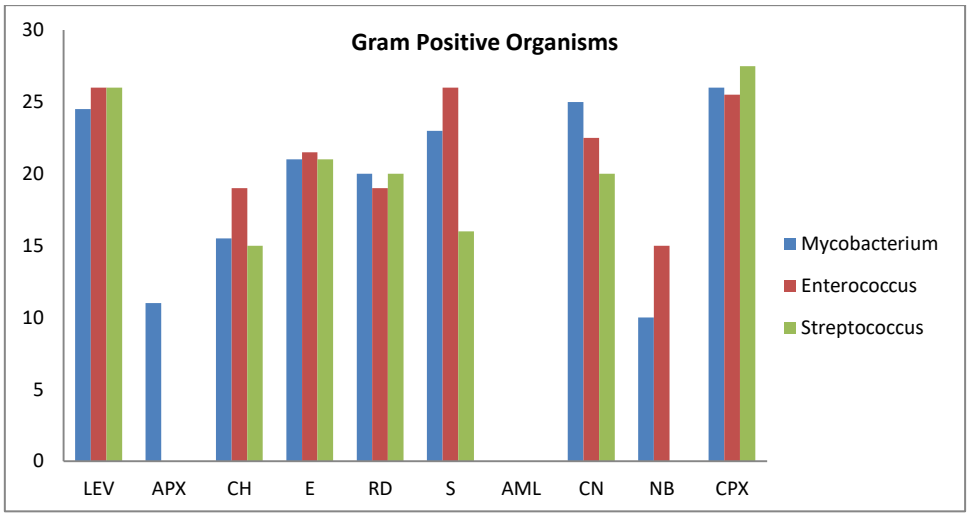

Figure 2: Shows the zone of inhibition of the gram-negative and gram positive bacteria against the various antibiotics

LEV: Levofloxacin; APX: Ampliclox, CH: Chloramphenicol, E: Erythromycin, RD: Rifampicin, S: Streptomycin, AML: Amoxicillin, CN: Gentamicin, NB: Norfloxacin, CPX: Ciprofloxacin

DISCUSSION

It is a fact that contaminated food is the primary source of transmission for many pathogenic bacteria and it is the major cause of enteric diseases in developing countries.[3] This study describes the incidence of bacterial pathogens in aprons and tables used in abattoir in Abraka and Obiaruku environs, Delta state. Out of the 50 apron samples collected, majority of the organism sampled were gram-negative. This finding corroborates earlier findings in which majority of the organism identified were gram-negative.

The microorganisms isolated from aprons are listed and arranged in descending order from the most to the least incident. *Proteus spp.* and *Citrobacter spp.* were the most predominant organisms. Contrary result was obtained by Ugwu *et al.*[3] In another related study, *Escherichia coli* was the predominant organisms.[6] The presence of these microbes on the aprons goes a long way to show the poor hygienic practices by butchers and meat vendors. Several studies have shown that meat handlers harbor microorganisms especially bacteria asymptomatically.[9,10] Meat handlers with poor personal hygiene and inadequate knowledge working in

establishments like abattoir could be possible sources of infections of many enteropathogenic bacteria.[11] Similarly, meat handlers who harbor pathogenic bacteria may contaminate foods with their fingers/aprons during food processing and finally lead to infection of consumers.[12]

The gram-negative bacteria species isolated from the tables are similar to those isolated from aprons with the exception of Pseudomonas. *Proteus spp. and Citrobacter spp.* were the most prevalent bacteria isolate. However, recent study identified Escherichia coli as the predominant bacteria isolate.[4] The presence of these microbes on the tables used for sectioning carcasses and displaying meat is an indication that meat placed on such tables could have been contaminated in the process.[3] Other factors such as unhygienic practices may also account for the contaminations of the tables after the daily normal sales.[3] *Proteus spp., Citrobacter spp.,* and *Salmonella spp*. were the most prevalent bacteria isolate from both the apron and table samples. Bacteria of the genus Proteus are part of the normal flora of the intestinal tract of humans and animals and are widespread in the environment.[13,14] Proteus species are members of the bacteria family called Enterobacteriaceae and are usually regarded as commensals in the gut and they are mostly recognized clinically as a cause of urinary tract infections.[15,16] Proteus spp. has been recently identified as potential pathogens in Crohn's disease recurrence after intestinal resection.[17,18] They possess many virulence factors which are potentially vital to gastrointestinal pathogenicity, they include: motility; adherence; the production of urease, hemolysins and IgA proteases; and the ability to acquire antibiotic resistance.[15] Gastrointestinal disease conditions which have been linked to Proteus spp. include: gastroenteritis (both spontaneous and foodborne), nosocomial infections, appendicitis, colonization of devices such as nasogastric tubes and Crohn's disease (the association of Proteus spp. with Crohn's disease was very strong in particular).[15]

Citrobacter spp. are commensal inhabitants of the intestinal tract of humans and other animals.[19] They have also been recovered from water, sewage and soil.[20,21] *Citrobacter spp.* are opportunistic pathogens of humans and have been associated with a variety of infections which include: urinary tract infections (UTIs) gastroenteritis, wound infections, pneumonia, brain abscesses, septicemia, meningitis and endocarditis, in particular in neonates and immunocompromised hosts.[22] As bacterial contaminants, they have been partly responsible for the cause of foodborne diseases and are often transmitted through food and water.[23,24] Consequently, food-handlers with poor personal hygiene could be potential sources of infections by these microorganisms.[23]

The Presence of Shigella among the Bacterial isolates points to the fact that the sanitary practices among meat vendors are not satisfactory[3], this is because Shigella organisms do not have any natural reservoirs in animals and can only spread from person to person.[25] The Centers for Diseases control and Prevention (CDC) estimates that 48million cases of food-borne illness occur in the United States annually and many of these illnesses are caused by *Salmonella spp.*[3] Biological contaminants such as bacteria, viruses, fungi, protozoa and helminths constitute the major cause of food-borne disease with varying degree of severity ranging from mild indisposition to chronic or life-threatening illness or both. In developing countries, such contaminants are responsible for food-borne diseases such as cholera, campylobacteriosis, E. coli, gastroenteritis, salmonellosis, Shigellosis among others.[3,26] The Unhygienic condition of the tables on which the meat is displayed for sale may also contribute to the contamination of meat. Often, the knives and cutlass used in cutting meat are also important contaminants since they are rarely sterilized and the lack of facilities for sterilization of tools at the slaughter houses and meat stalls can be a major cause of this. In addition, the indiscriminate use of these tools in the cutting of offal like the intestines constitute another possible source of meat contamination as the gut contents can easily be spread to the table and the entire meat to be sold. The microbiological safety of food is achieved by as far as possibly ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication.

The isolates showed varying degree of susceptibility to the different antibiotics used. The antibiotic susceptibility test results showed that all the gram-positive bacteria isolates were sensitive to gentamicin, levofloxacin, chloramphenicol, erythromycin, rifampicin, streptomycin and ciprofloxacin. This may be as a result of the presence of thick peptidoglycan layer which absorbs antibiotics more than gram-negative isolates. Majority of the gram-positive isolates were resistant to norfloxacin except Enterococcus. The gram-positive bacteria isolates were all resistant to amoxicillin and Ampiclox (ampicillin/cloxacillin) with no zone of inhibition. *Streptococcus spp.* was resistant to amoxicillin and Ampiclox. This result is in tandem with similar results.[3] The susceptibility result of bacteria isolated showed that the gram-negative organisms are more resistant than the gram-positive organism; which is expected due to the intrinsic nature of gram-negative cell wall.[3,27]

CONCLUSION

This study reveals that the aprons and tables of butchers and meat vendors at the abattoirs are a reservoir of various bacteria, some of which are food borne pathogens and are multidrug resistant.The high prevalence of some of these organisms in this study coupled with their high antibiotic resistance profile is reflective of the poor hygiene practices carried out at the abattoirs in Abraka and Obiaruku and thus pose a serious public health concern to the consumers of meat from such abattoirs.

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