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Isolation and Antibiogram of Bacteria Isolated from Processed and Unprocessed Cow-Skin (Ponmo) in Ogbese Market

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Authors' contributions

This work was carried out in collaboration between all authors. Author TAO designed the study, performed the statistical analysis wrote the protocol and the first draft of the manuscript. Authors FCA and BSA managed the analyses of the study. Author SCA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The use of ponmo as major meat source to average Nigerians if processed under unhygienic condition might result in disease outbreak. Inadequate and under-utilization of food nutrients needed by human for normal body functions has resulted in malnutrition and outbreak of diseases. Fifty samples of cowhides comprising forty processed (ponmo) and ten unprocessed were sourced from Ogbese Market, Ondo State, Nigeria. The microbial analyses were carried out using serial dilution and pour plate methods. The 0.1ml of the diluents was aseptically plated on molten nutrient agar and incubated temperature. Pure cultures were obtained by repeated streaking on the appropriate microbiological media. Antibiotics sensitivity pattern on the bacterial isolates were carried out using commercial antibiotics. The highest microbial load from unprocessed Ponmo with fur was 3.5 x10^5 while the processed Ponmo was 1.5 x 10^5cfu/ml. *Staphylococcus aureus, Streptococcus mitis, Salmonella typhimorium*, *Bacillus subtilis*, *Escherichia coli, Staphylococcus epidermidis, Shigella dysenteriae* and *Micrococcus leteus* were isolated. Ciprofloxacin showed inhibitory potency on the entire isolates with *Staphylococcus aureus* showing high resistance to most of the antibiotics.

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Keywords: Cow meat (Ponmo); Staphylococcus aureus; Salmonella typhimorium; Bacillus subtilis; antibiotic resistance.

1. INTRODUCTION

The global demand for meat and dairy products like milk, yoghurt and egg as part of human diets in recent time with rapid growth has reflected in its demand in livestock industry around the world [1]. The nutritional demand obtained from livestock products containing essential component has tremendously contributed to its daily diet intake [2]. The nutritional component of animal product containing protein are of more biological values with essential amino acids, vitamins, niacin, phosphorus, zinc and iron which help in nourishing body cell, promote growth, repair and maintenance of worn out tissues [3].

Raw hides are by-product obtained from farm animals in meat industry used for leather production. It protects the body from injuries, climatic and environmental influences, and body temperature regulation [4]. The tanning industry and the downstream industries such as footwear, furniture automotive, clothing, leather goods, and saddler are entirely dependent for their raw material on suppliers of cattle hides, sheep skin and small number of goat and other skins [5]. Ponmo is known to contain less nutritional value despite its appeal taste deliver to the taste buds when consumed [2].

Ponmo is a meat product obtained from the tenderization of hide of beef or cattle in hot water followed by scrapping with sharp object like razor blade [1]. Ponmo is usually off-white or brown in colour depending on the type of animal used. In Nigeria, the unprocessed cow-skin usually sold in the market is mostly transported from the Northern part to the West. Processing of ponmo for human consumption involves different stages such as: Drying, singe, scraping, boiling and washing. Ponmo is used as major delicacy and important food ingredients in the preparation of several stews in various cultures in Nigeria especially at several public outings [6]. Consumption of ponmo has been found high among the low class people in Nigeria depending on the financial strength and interest. The high poverty level in Nigerians and other developing countries have made more people opting for cow-skin and its product because of economic reasoning. For this reason, most Nigerians love to eat ponmo as much, believing that, daily meal without ponmo is incomplete [7].

In some abattoir site and ponmo processors, singe is a common practice to get rid of the fur [2]. In recent time, the scarcity of firewood has resulted in local butchers using scrap tyres, plastics and spent engine oil mixed with fire-wood [6]. These materials confer potentially toxic organic substances such as benzene, lead and dioxin to the hide which can contaminate, render them unfit for human consumption and cause disease [1]. Therefore, this work is targeted in assessing the quality of processed and unprocessed cow-skin (Ponmo) and associated microorganisms possibly of causing diseases in man which can be prevented through adequate quality control measures by the processors.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total number of 50 samples containing forty processed ponmo and ten unprocessed cowhides obtained from cow slaughtering house and cowhide processors under hygienic condition in Ogbese Market, Ondo State, Nigeria were collected in batches in an ice pack bag between June and August, 2015 and then transported to laboratory for further analyses.

2.2 Microbial Counts Enumeration

Serial dilution method was used for the isolation of microorganisms associated with the ponmo samples as described by Cheesbrough [8]. One gram of the ponmo sample was dispensed into 9 ml of sterile distilled water to form a stock solution. One millilitre (1 ml) of the suspension from the sample was dispensed into 9 ml sterile distilled water and serially diluted. An aliquot of 0.1 ml was inoculated into a sterile Petri dishes, pour plate with sterilized molten medium (nutrient agar and MaCankey agar), then incubated for 24 hours at 37℃ in an incubator. The colony forming counts per millilitre of sample was determined using standard microbiological method. The pure culture of the microbial isolates was obtained by repeated streaking on nutrient agar and MacCankey agar medium. The bacterial isolates were characterized and identified using standard biochemical methods [9].

2.3 Susceptibility Test

The antimicrobial susceptibility test of the bacterial isolates was determined using disc diffusion method [10]. The antibiotics sensitivity test was also investigated using GBMTS discs (Abtek biological Ltd.) containing the following antibiotics: ampicillin (SP), gentamycin (CN), ampiclox (APX), ciprofloxacin (CPX), pefloxacin (PEF), lexoflacin (Z), amoxil (AM), rifampin (R), streptomycin (S), septrin (SXT), erythromycin (E), chloraphenicol (CH), ampicillin (SP), augmentin (AU) and ofloxacin (OFX). The commercial antibiotics were placed on nutrient agar plates previously seeded with an 18 - 24 hours aged culture of each test microorganisms using sterile glass spreader. The plates were incubated at 37℃ for 48 hours, the antibacterial susceptibility pattern was measured by the zones of inhibition, examined and interpreted accordingly [11].

3. RESULTS

3.1 Total Bacteria Counts

The total bacteria count isolated from ponmo samples are represented (Table 1). The highest microbial load of 3.2 was obtained from unprocessed ponmo with fur while the lowest counts 2.0 was obtained from processed ponmo boiled and processed ponmo boiled from market women and hawkers respectively. The microorganisms isolated were *Bacillus subtilis*, *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus mitis, Micrococcus leteus*, *Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhimorium* (Table 2). The occurrence of the microorganisms showed that *Bacillus subtilis*, *Staphylococcus aureus, Salmonella typhimorium* and *Micrococcus leteus* was observed to occur in all the samples assessed. *Staphylococcus aureus* showed the highest percentage occurrence of 44% while *Shigella dysenteriae* showed the lowest percentage of occurrence of 4% (Table 3).

3.2 Antibiotics Sensitivity Pattern of the Bacteria Isolates

Figs. 1 and 2 show the results of antibiotic sensitivity test of both the gram positive and gram negative microorganisms. The antibiotic sensitivity pattern on *Staphylococcus epidermis* showed its high susceptibility to gentamycin (CN), amoxil (AM), ciprofloxacin (CPX) and erythromycin (E) with zones of inhibition of 21 mm, 11 mm, 18 mm and 22 mm while *Staphylococcus aureus* show resistant to ampicillin (SP), gentamycin (CN), ciprofloxacin (CPX), pefloxacin (PEF), lexoflacin (Z), amoxil (AM), rifampin (R), septrin (SXT), erythromycin (E), chloraphenicol (CH), ampicillin (SP), augmentin (AU) and ofloxacin (OFX) (Fig. 1). The gram negative microorganisms isolated such as *Salmonella* spp was susceptible to amoxil (AM), streptomycin (S), septrin (SXT), chloraphenicol (CH) and augmentin (AU), Shigella spp. was susceptible to ciprofloxacin (CPX) and pefloxacin (PEF) while E. coli was resistant to pefloxacin (PEF) (Fig. 2).

Table 1. Total bacterial count associated with ponmo samples

Samples	Microbial load (cfu/ml) x 10^6
Processed ponmo (market women)	2.5
Processed ponmo boiled (market women)	2.0
Processed ponmo (hawkers)	2.1
Processed ponmo boiled (hawkers)	2.0
Unprocessed ponmo with fur	3.2

4. DISCUSSION

Raw meat remains an important and probably the major source of human food borne infection with pathogenic bacteria. In spite of decades' effort, it has been difficult to obtain food animals free of pathogenic bacteria [12]. The microorganisms isolated from processed ponmo and unprocessed ponmo samples were *Bacillus subtilis*, *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus mitis, Micrococcus leteus*, *Escherichia coli*, *Shigella dysenteriae* and *Salmonellatyphimorium.* The presence of bacteria in meat and meat products have been widely reported from different parts of the world [12,13]. The occurrence of these microorganisms may be due to lack of proper quality control measures in handling and processing of the meat. Unhygienic practices of meat processing, busily conversing, coughing and sneezing on the meat as source of contaminant has been reported [14]. Okonko et al. [15] and Koffi-Nevry et al. [16] reported that food can be infected with microorganisms as a result of coughing and careless sneezing among the butchers, food handlers and processors. Iroha et al. [17] reported similar microorganisms from

contaminated raw meat. The results obtained from this study corroborates the findings of Moshood et al. [18] and Ibrahim et al. [19] who reported common bacteria such as *Staphylococcus aureus, Salmonella typhimorium* and *Bacillus subtilis* from fresh and smoke fish.

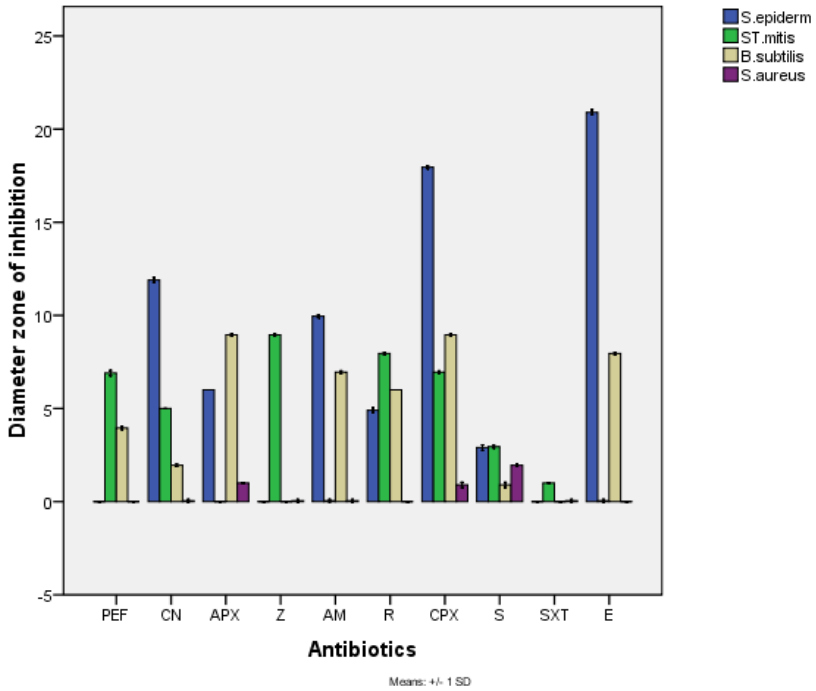

Fig. 1. Antibiotics sensitivity graph for gram positive bacteria

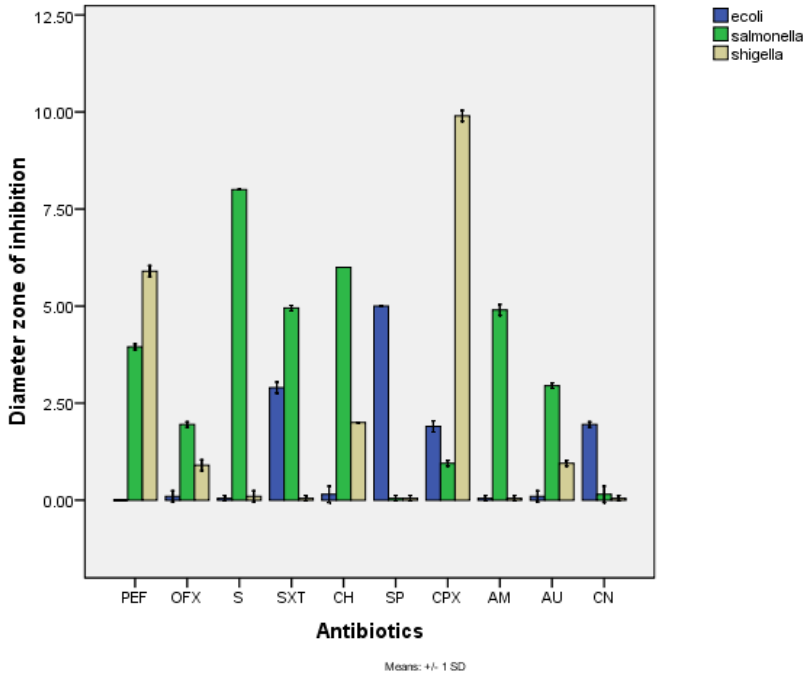

Fig. 2. Antibiotics sensitivity graph for gram negative bacteria

Table 2. Morphological and biochemical characteristics of the bacteria isolated from ponmo

Isolate code	G. reaction	Cell shaoe	Cell arrangement	Cell colour	Cell texture	Catalase test	Oxidase test	Coagulase test	Citrate test	Motility	Indole	Glucose	Galactose	Lactose	Sucrose	Maltose	Probable microorganisms
Pm	+	C	Cl	CR	S	+	-	+	+	-	-	A	AG	A	A	A	*Staphylococcus aureus*
upf	+	R	Cl	CR	S	+	-	+	+	+	+	A	AG	A	A	A	*Bacillus subtilis*
Pm	+	C	Ch	LG	S	-	-	-	+	-	-	A	-	-	AG	-	*Streptococcus mitis*
Pmb	-	R	CH	CR	S	+	-	-	+	-	-	A	-	A	-	-	*Escherichia coli*
Pmb	-	R	Ch	MT	S	+	-	-	+	-	-	-	A	A	A	A	*Salmonella typhimorium*
Pm	-	C	Cl	CR	S	+	-	-	+	-	-	AG	A	A	A	A	*Staphylococcus epidermis*
Pmb	-	C	S	CR	R	+	-	-	+	-	-	-	A	A	A	A	*Shigella dysenteriae*
upf	+	R	Cl	YL	R	+	+	+	-	-	-	-	+	+	-	-	*Micrococcus leteus*

Key: pm- processed ponmo, pmb – processed ponmo boiled, upf – unprocessed ponmo with fur, CR – creamy, Cl – cluster, R – rod, A – gas production, AG – acid and gas production, S – spherical, C - cluster

Table 3. Occurrence of bacterial isolates associated with ponmo samples

Microorganism	Pp(market women)	Pmb(market women)	Pp (hawkers)	Ppb (hawkers)	Upf	%
Staphylococcus aureus	+	+	+	+	+	44
Bacillus subtilis	+	+	+	+	+	14
Staphylococcus sp.	+	+	-	-	-	12
Staphylococcus mitis	+	-	-	-	-	12
Salmonella typhimorium	+	+	+	+	+	10
Escherichia coli	+	+	+	+	+	8
Shigella dysenteriae	-	-	-	-	+	4
Micrococcus leteus	+	+	+	+	+	8

Key: pm- processed ponmo, pmb – processed ponmo boiled, upf – unprocessed ponmo with fur

The occurrence and increase in the microbial load of the unprocessed ponmo with fur and processed ponmo samples might be due to the mass pollution from the environment at the point of selling [11], contamination acquired during transportation, storage, processing, packaging materials, poor handling by the handlers and poor preservation of cowhide [20]. The occurrence of *Staphylococcus epidermis, Micrococcus leteus*, *Bacillus subtilis* and *Escherichia coli* from the unprocessed cowhide with fur had been reported [21]. The presence of *Micrococcus* spp could be the natural microflora in the samples [22]. *Salmonella typhimorium, Shigella dysenteriae* and *Escherichia coli* in the ponmo might arise from feacal contaminant due to the poor personal hygiene by the food handler and marketers and dust from post contamination of processed ponmo [23]. Clarence et al. [24] reported the predominance of gram-negative organisms such as *Salmonella typhimorium, Shigella dysenteriae* and *Escherichia coli, Staphylococcus aureus, Bacillus subtilis*, *Enterobacter* spp*, Pseudomonas aureginosa* and *Klebsiella pneumoniae* in meat pie. Iddrisu [25] and Kutah [26] also reported the occurrence *Escherichia coli*, *Streptococcus pyogenes and Staphylococcus aureus* from raw and fried cheese samples. Moshood et al. [18] reported the presence of *Salmonella* specie in roasted meat. These results obtained from this study conform to the findings of Obeng et al. [27] who isolated similar microorganisms.

The presence of great number of pathogenic bacteria especially *Salmonella* specie in foods has caused great concern in relation to public health. The use of antibiotics has been proven to be an effective means for the prevention and control of infection associated with the pathogens. Indiscriminate use of these antibiotics can result in adverse consequences by promoting the selection and prevalence of drug resistant microbial populations Iddrisu [25]. This problem may be attributed to the natural resistance of certain species of microorganisms to antibiotics, possible transfer of antibiotic resistance among species, and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, which could also select for resistant strains Obeng et al. [27]. The antibiotic susceptibility profile showed the prevalence of gentamycin (CN), amoxil (AM), ciprofloxacin (CPX) and erythromycin (E) on *Staphylococcus epidermis.* The resistance of *Staphylococcus aureus* to antibiotics may be due to the presence of peptidoglycans in its cell wall, certain enzymes among other virulence factors [28]. The resistance of gram negative strains to most antibiotics more than gram positive is attributed due to the intrinsic nature of their cell wall [28]. Nafisa et al. [29] reported the prevalence antibiotics susceptibility profile of cefazolin, lincomycin, streptomycin and tetracycline resistance against some potential bacterial pathogens. Iroha et al. [17] reported the antibiotic resistance of gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae,*

Salmonella typhimorium and *Shigella dysenteriae*) isolated from chicken meat to ofloxacin and gentamycin. The antibiotic susceptibility of *Bacillus subtilis* isolated raw meat sample to gentamycin has been reported [30].

5. CONCLUSION

The result obtained from this study showed that the ponmo samples serve as a reservoir for both gram positive and gram negative bacteria. The presence of these microorganisms showed an indication of contaminant from our environment which could cause infectious diseases. The susceptibility of the isolates to the commercial antibiotics shows the safety level of the processed ponmo. Therefore, adequate food processing, proper hygiene and the use of antibiotics are very essential to control the emergence of antibiotic resistance strains.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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