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Presence of Pathogenic Bacteria in Butchering Tables, Slaughtering Pavements and Meat Samples Collected from Slaughterhouses in Ogun State (Western Region), Nigeria

Adeyemi Kayode[1]

[1]Department of Microbiology, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara-144411, India

Abstract: ***Aim:*** ***Bacterial assessment of slaughterhouses (abattoir) in Ogun state: Kara (Berger) and Odo-eran (Abeokuta) was conducted.*** ***Methods and result:*** ***Swabs of butchering tables, slaughtering pavements and meat samples were obtained from both sites over a period of three months, and examined for their bacterial contents. Both slaughterhouses were found to contain suspected pathogenic bacteria as well as spoilage organisms. The microbial load from samples obtained from Kara abattoir were generally higher than that obtained from Odo-eran abattoir however, pathogenic bacteria isolated from both abattoirs were species of Enterobacter, Staphylococcus, Klebseilla, Pseudomonas, Bacillus, Streptococcus, Salmonella, Proteus, Clostridium, Shigella and Escherichia coli.*** ***Conclusion****: These findings pose health concerns to meat consumers in Ogun state. However, proper cooking of the meat would destroy most of these pathogens.* ***Significance of Study****: This study reflects the need for the Ogun state government's intervention in the rehabilitation of these abattoirs and ensuring that butchers follow already designed international guidelines of slaughtering animals, which would have a significant positive effect on the microbial population and load of meat and meat products obtained from these abattoirs.*

Keywords: Abattoirs; pathogenic bacteria in meat; foodborne diseases; butchering tables; slaughtering pavements.

1.Introduction

According to the Oxford dictionary (2007), a slaughterhouse (also called an abattoir) is a facility where animals are killed and processed into meat for food. The animals commonly slaughtered for food are cattle (for beef and veal), sheep (for lamb and mutton), pig (for pork), horse (for horsemeat), goat (for chevon) and fowl, largely chickens, turkeys, and ducks, for poultry meat (USDA, 2000). In the United States, around ten billion animals are slaughtered every year in 5,700 slaughterhouses and processing plants employing 527,000 workers (Williams *et al*. 2007). In 2007, 28.1 billion pounds of beef were consumed in the U.S. alone (Torres, 2007). In Canada, 650 million of animals are killed for meat annually (Torres, 2007). In the European Union, the annual figure of animals killed for meat is 300 million cattle, sheep, pigs, and 4 billion chickens (Global Action Network, 2008). In Nigeria (as in many developing countries), the discharge of untreated wastewater from slaughterhouses is still a major problem. Wastewaters from abattoirs contain a variety of pathogenic microorganisms, and are directly introduced into neighboring water bodies (streams, ponds and rivers). These water bodies are used by local communities for domestic activities such as cooking, washing and bathing (Adeyemo, 2003). It is as a result of this pollution that environmental pollution and related diseases (especially zoonotic diseases) are caused and transferred (Salami, 1998). Furthermore, the consumption of meat containing various pathogens has shown to be problematic (Jay, 2005; William and Dennis, 2008). These pathogens not only cause various foodborne diseases but also cause spoilage of meat provided the intrinsic and extrinsic conditions are favorable.

Itah *et al*. (2005) reported the presence of *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Staphylococcus epidermidis, Micrococcus roseus, Bacillus subtilis*, and *Streptococcus* sp. in meat samples and flies obtained from Uyo abattoir (Nigeria). They further indicated the predominant presence of species of *Klebsiella*, *Pseudomonas* and *Salmonella* in samples obtained from this abattoir which is indicative of gross contamination and constitute potential health hazard to consumers. In Morocco (East Africa), Cohen *et al.* (2007) reported that pathogenic bacteria such as *Salmonella sp., Listeria monocytogens, Staphylococcus aureus* and *Clostridium perfringens* were present in poultry meats. Adesemoye *et al.* (2006) also reported the predominant presence of *Bacillus sp., Clostridium perfringens, Pseudomonas aeruginosa, Micrococcus luteus, Vibrio sp., Lactobacillus plantarum* in some Nigerian abattoirs. Methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated from sheep, bovine, camel and poultry meat in Jordan from samples collected in retail shops and slaughterhouses in Amman area (Samar *et al.* 2007). In another study, Al-sheddy *et al.* (2001) isolated *Salmonella sp*., *Escherichia coli* O157:H7 and *Listeria monocytogens* in meat and lamb meats.

Many of these microorganisms isolated from meat samples are highly pathogenic and cause various diseases. For instance, *Clostridium perfringens* is a common cause of gas gangrene, food poisoning as well as the bowel disease called necrotizing colitis (Loir *et al.* 2003). It is also known to cause sporadic diarrhea in children aged five years and below (Efuntoye and Adetosoye, 2004). Also, *Vibrio* species such as *Vibrio cholerae* causes cholera in humans. Other Vibrios causes sepsis or enteritis (Brooks *et al.* 2004). Also *Salmonella* species are known to cause enteric fevers

(typhoid and paratyphoid), which are caused by *Salmonella typhi* and *Salmonella paratyphi* respectively and septicemiasis which is caused by *Salmonella choleraesuis* (Brooks *et al.* 2004). *Listeria monocytogens* may cause meningoencephaltis with or without bacteraemia in humans (Brooks *et al.* 2004). In this study, the presence of these pathogenic microorganisms was screened for in samples obtained from slaughterhouses in Ogun state using conventional microbiological techniques.

2.Materials and Method

2.1 Materials

All materials and chemicals used in this investigation were of analytical grade and purchased from Hi-Media Pvt. Ltd, Mumbai, India.

2.2 Selection of Slaughterhouses

Two slaughterhouses were used in this study. These were Kara abattoir (Berger, Lagos, Nigeria) and Odo-eran abattoir (Abeokuta, Ogun, Nigeria). The choice of these slaughterhouses was majorly due to their high patronage by meat consumers (i.e., high commercial activities), and their close proximity to the laboratory (Redeemers' University, Ogun, Nigeria). Furthermore, the differences in the construction of these abattoirs played a crucial part to their eventual selection. Both abattoirs were local abattoirs as slaughtering of animals was done manually (not mechanically) on slaughtering pavements however, Odo - eran abattoir had tarred road unlike Kara abattoir which was covered with bare soil.

2.3 Collection of samples

Butchering tables and slaughtering pavements swabs, as well meat samples were aseptically collected from slaughterhouses. Sterile cotton swabs (Hi-Media Pvt. Ltd, PW009) were used to aseptically swab both tables and pavements after which the cotton swabs were placed into sample bottles (McCartney bottles) containing 10.0ml of pre-enrichment broth (peptone water)(Hi-Media, Pvt. Ltd, M028). Small cuts of meat were aseptically transferred into sample bottles as well. Samples were collected over a period of three (3) months, at an interval of 27days from each abattoir, and labeled appropriately. Samples were collected in duplicates.

2.4 Isolation of Bacteria

Samples were serially diluted according to standard protocol given by Atlas *et al.* (1995). Culture media used in this research work were Nutrient Agar (NA)-for isolating aerobic mesophilic bacteria (Hi-Media Pvt. Ltd, M1210), Violet red bile Agar (VRBA)-for enteric bacteria(Hi-Media Pvt. Ltd, M049A), Eosin-methylene blue Agar (EMBA)-for confirming *E.coli* (Hi-Media Pvt. Ltd, M317) and Salmonella-Shigella Agar (SSA)-for isolating *Salmonella* and *Shigella* (Hi-Media Pvt. Ltd, M108) and were prepared and sterilized according to their respective manufacturers instructions. Inoculation was done by spread plate method (Atlas *et al.* 1995). Incubation of samples was done at appropriate temperatures and duration (2-5days); NA-30^{0}C (Ercolini *et al.* 2009), VRBA-37^{0}C (Sawant *et al.* 2007) and SSA-43^{0}C (Kafel and Bryan, 1977). For anaerobic cultures anaerobic bags (Hi-media, Pvt. Ltd, LE008) were used. Sub-culturing of distinct colonies was done to obtain pure-cultures.

2.5 Identification of pure cultures

Identification of bacteria pure cultures was based on colony morphology examination, cellular morphology examination (by gram staining) (Atlas *et al.* 1995) and biochemical tests. Biochemical tests such as coagulase test, urease activity test, oxidase test, carbohydrate fermentation (sugar fermentation), catalase test, indole production test and citrate utilization test were all performed following the standard protocol given by Atlas *et al.* 1995.

3.Results and Discussion

The abattoirs in Ogun state, Nigeria were examined for the presence of pathogenic bacteria from slaughtered animals (meat) and the environment (Butchering tables and slaughtering pavements). Generally, the microbial load obtained from samples of Kara abattoir was higher than that of Odo-eran abattoir (Table 1 and Table 2). A total of 76 pure isolates were obtained from Kara abattoir while a total of 38 pure cultures were obtained from Odo-eran abattoir. Biochemical tests carried out on all pure cultures obtained from samples gotten from both abattoirs resulted in the identification of suspected species of *Enterobacter, Staphylococcus, Klebseilla, Streptococcus, Escherichia coli, Clostridium, Bacillus, Salmonella, Shigella, Proteus*, and *Pseudomonas*. Table 3 shows the summarized results of biochemical tests used in identifying these bacteria. From Kara abattoir, suspected species of *Enterobacter, Staphylococcus, Klebseilla, Streptococcus, Escherichia coli, Clostridium, Bacillus, Salmonella, Pseudomonas, Shigella* and *Proteus* were all isolated and identified while from samples collected from Odo-eran abattoir species of *Klebseilla, Streptococcus, Enterobacter, Pseudomonas, Escherichia coli, Proteus* and *Salmonella* were isolated and identified. In Kara abattoir, it was observed that of the 76 pure isolates obtained, *Escherichia coli* had the highest frequency of 16. This was followed by *Staphylococcus sp., Enterobacter sp.,* and *Klebsiella sp.* which had frequencies of 12, 11 and 9 respectively. *Pseudomonas sp*. had a frequency of 7 while *Salmonella sp*., *Bacillus sp*., and *Streptococcus sp.* all had a frequency of 5 respectively. Furthermore, *Clostridium* sp., *Shigella sp*., and *Proteus sp*. had the lowest frequencies of occurrence of 2 respectively. In Odo-Eran abattoir, it was observed that of the 38 pure isolates obtained, *Klebsiella sp.* had the highest frequency of occurrence of 9. *Streptococcus sp*., *Enterobacter sp*. and *Pseudomonas sp.* had frequencies of 6, 5 and 5 respectively. *Escherichia coli* and *Proteus sp.* had frequencies of occurrence of 4 while Shigella sp. had 3. Finally, *Salmonella* sp. had the lowest frequency of 2.

Table 1: Mean bacteria count of samples obtained from Kara abattoir

Parameter	Meat			Table			Pavement		
Medium, ^{o}C	NA,30	VRB,37	SSA,43	NA,30	VRB,37	SSA,43	NA,30	VRB,37	SSA,43
Sample 1(cfu)	380	41	35	265	34	23	245	29	22
Sample 2(cfu)	369	40	32	205	29	27	213	28	23
Mean	374.5	40.5	33.5	235	31.5	25	229	28.5	22.5
Dilution factor	10^{-6}	10^{-6}	10^{-6}	10^{-5}	10^{-5}	10^{-5}	10^{-4}	10^{-4}	10^{-4}
Volume, ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MBC, $cfuml^{-1}$	$7.5x10^{8}$	$8.1x10^{7}$	$6.7x10^{7}$	$4.7x10^{7}$	$6.3x10^{7}$	$5.0x10^{6}$	$4.6x10^{6}$	$5.7x10^{5}$	$4.5x10^{5}$

MBC: mean bacteria count, CFU: Colony forming unit

Table 2: Mean bacteria count of samples obtained from Odo-eran abattoir

Parameter	Meat			Table			Pavement		
Medium, ^{o}C	NA,30	VRB,37	SSA,43	NA,30	VRB,37	SSA,43	NA,30	VRB,37	SSA,43
Sample 1(cfu)	180	23	14	90	26	07	73	17	03
Sample 2(cfu)	153	17	10	121	20	09	70	14	06
Mean	166.5	20	12	105.5	23	08	71.5	15.5	4.5
Dilution factor	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-3}	10^{-3}	10^{-3}
Volume, ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MBC, $cfuml^{-1}$	$3.3x10^{6}$	$4.0x10^{5}$	$2.4x10^{5}$	$2.1x10^{6}$	$4.6x10^{5}$	$1.6x10^{5}$	$1.4x10^{5}$	$3.1x10^{4}$	$9.0x10^{3}$

MBC: mean bacteria count, CFU: Colony forming unit

Table 3: Summary of biochemical test used in identifying bacterial pure cultures obtained

Gram stain result	Shape	Growth in air	Acid from glucose	Gas from glucose	Acid from mannitol	Gas from mannitol	Acid from Lactose	Gas from Lactose	Catalase	Oxidase	Indole	Coagulase	Urease	Citrate	Suspected Bacteria
-	R	+	+	+	-	-	+	+	+	-	+	-	-	+	*Enterobacter sp.*
+	C	+	+	-	+	+	+	+	+	+	-	+	-	-	*Staphylococcus sp.*
-	R	+	+	+	-	-	+	+	+	-	-	-	+	+	*Klebseilla sp.*
+	*C*	+	+	-	-	-	-	-	+	+	-	-	-	-	*Streptococcus sp.*
-	R	+	+	+	-	-	+	+	+	-	+	-	-	-	*Escherichia coli*
+	R	-	+	+	-	-	-	-	-	-	-	-	-	-	*Clostridium sp.*
+	R	+	+	-	-	-	-	-	+	+	-	-	-	-	*Bacillus sp.*
-	R	+	+	+	-	-	-	-	+	-	-	-	-	+	*Salmonella sp.*
-	R	+	+	+	-	-	-	-	+	-	-	-	-	-	*Shigella sp.*
-	R	+	+	+	-	-	-	-	+	-	+	-	+	+	*Proteus sp.*
-	R	+	-	-	-	-	-	-	+	+	-	-	+	-	*Pseudomonas sp.*

Interpretation: (+) Positive test, (-) Negative test, (R) Rod- shaped, (C) Cocci- shaped

In this study, the predominant bacteria isolated were *Escherichia coli, Salmonella sp., Proteus sp., Klebseilla sp., Pseudomonas sp., Enterobacter sp., Streptococcus sp., Shigella sp., Staphylococcus sp., Bacillus sp.* and *Clostridium sp*. This is similar to that reported by Itah *et al.* (2005), where they observed in Uyo abattoir (Nigeria), that *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Staphylococcus epidermidis, Micrococcus roseus, Bacillus subtilis,* and species of *Streptococcus, Klebseilla, Pseudomonas* and *Salmonella* were isolated from meat samples. Also, Adesemoye *et al.* (2006) had earlier reported that *Bacillus sp., Clostridium sp.,* and *Pseudomonas aeruginosa* were predominant in some Nigerian abattoirs.

Six of the bacteria isolated from both abattoirs were members of the family *Enterobacteriacea* (*Escherichia coli, Enterobacter sp., Salmonella sp., Klebseilla sp., Shigella sp.* and *Proteus sp.*). This is not surprising as they are enteric organisms and would have come from the intestinal tract and fecal matters of the slaughtered animals (Jay, 2005). They are also likely to be present in the wash waters as reported by Adesemoye *et al.* (2006). Several studies have shown the pathogenic effect of most of these enteric microorganisms especially when consumed with poorly cooked meat such as barbequed meat (Loir *et al.* 2003). Thus the presence of these pathogenic microorganisms in samples obtained from these abattoirs pose a great threat to consumers of meat from these abattoirs in situations of undercooked meat. The ability of some of these pathogens such as *E.coli* to produce heat stable toxins is of great concern as it increases the possibility of consumers suffering from food poisoning. However, individuals who consume properly cooked meats are less likely to suffer from foodborne diseases associated with the presence of these pathogens (Jay, 2005). *Pseudomonas sp.* is a versatile microorganism, having varieties of enzymes that enable it to decompose a variety of compounds found in nature. *Pseudomonas sp.* are generally regarded as part of the microbiota of the soil (however, they are ubiquitous in nature), and its presence in meat samples as well as butchering table and slaughtering pavement indicates contaminations by soil. Although, *Pseudomonas sp.* are generally not known to cause any foodborne diseases, their presence in these samples from both abattoirs however, is

undesirable as it causes spoilage of meat and serve as an indication of soil contamination and as a result, possible contamination with known human pathogen present in the soil. Examples of such soil dwelling pathogens are *Clostridium sp.* and *Bacillus sp.* both of which were isolated in this study. *Clostridium sp.* is a common cause of gas gangrene and food poisoning as well as the bowel disease called necrotizing colitis (Loir *et al.* 2003). It is also known to cause sporadic diarrhea in children aged 5 years and below (Efuntoye and Adetosoye, 2004). *Bacillus sp.* is also known to cause food poisoning (*Bacillus cereus*) (Loir *et al.* 2003). It is however important to note that both of these soil dwelling pathogens were only isolated from Kara abattoir and not Odo-Eran abattoir. The possible reason for this is most likely due to the construction design of these abattoirs. Unlike Odo-eran abattoir where roads were properly tarred and proper drainage systems were constructed, Kara abattoir was un-tarred, lacking proper drainage and as a result, the animals, butchering equipments, wash water were all directly exposed to the soil thus it is most likely that these pathogens were from the soil, or slaughtering equipment (which must have come in contact with the soil), rather than the meat (slaughtered animal).

Food poisoning caused by *Staphylococcus* species is one of the most common causes of foodborne illness due to the widespread occurrence of *S. aureus* and the ability of many strains to produce enterotoxins (Jay, 2005). The presence of *S. aureus* in food and other food products usually indicates contamination by food handlers and in this case animal butchers that may be hosting the pathogen and releasing it the meat through skin lesions containing *S. aureus,* or releasing the pathogen into the meat either by sneezing or by coughing (Jay, 2005).

Salmonella sp. and *Shigella sp.* are known pathogen of humans usually caused when food contaminated with the appropriate strain (in significant numbers) is ingested resulting in food poisoning called Salmonellosis and Shigellosis (Jay, 2005). It has been established that eggs, poultry, meat and meat products are the most common food vehicles of Salmonellosis to humans (Jay, 2005) thus the presence of *Salmonella sp*. in samples obtained from both abattoirs is of great health concern. Foodborne shigellosis is a common foodborne disease and perhaps second only to those caused by *Staphylococcus* and *Salmonella* (Jay, 2005). The ability of some species of this pathogen to produce heat stable enterotoxins (Shigga toxins) has also led to numerous reported cases of food poisoning, especially when meat is poorly cooked or undercooked (Jay, 2005). Consequently, consumption of meat from these abattoirs, in undercooked form is hazardous. Furthermore, the presence of spoilage organisms isolated from meat samples of both abattoirs is also of great concern. In this study, known meat spoilers such as *Klebseilla sp., Bacillus sp., Pseudomonas sp*., *Clostridium sp*., and *Proteus sp.* were isolated from meat samples obtained from both abattoirs. It has been reported that species of *Pseudomonas* and *Bacillus* can cause surface slime; aerobic meat spoilage (Williams and Dennis, 2008). Furthermore, species of *Clostridium* has been seen to cause souring of meat (Williams and Dennis, 2008). Putrefaction which is an anaerobic decomposition of protein with the production of foul-smelling compounds such as H_2S can be caused by species of *Clostridium*, *Pseudomonas* and *Proteus* (Williams and Dennis, 2008).

4.Future Scope of Present Study

The present study is one with a major limitation as anti-microbial susceptibility of the pathogens isolated using known antibiotics, was not done due to time and financial constraints. Had this step been done, the information obtained would have shed more light on the possible risk of consuming antibiotic resistant pathogens in meat obtained from these abattoirs and with the advent of multi-drug resistance in various known human pathogens, the importance of this step in subsequent investigations of this manner is important.

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Author Profile

Kayode Tolulope Adeyemi, B.Sc. Microbiology (Redeemers' University, Nigeria), M.Sc. Microbiology (LPU, India).