Microbial Assessments of Raw Beef Meat Products from Market Sources in Benin City

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Abstract- This study was aimed at investigating the microbial composition of raw beef meats sourced from markets with a view to assessing thequality, quantity and diversity of microorganisms. The results of this study revealed that the total viable bacterial counts of raw beef meat ranged between $35.58\times10^3\pm04.56$ cfu/g and $48.00\times10^3\pm06.4$ cfu/g, while the fungal counts ranged between $10.36\times10^3\pm02.03$ cfu/g and $16.80\times10^3\pm03.37$ cfu/g. The mean microbial counts revealed that the total viable bacterial counts had its highest and least counts recorded in the open market and cold room while the fungal counts had its highest and least counts recorded in the abattoir and cold room. Eleven (11) bacterial and six (6) fungal isolates were identified from the market sources. Species of Penicillium, *Aspergillus niger*and *Aspergillus flavus* recorded the highest frequency of occurrence of 41.18%, 32.00% and 45.45% in the abattoir, open market and cold room respectively. The total viable bacterial counts and fungal counts revealed no significant difference ($p>0.05$) across the market sources. There were high levels of antibiotic susceptibility of the bacterial isolates to ciprofloxacin (CPX), gentamicin (CN) and ofloxacin (OFX), while most bacterial isolates were resistant to cloxacillin (APX), co-trimoxazole (SXT) and chloramphenicol (CH). The methicillin resistant status of all the bacterial isolates showed 100% susceptibility to methicillin (AAN). The plasmid profile result showed the bands of resistant bacterial isolates making them plasmid mediated. Therefore, microbial assessments of raw beef meat products should be encouraged by the relevant health sectors to checkmate microbial load in raw beef meats.

Index Terms- Raw beef meat, market sources, antibiotic susceptibility, plasmid profiles.

I. INTRODUCTION

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, it causes spoilage and food-borne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). Although the muscles of healthy animals do not contain microorganisms, meat tissues get contaminated during the various stages of processing ranging from slaughter to transportation (Ercolini *et al.*, 2006). A great diversity of microbes inhabits fresh meat, but different types may become dominant depending on pH, composition, textures, storage temperature and transportation means of raw meat (Ercolini *et al.*, 2006; Li *et al.*, 2006; Adu-Gyamfi *et al.*, 2012). Meat, a rich source of protein and fat, low in carbohydrate contents with sufficient water activity supports the growth of both spoilage and pathogenic bacteria. Major spoilage organisms in raw meat and poultry includes *Pseudomonas* spp., others are *Shewanella* sp., *Brochothrix* sp.and members of enterobacteriaceae. Growth of yeasts and molds is essentially slow on fresh meat as compared to bacteria; therefore, they are not major component of spoilage flora. Raw meat may harbour many important pathogenic microbes such as *Salmonella* spp., *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and to some extent, *Listeria monocytogenes* making the meat a risk for human health,as without the proper handling and control measures of these pathogens, food borne illnesses may occur (Nørrung *et al.*, 2009). Beef is processed in abattoir, and the design of abattoirs varies from person to person and from state to state including ownership but they are principally a place where livestock are slaughtered (Marriott, 2004). A number of slaughter facilities are found in an abattoir, whether stationary or mobile. These facilities could be a source of contamination to the slaughtering processes. It has been reported that abattoirs are not 100% hygienic (Gill *et al.*, 2005). Different factors could contribute to the contamination of beef products processed in abattoirs especially during processing and manipulations such as skinning, evisceration, storage and distribution at slaughter houses and retail establishment. In most developing countries, their traditional methods of handling, processing and marketing of meat undermine quality whereas poor sanitation leads to considerable loss of product as well as the risk of food-borne disease (Garcia, 2007). Bacteria which are responsible for food borne diseases contaminate meat directly and indirectly especially from animal excreta at slaughter process (Emswiller *et al*., 1976). They can also be transferred from beef-contact surfaces, utensils and other slaughtering equipment (Yen, 2003). The external contamination of meat constitutes a major problem in most developing countries'abattoirs where they are potential sources of infection as microbial surface contamination of carcasses has been repeatedly reported to have a significant effect on the meat shelf life of beef. Moreover, contaminants may also include pathogens such as *Salmonella* sp., *Vibrio cholerae*, *Escherichia coli* and *Listeria* sp. which can contaminate the meat thereby causing severe problem for consumers (Elmossalami, 2003). Faecal matter is a major

source of contamination and could reach carcasses through direct deposition as well as by indirect contact through contaminated and unclean carcasses equipment, surfaces, workers, installations and air (Burch *et al*., 2002). Fortunately, most of the bacterial colonies which have been isolated from beef carcasses are said to be non-pathogenic, except for few human pathogens such as *Salmonella* sp. *Campylobacter* sp. and *Listeria* sp. which have been isolated in a number of cases (Emswiller *et al.,* 1976).

The beef meat available for sales comes through a long chain of slaughtering and transportation from market sources (abattoir, open markets and closed markets), where each source may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environments are major factors contributing to bacterial contamination of meat (Gill *et al.*, 2000). Contaminations can be aggravated during transportation, storage and handling of meat at butcher shops. In accordance with HACCP (Hazard analysis critical control point), food safety requirements should be followed strictly to control the food-borne illnesses and to keep the microbial load of raw meat in checked. Meat is an important source of protein, but however can be an important source of human infection. About 75% of emerging human infectious diseases are thought to have come from animals, including wildlife. Considering the processing of meats from unhygienic environments in developing nations, this study therefore seek to investigate the microbial composition of meats sourced from markets, particularly those implicated in disease forms in humans. Infection is defined as the entry of harmful microbes into the body and its multiplication in the tissues / bloodstream (Jennie, 2001) resulting to complications. The infection may remain localized, subclinical and temporary if the body's defensive mechanisms are effective. A local infection may persist and spread by extension to become an acute, sub-acute or chronic clinical infection or disease state. The study also seeks to assay possible contaminants or disease-causing microbes and the healthy state of meat products for human consumption, to determine at what stage the meat products gets contaminated, either at the abattoir, at sales points or cold room as well as the virulent nature and possible way to check the menace. The aim of the study was to investigate the microbial composition of meats products from market sources with a view to assessing the quality, quantity and diversity of microorganisms in Benin City. The specific objectives of our study was to enumerate the microbial population of meat products, isolate, characterize and identify microorganisms from meat sources in Benin City, Nigeria, determine the antibiotic susceptibility pattern of the bacterial isolates and the frequency of occurrence of the microbial isolates, to determine the methicillin resistant status (susceptibility pattern) of the bacterial isolates and the plasmid profile of the bacterial isolates.

II. MATERIAL AND METHODS

This research work was carried out in Benin City, Edo State, Nigeria. Meat products were purchased from Bob Izua Slaughter House, Ikpoba Hill Market and Cold Room, all in Benin City. The experimental analyses of this research were carried out in Department of Microbiology, Faculty of Life Sciences, University of Benin, and the plasmid profiling of the bacterial isolates were carried out at the Molecular and Virology Laboratory of the International Institute of Tropical Agriculture, Ibadan. Beef meat samples were collected from Bob Izua Slaughter House, Ikpoba Hill Market and meat cold room in Ikpoba environs, Benin City. Samples were collected twice every month, for a period of six months between 9am and 11am. Slaughtering is usually carried out in the mornings, apart from samples which were collected from the closed markets, which are the leftover of meat sample from the sales of previous days and were preserved in refrigerators for the next day sales. Samples were collected on a market day and on a non-market day to check for the possible increase or decrease in the microbial load of the meat samples. The market sources include; the Abattoir (Bob Izua Slaughter House), Open Market (Ikpoba Hill Market, where the meats are kept in the tray), and Closed Market (where the meats are in the freezer/cold room), for example, a cold room. The meat products from the Abattoir are transported to the Open Market by vehicles or Wheel Barrows/Keke Nape. In the Open Market, meat products are sold from morning to evening, the leftover are kept in the freezer for the next day market sales. The leftover of the meat sample from the sales of previous days are preserved in refrigerators in the Closed Market for the next day sales. A total of 36 samples were collected from the market sources (Abattoir, Open Market and Closed Market) which comprises of 6 samples per month for the sampling period of 6 months.

A. Enumeration of Microorganisms**:**

Ten grams (10 g) of the raw beef meat samples was homogenized in a mortar and transferred to 90.0 ml of sterile distilled water. This served as the stock culture (Cheesbrough, 2000). From the stock suspension, 1.0 ml of the sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterilized distilled water to obtain 10^{-1} dilution. The contents were mixed thoroughly and further serial dilution was carried out to obtain 10^{-3} and 10^{-5} dilutions (Cheesbrough, 2000). Aliquot of 0.1 ml from the 10^{-1}, 10^{-3} and 10^{-5} dilutions was aseptically pipetted and transferred into correspondingly-labeled Petri dishes. This was followed by pouring of prepared, cooled but molten nutrient agar, MacConkey agar, blood agar and potato dextrose agar (oxoid) using pour plate techniques. The contents were gently swirled and allowed to solidify at room temperature (Ogunledun, 2007).

B. Preparation of Culture Media:

The following media was used in this study and was prepared according to manufacturer's instruction; nutrient agar, blood agar, MacConkey agar, potato dextrose agar and Muller Hinton Agar.

Microbiological Analyses:

The microbial isolates were characterized and identified based on the cultural, morphological and biochemical characteristic features according to the methods of (Cheesbrough 2000, 2003; Oyeleke *et al.,* 2008) and the methods of Bergey's Manual for Determinative Bacteriology (Buchanan *et al.,* 1974). A loopful of the culture was picked and inoculated into sterilized slant bottles containing prepared nutrient agar (oxoid) medium using streaking technique. The slants were incubated at 37ºC for 24-48 h after which Gram's staining, spore staining, biochemical tests and sugar fermentation tests were carried out to authenticate the identity of the microbial isolates. The cultural and morphology characteristics of the fungal isolates were identified with the features described by Barnett *et al.,* (1972).

Antibiotic Susceptibility Test:

The agar diffusion techniques as described by (Bauer *et al.,* 1966) were used for the test. Bacterial colonies from the pure culture were transferred into nutrient broth and incubated overnight, thereafter the test organisms were streaked on agar plates using sterile inoculating wire loop. The appropriate multi – disc depending on whether the test organism plated was Gram-negative organism or Gram-positive organism was then placed firmly onto the surface of the dried plates, using sterile forceps. The plates were kept at room temperature for one hour, which allowed diffusion of different antibiotics from the disc into the medium. The plates then incubated at 37^0C for 18 – 24 h. Record and interpretations of results were done using the zone sizes according to the methods of CLSI, (2005).

C. Methicillin Antimicrobial Susceptibility Test:

The methicillin antibiotic susceptibility pattern of the isolates was determined using Kirby-Bauer-NCCLS modified disc diffusion techniques (Cheesbrough, 2002). Standardized overnight culture of each isolate were flooded on the surface of Mueller Hinton agar (MHA) plates, the excess drained off, allowed to dry, while the lid was in plate. Standard methicillin antibiotic disc was aseptically positioned at a reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h. The plates in duplicates, were incubated at 37°C for 24 h, diameters of the zones of inhibition produced by the antibiotic disc were measured and recorded.

Isolation of Plasmid DNA and Agarose Gel Electrophoresis:

Plasmid isolation was carried out using the alkaline lysis method as described by Birnboim *et al.,* (1979). Agarose gel electrophoresis of the isolated plasmid DNA was carried out in tris-borate buffer system using 1.2% agarose, for 1 h at 75v. The plasmids DNA were isolated using lysing solution (200mM NaOH, 1% Sodium deodecylSulphate). The lysate was kept in ice for 30 min and centrifuged for 5 min, then phenol: chloroform (1:1) treatment was followed with the clear supernatant. Plasmid DNA were precipitated with equal volume of chilled isopropyl alcohol and DNA pellet was dissolved in 100 µl of TE buffer (diethylether). A 0.8% agarose gel was used to resolve DNA fragment and it was prepared by combining 0.8 g agarose in ten times concentration of Tris acetate ethylene diamine tetraacetate (10 ml 10XTAE) buffer and 90 ml distilled water in a 250 ml beaker flask and heating in a microwave for 2 min until the agarose was dissolved. A volume of 2.5 ml ethidium bromide (5.0 mg/ml) was added to the dissolved agarose solution with swirling to mix, the gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs were inserted. The gel was allowed to stand for 30 min and the casting comb was carefully removed after the gel had completely solidified. A one times concentration (IX) of Tris acetate ethylene diamine tetraacetate was added into the reservoir, till the buffer covers the agarose gel, 0.5 µl of gel tracking dye (bromophenol blue) was added to 20 µl of each sample with gentle mixing. Twenty (20) µl of the sample was then loaded onto the wells of the gel, the mini horizontal electrophoresis gel set-up was covered and the electrodes connected. Electrophoresis was carried out at 100 - 120 mA for 1 hr. At the completion of the electrophoresis, the gel was removed from the buffer and viewed at a wavelength of 260nm under a UV-light box. The band pattern of the DNA fragments was then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system. "The molecular sizes of each plasmid were determined by comparison with plasmids of known mass (Datta *et al.,* 1971).

D. Plasmid Curing Procedure:

The isolates that showed resistance to the antibiotics used were subjected to standard plasmid curing methods (Sijhary *et al.,* 1984). The isolates that showed resistance to some standard antibiotics due to presence of plasmids were selected and subjected to SDS plasmid curing treatments. Briefly, 1g of sodium dodecyl sulphate (SDS) was added to 100 ml of nutrient broth. The solution was autoclaved at 121 ^{0}C for 15 min, the pH was adjusted to 7.6, and thereafter steamed for 1 h. Fresh 24 h old cultures of the samples were standardized and 0.5 ml was pipetted into the 100 ml broth. Control broth containing no SDS was also subjected to similar treatments. Cultures were incubated with aeration at 37 ^{0}C for 24 hr. The cells including those from the control broth were then tested for antibiotic susceptibility. The reason for carrying out susceptibility test was to determine whether the eliminated plasmid was responsible for resistance to the used antibiotic.

E. Statistical Analyses:

The experimental procedure was done in triplicate. The mean values ± standard errors were calculated for both bacterial counts and fungal counts of the samples. The data obtained was analyzed using the parametric test of analysis of variance (ANOVA), at $P<0.05$ confidence limits for all parameters (Ogbeibu, 2005).

III. RESULTS

The results presented in Table 1 showed the mean microbial counts of meat from market sources. The results of total viable counts ranged from $48.00\times10^3\pm06.41$ to $35.58\times10^3\pm04.56$ cfu/g with the count recorded at the open market (B) showing the highest bacterial counts while cold room (C) had the lowest counts. Total coliform counts also ranged from $32.97\times10^3\pm04.58$ to $16.69\times10^3\pm03.60$. Total coliform counts for beef samples was least at cold room (C) and highest in open market (B). The staphylococcal counts of the raw beef meat samples ranged from $23.83\times10^3\pm03.66$ to $20.16\times10^3\pm03.29$ cfu/g with the counts recorded at open market (B) showing the highest counts and counts recorded at the cold room having the least counts. The fungal counts of raw beef meat ranged from $16.80\times10^3\pm03.37$ to $10.36\times10^3\pm02.03$ cfu/g. The highest fungal counts of raw beef meat were recorded at the abattoir while cold room had the least fungal counts. The coliform counts showed significant difference ($p<0.05$) in the mean microbial counts of meat samples in the open market. The total viable counts, staphylococcal counts and fungal counts revealed no statistically significant difference ($p>0.05$) across the market sources (Table 1).

Table 1: Mean microbial counts of raw beef meats from Market Sources.

	Market Sources		
Counts	Abattoir	Open Market	Cold Room
Total Viable Bacterial Counts	$43.75\times10^{3a}\pm06.41$	$48.00\times10^{3a}\pm06.41$	$35.58\times10^{3a}\pm04.56$
Coliform Counts	$24.80\times10^{3a}\pm03.84$	$32.97\times10^{3ab}\pm04.58$	$16.69\times10^{3a}\pm03.60$
Staphylococcal Counts	$20.88\times10^{3a}\pm03.63$	$23.83\times10^{3a}\pm03.66$	$20.16\times10^{3a}\pm03.29$
Fungal Counts	$16.80\times10^{3a}\pm03.37$	$16.47\times10^{3a}\pm02.65$	$10.36\times10^{3a}\pm02.03$

Values are mean ± SE; Colony forming unit per gram= cfu/g.

Mean bearing different superscript (a and b) between column differ significantly ($p<0.05$).

The bacterial isolates identified during this study were *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Streptococcus pyogenes*. Cultural, morphological and biochemical features of the bacterial isolates are presented on Table 2.

Table 2: Cultural, Morphological and Biochemical Features of the bacterial isolates

Features	1	2	3	4	5	6	7	8	9	10	11
Cultural											
Elevation	Convex	Flat	Convex	Low convex	Convex	Low convex	Convex	Convex	Convex	Convex	Low convex
Margin	Entire	Undulated	Entire	Entire	Entire	Entire	Entire	Entire	Smooth	Entire	Entire
Colour	Yellow	Cream	Cream	Cream	Cream	Cream	Red	Yellow	White	Cream-white	Cream
Shape	Circular	Irregular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Size	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Small	Small	Small	Medium
Morphological											
Gram staining	+	+	-	-	-	-	-	+	+	+	-
Cell type	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Cocci	Cocci	Cocci	Rod
Cell arrangement	Single	Chains	Single	Single	Single	Single	Single	Cluster	Chains	Cluster	Singles
Spore staining	-	+	-	-	-	-	-	-	-	-	-
Biochemical											
Catalase	+	+	+	+	+	+	+	+	-	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-
Coagulase	-	-	-	-	-	-	-	+	-	-	-
Urease	+	+	-	-	+	+	-	+	-	+	-
Indole	-	-	-	+	-	+	-	-	-	-	-
Cittrate	+	+	+	-	+	+	+	+	+	+	+
Sugar Fermentation											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	+	+	-	-	-	-	-	+
Possible Isolates	*Micrococcus* sp.	*B. subtilis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*Serratia* sp.	*S. aureus*	*S.pyogenes*	*S. epidermidis*	*E. aerogenes*

Key: + = Positive, - = Negative.

Key: F1= Fungal Isolate 1, F2= Fungal Isolate 2, F3= Fungal Isolate 3, F4= Fungal Isolate 4, F5= Fungal Isolate 5, F6= Fungal Isolate 6.
A total of six (06) fungal isolates were isolated from the raw beef meat samples obtained from abattoir, open market and cold room. The fungal isolates are *Penicillium* sp., *Aspergillus flavus*, *Mucor* sp., *Saccharomyces* sp., *Rhizopus stolonifer*, and *Aspergillus niger*. Cultural and microscopic features of the fungal isolates are shown on Table 3.

Table 3: Cultural and Microscopic Features of the fungal isolates

Features	F1	F2	F3	F4	F5	F6
Cultural	White fluffy colony with reverse side cream	Black fluffy colony with reverse side yellow	Greenish yellow colonies with reverse side yellow	White flat colony with reverse side cream	Medium creamy colony with convex elevation and entire margin	Greenish colony with white periphery
Microscopic						
Nature of hyphae	Non-Septate	Septate	Septate	Non-septate	Pseudohyphae	Septate
Colour of spore	Cream	Brown	Yellow	Cream	Cream	Greenish
Type of spore	Sporangiophore	Conidiophores	Conidiophores	Sporangiophore	Chlamydospore	Conidiophores
Appearance of special structure	Rhizoid	Foot cells	Foot cells	Sporangium	Budding	Brush-like conidia
Possible isolates	*Rhizopus* sp.	*Aspergillus niger*	*Aspergillus flavus*	*Mucor* sp.	*Saccharomyces* sp.	*Penicillium* sp.

Table 4 shows the frequency of distribution of bacterial isolates, with *Staphylococcus aureus* showing the highest frequency of occurrence (18.07%), while *Serratia marcescens* had the least frequency (02.41%) at the open market.

Table 4: Percentage Frequency of occurrence (%) of bacterial isolates

	Market Sources		
Isolates	Abattoir (A)	Open Market (B)	Cold Room (C)
Staphylococcus aureus	10 (19.61)	15 (18.07)	08 (25.00)
Escherichia coli	11 (21.57)	13 (15.66)	05 (15.63)
Pseudomonas aeruginosa	07 (13.73)	11 (13.25)	09 (28.13)
Streptococcus pyogenes	05 (09.80)	09 (10.84)	Nil
Staphylococcus epidermidis	03 (05.88)	08 (09.64)	05 (15.63)
Bacillus subtilis	04 (07.84)	07 (08.43)	Nil
Micrococcus luteus	Nil	06 (07.23)	05 (15.63)
Klebsiella pneumonia	03 (05.88)	05 (06.02)	Nil
Enterobacter aerogenes	08 (15.69)	04 (04.82)	Nil
Proteus vulgaris	Nil	03 (03.61)	Nil
Serratia marcescens	Nil	02 (02.41)	Nil
Total	51 (100)	83 (100)	32 (100)

Table 5 shows the frequency of occurrence of fungal isolates with *Aspergillus niger* showing the highest frequency of occurrence (32.0%) and *Saccharomyces* sp. having the least frequency (4.0%).

Table 5: Percentage Frequency of occurrence (%) of fungal isolates

	Market Sources		
Isolates	Abattoir (A)	Open Market (B)	Cold room (C)
Aspergillus niger	05 (29.41)	08 (32.00)	03 (27.27)
Mucor sp.	02 (11.76)	05 (20.00)	01 (09.09)
Rhizopus stolonifer	03 (17.65)	05 (20.00)	Nil
Aspergillus flavus	Nil	03 (12.00)	05 (45.45)
Penicillium sp.	07 (41.18)	03 (12.00)	02 (18.18)
Saccharomyces sp.	Nil	01 (04.00)	Nil
Total	17 (100)	25 (100)	11 (100)

Table 6 shows the results obtained from the antibiotic susceptibility pattern of the bacterial isolates before curing. It revealed that *M. luteus, S. aureus, Serratia sp.* and *E. aerogenes* were sensitive to gentamycin. Again, *M. luteus, S. pyogenes, S. epidermidis, Serratia sp., P. aeruginosa, E. coli, K. pneumonia* and *E. aerogenes* were sensitive to ciprofloxacin. However, *M. luteus, B. subtilis S. aureus, S. pyogenes, S. epidermidis, Serratia sp., P. vulgaris, P. aeruginosa, E. coli, K. pneumonia and E. aerogenes* were revealed to be resistant to pefloxacin, amoxacillin and streptomycin.

Table 6: Zone of inhibition of commercial antibiotics (standard sensitivity disc) in mm on the bacterial isolates prior to curing

Gram+ve	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
M. luteus	0.0(R)	20.0(S)	0.0(R)	13.0(I)	10.0(R)	10.0(R)	19.0(S)	11.0(I)	0.0(R)	18.0(S)
B. subtilis	0.0(R)	17.0(I)	0.0(R)	0.0(R)	0.0(R)	0.0(R)	15.0(I)	08.0(R)	0.0(R)	24.0(S)
S. aureus	0.0(R)	18.0(S)	0.0(R)	0.0(R)	0.0(R)	0.0(R)	13.0(I)	0.0(R)	0.0(R)	15.0(I)
S. pyogenes	0.0(R)	15.0(I)	0.0(R)	0.0(R)	0.0(R)	0.0(R)	20.0(S)	0.0(R)	0.0(R)	20.0(S)
S. epidermidis	0.0(R)	16.0(I)	0.0(R)	0.0(R)	0.0(R)	0.0(R)	18.0(S)	0.0(R)	0.0(R)	13.0(I)
G-ve	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Serratia sp.	0.0(R)	0.0(R)	12.0(I)	22.0(S)	0.0(R)	0.0(R)	19.0(S)	0.0(R)	20.0(S)	0.0(R)
P. vulgaris	0.0(R)	0.0(R)	0.0(R)	12.0(I)	0.0(R)	0.0(R)	16.0(I)	0.0(R)	17.0(I)	0.0(R)
P. aeruginosa	0.0(R)	0.0(R)	0.0(R)	18.0(S)	0.0(R)	0.0(R)	10.0(R)	0.0(R)	22.0(S)	0.0(R)
E. coli	0.0(R)	0.0(R)	0.0(R)	20.0(S)	0.0(R)	0.0(R)	16.0(I)	0.0(R)	24.0(S)	0.0(R)
K. pneumoniae	0.0(R)	0.0(R)	0.0(R)	24.0(S)	0.0(R)	0.0(R)	14.0(I)	0.0(R)	26.0(S)	0.0(R)
E. aerogenes	0.0(R)	0.0(R)	0.0(R)	18.0(S)	0.0(R)	0.0(R)	20.0(S)	0.0(R)	28.0(S)	0.0(R)

KEY: PEF = Pefloxacin (10µg), CN= Gentamycin (10µg), CH= Chloramphenicol (30µg), APX= Cloxacillin (30µg), Z= Zinnacef (20 µg), AM= Amoxacillin (30µg), AU= Augmentin (30µg), R= Rocephin (25µg), OFX= Tarivid/Ofloxacin (10µg), CPX= Ciprofloxacin (10µg), S= Streptomycin (30µg), SP= Sparfloxacin(10µg), SXT= Co-trimoxazole (30µg), E= Erythromycin (10µg). S= Susceptible, R= Resistant, I = Intermediate, R= < 11 mm, I =11 – 17 mm, S= ≥ 18 mm.

Table 7 shows the results obtained from the antibiotic susceptibility pattern of the bacterial isolates after curing. After curing, it was observed that Erythromycin and Tarivid became sensitive (effective) against multidrug resistant bacterial isolates. The loss of resistance after curing indicates the presence of the resistance properties in the plasmid. However, *Serratia sp., P. vulgaris, P. aeruginosa, E. coli, K. pneumonia and E. aerogenes* were resistant to amoxacillin, co-trimoxazole and augmentin suggesting that the resistant properties shown by bacterial isolates were not plasmid mediated.

Table 7: Zone of inhibition of commercial antibiotics (standard sensitivity disc) in mm on the microbial isolates after curing

Gram+ve	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
M. luteus	19.0(S)	24.0(S)	16.0(I)	18.0(S)	15.0(I)	18.0(S)	21.0(S)	15.0(I)	13.0(I)	20.0(S)
B. subtilis	11.0(I)	20.0(S)	12.0(I)	0.0(R)	0.0(R)	0.0(R)	18.0(S)	11.0(I)	10.0(R)	28.0(S)
S. aureus	15.0(I)	22.0(S)	11.0(I)	0.0(R)	0.0(R)	13.0(I)	16.0(I)	10.0(R)	11.0(I)	20.0(S)
S. pyogenes	12.0(I)	20.0(S)	15.0(I)	10.0(R)	14.0(I)	10.0(R)	24.0(S)	16.0(I)	10.0(R)	30.0(S)
S. epidermidis	15.0(I)	16.0(I)	11.0(I)	13.0(I)	10.0(R)	14.0(I)	22.0(S)	18.0(S)	12.0(I)	26.0(S)
G-ve	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Serratia sp.	10.0(R)	13.0(I)	18.0(S)	26.0(S)	05.0(R)	10.0(R)	22.0(S)	10.0(R)	24.0(S)	13.0(I)
P. vulgaris	07.0(R)	10.0(R)	10.0(R)	18.0(S)	0.0(R)	0.0(R)	20.0(S)	13.0(I)	20.0(S)	14.0(I)
P. aeruginosa	06.0(R)	14.0(I)	10.0(R)	22.0(S)	04.0(R)	0.0(R)	15.0(I)	10.0(R)	25.0(S)	0.0(R)
E. coli	10.0(R)	19.0(S)	15.0(I)	25.0(S)	06.0(R)	0.0(R)	21.0(S)	15.0(I)	28.0(S)	17.0(I)
K. pneumoniae	08.0(R)	13.0(I)	11.0(I)	28.0(S)	0.0(R)	08.0(R)	18.0(S)	11.0(I)	28.0(S)	13.0(I)
E. aerogenes	10.0(R)	15.0(I)	13.0(I)	22.0(S)	10.0(R)	0.0(R)	24.0(S)	12.0(I)	30.0(S)	10.0(R)

KEY: PEF = Pefloxacin (10µg), CN= Gentamycin (10µg), CH= Chloramphenicol (30µg), APX= Cloxacillin (30µg), Z= Zinnacef (20 µg), AM= Amoxacillin (30µg), AU= Augmentin (30µg), R= Rocephin (25µg), OFX= Tarivid/Ofloxacin (10µg), CPX= Ciprofloxacin (10µg), S= Streptomycin (30µg), SP= Sparfloxacin (10µg), SXT= Co-trimoxazole (30µg), E= Erythromycin (10µg). S= Susceptible, R= Resistant, I= Intermediate, R= < 11 mm, I = 11 – 17 mm, S= ≥ 18 mm.

The antimicrobial sensitivity testing (Table 8) of the bacterial isolates from the meat samples using some broad and narrow spectra antibiotics showed 100% susceptibility (sensitive) to some antibiotics like ciprofloxacin, gentamicin, ofloxacin, while most of the bacterial isolates were found to be resistant to ampiclox, septrin and chloramphenicol.

Table 8: Antibiotic susceptibility pattern using single disc methicillin drug

S/N	Microbial isolates	Zone of inhibition in mm	Susceptibility pattern
1	*M. luteus*	31.0	S
2	*B. subtilis*	30.5	S
3	*S. aureus*	23.0	S
4	*S. pyogenes*	31.5	S
5	*S. epidermidis*	31.0	S
6	*Serratia* sp.	33.0	S
7	*P. vulgaris*	30.5	S
8	*P. aeruginosa*	28.0	S
9	*E. coli*	30.0	S
10	*K. pneumonia*	31.0	S
11	*E. aerogenes*	32.0	S

Key: S=Susceptible

The result of the plasmid curing revealed that resistance to some commonly used antibiotics such as septrin, streptomycin and ampiclox by the bacterial isolates were plasmid mediated.

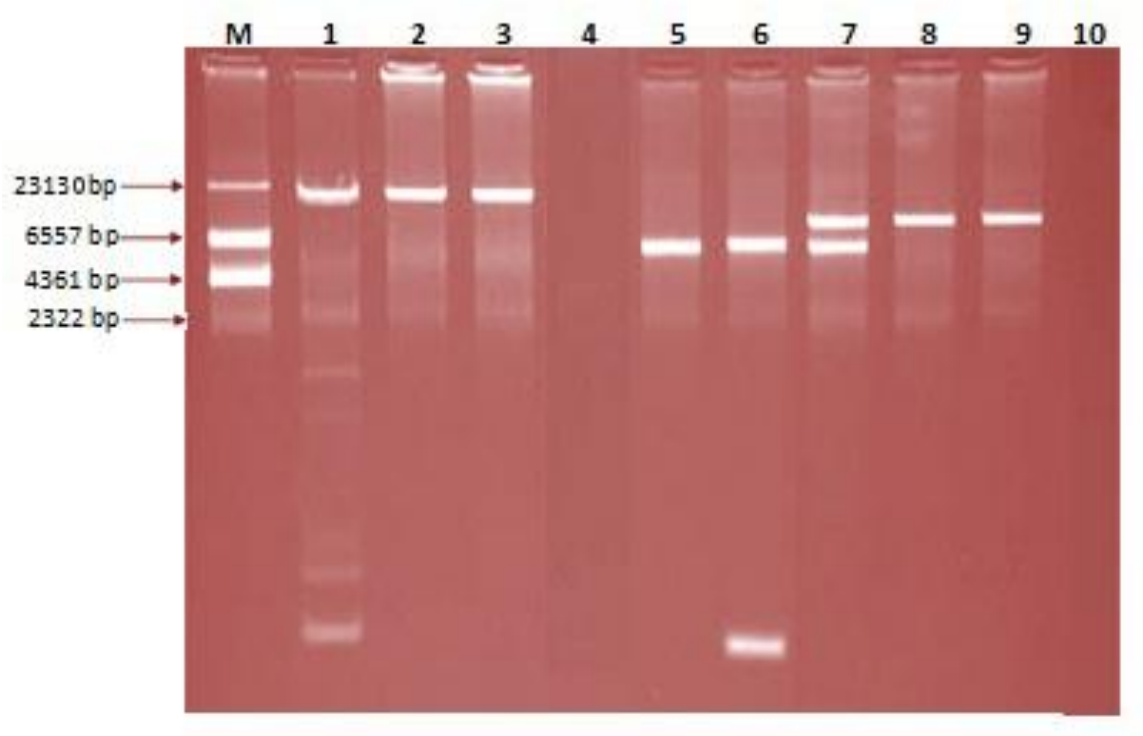

Plate 1: Agarose gel electrophoresis of plasmids harbored by the bacterial isolates. B_1= *Staphylococcus aureus*, B_2= *Bacillus subtilis*, B_3= *Escherichia coli*, B_4= *Enterobacter* sp, B_5= *Micrococcus luteus*, B_6= *Streptococcus* sp., B_7= *Pseudomonas aeruginosa*, B_8= *Staphylococcus epidermiidis*, B_9 = *Proteus* sp., B_{10}= *Serratia* sp.

IV. DISCUSSION

Most of fresh food especially that of animal origin like beef is highly vulnerable to microbial invasion and food poisoning since meat is an ideal medium for growth of a number of microorganisms due to its nutritive value (Soyiri *et al.,* 2008). Meat samples from abattoir, open market and cold room yielded marked growth of bacteria and fungi. The presence of these organisms in raw beef meats could mean that these meats contain essentially all nutrients needed for bacteria proliferation in adequate quantity. High microbial load revealed in the raw beef meat from abattoir and open market in this study may be due to the condition of handling, the tables, equipment and machines, which had been used during processing, preparing, and the hygienic practice, employed by butchers.

Adzitey *et al.,* (2011b), reported that the butchering activity is more dominated by the youth and middle-aged men who are more energetic as the butchering business requires much physical strength. During the study, it was revealed that, the abattoir and open market workers had low level of education and this could make difficult in acceptability of modern slaughtering practices as well as adherence to strict hygienic and standard slaughtering practices that contribute to microbial contamination.

It was also discovered that abattoir was often congested with people who were not directly involved with slaughtering and processing of the carcasses which may contribute to contamination of meat with microbes. Most of the vehicles used to transport meat from abattoir to retail meat outlets were taxi, tricycles, wheel barrow and various types of vehicles. The meats were carried in sack bags, open bowls and basins, as well as with bare hands. Sometimes abattoir workers stepped on the meat. This may also contribute to bacterial contamination of meat. The slaughtering of beef carcasses on the bare floor as result of lack of hoists in the abattoir also created room for contamination. Adeyemo *et al.,* (2009), found that the animals were often slaughtered and eviscerated on the floor because of the absence of mechanical or manual hoists a factor which contributed to a major source of contamination.

James *et al.,* (2005), reported that the longer meat surface is exposed to the environment, the higher the microbial load. The meat samples from open market had the highest mean of total viable counts, followed by those from abattoir and finally the meat from cold room had least contamination. The low microbial load in raw beef from cold room could be due to the effect of cooled storage in the refrigerator which was not conducive for microbial proliferation and growth. When comparing the microbial counts of raw meats purchased from the three market sources, results revealed that the open market had the highest bacteria counts ($48.00\times10^3\pm06.41$cfu/g) for total viable bacterial counts. The high total viable bacteria counts recorded in this study showed the microbial diversity (differences in form or species) in the market sources, condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination.

Fresh meats sold to the public in the open market were grossly contaminated with coliform bacteria ($32.97\times10^3\pm04.58$ cfu/g) as well as other bacterial forms. However, no significant difference was found between the mean total coliform counts of samples in abattoir and cold room. The mean staphylococcal counts and fungal counts of raw meat samples recorded no significant difference among the three market sources. According to statistical analysis, it was noted that the microbiological quality of most of the raw meat and meat products of Benin City was significantly poor. The finding showed that raw meat and meat products, which were collected from different market sources, were contaminated with pathogenic microorganisms. A total of eleven bacterial isolates covering nine genera were isolated and characterized as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus epidermidis, Bacillus subtilis, Micrococcus luteus, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus vulgaris* and *Serratia marcescens.*

The predominance of *Escherichia coli* (21.57%) in the abattoir, *Staphylococcus aureus* (18.07%) in the open market and *P. aeruginosa* (28.13%) in the cold room were attributed to the handling process such as killing equipment and the water used in washing the meat. Other researchers have reported the presence of *Pseudomonas*, *Escherichia coli* and other gram-negative bacteria in raw meat samples (Lin *et al.,* 2004). No *Salmonella* and *Shigella* was determined, this may be as a result of some preventive measures adopted by the meat handlers to prevent contamination.

In contrast to the results of this study, Hayes *et al.,* (2003) found *Enterococcus spp.* to be the most dominant bacteria on 971 of the 981 samples (99%) of all meat (chicken, turkey, pork and beef) in the state of Iowa. Although most of organisms found in this study are normal flora of different parts of man and animal body, some of them have been associated with many disease problems. They might cause disease in their presence in the animal body or by contamination of food. The micro-flora of meats available to consumers is the total sum of microorganisms acquired during processing of animal muscle food. Animal health, dressing skills, personnel hygiene, abattoir cleanliness, and adequate storage and holding temperature during distribution and retail influence the constitution and number of micro-organisms present (Hudson *et al.,* 1996).

This study agrees with Bhandare *et al.* (2007), due to improper/unhygienic handling and processing of the meat, meat is normally transported to the markets in unhygienic meat vans, taxis, motor cycles, motor kings, and sometimes on bicycles. Meat sellers were also observed busily conversing, coughing, and sneezing, which might result in contamination through introduction of saliva on the meat. Okonko *et al.,* (2008) stated that, food can be infected with microorganisms as a result of "coughing" and "sneezing" from those who handle and process these foods. Koffi-Nevry *et al.,* (2011) also stated that, careless sneezing and coughing among butchers can result in microbial contamination of raw beef.

Findings from this study revealed the open market as the most major source of meat contamination in Benin City. During selling in retail meat outlets further contamination can occur through contact with handling equipment (tables, logs, hooks, balances and knives), insects, air, personnel and even consumers (Mtenga *et al.,* 2000). Meat sold to the public in open markets or by streets vendors are grossly contaminated with coliform bacteria as well as other bacterial forms. The finding of this study revealed that meat was contaminated with pathogenic Gram-positive and Gram-negative bacteria. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards,

"since improper handling and improper hygiene might lead to the contamination of meat and this might eventually affect the health of the consumers (Okonko *et al.*, 2008, 2009 a, b). It is, therefore, suggested that meat processors and sellers should be educated on the adverse effect of contamination. However, the processors, handlers and sellers should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and fecal contamination of fresh meats and meat products.

The presence of these organisms in meat should receive particular attention, because their presence indicates public health hazards and give warning signal for the possible occurrence of food borne intoxication (Kabir, 2009). The susceptibility results of bacteria isolated from meat samples showed that they are highly resistant to some of the antibiotics tested such as cloxacillin, amoxacillin and co-trimoxazole. Gram-negative organisms are more resistant than the Gram-positives; this is expected because of intrinsic nature of Gram-negative cell wall. The Gram-negative micro-organisms isolated belongs to the *Enterobacteriaceae* family, this group of organisms are always resistant to various classes of antibiotics. They are known to harbour series of antibiotic resistance genes which can be transferred horizontally to other bacteria species. Their resistance to the cephalosporins may be due to the production of beta lactamase enzymes, these enzymes are known to inactivate antibiotics especially the beta lactams. Resistance observed in other antibiotics classes may also be by other mechanisms which may be by drug efflux where drug are forcefully pumped out of the cell thereby allowing a sub-inhibitory concentration to penetrate the cell wall of this organisms, it may be as a result of a point mutation that has occurred in these bacteria thereby allowing the organism to acquire additional structure that will inhibit drug action.

S. aureus showed some level of intermediate susceptibility to erythromycin, even at that, it still possesses some public health problems because when these antibiotics are misused, this organism will develop resistance against it. *Bacillus subtilis* was resistant to most of the antibiotics tested. The results of the curing experiment revealed that majority of bacterial isolates carried the antibiotics genes on transmissible plasmids. The plasmids analysis revealed that there were detectable plasmid DNA genes in Isolates 1, 2, 3, 5, 6, 7, 8 and 9. The plasmids DNA genes isolated in the 0.8% Agarose gel Electrophoresis might be the bacterial contents that confer plasmid resistance on the bacterial isolates. This is in accordance with the result of this study. The plasmid profile analysis examines the total bacterial plasmid content and is separated by electrophoresis. "This method is used to assess the spread of antibiotics plasmid DNA resistance determinates (Clowers, 1972). The use of antibiotics in growing animals and treating bacterial infections could be the reason for the observed resistance, which has a range of human health consequences ranging from protracted illness to death, as endangered patients are those with impaired immunity. In order to produce meat which is safe and wholesome there is a need for use of good animal husbandry practices, good processing practices, good handling practices, standard operating procedures, sanitation standard operating procedures and hazard analysis critical control point system along the beef production chain. The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is therefore emphasized and recommended to reduce possible contamination (Okonko *et al.*, 2009 a, b). The result of this study provides information to existing data on microbial contaminants of meat products. The data generated from this study revealed the contamination status of meat in abattoir, open market and cold room. This study showed that MRSA testing of meat samples is highly effective against microbial contaminants. This study also demonstrates the role of raw beef food acting as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem. This study has provided comparative data on the susceptibility of the bacterial isolates to commonly used antibiotics. Training and guidance programs should be set-up in order to develop awareness among butchers and meat sellers. Education and application of control measures suitable to prevent meat contamination, from slaughtering to selling, through to final consumption is highly recommended. To always present quality meat, health - and standards authorities should keep follows up through testing of samples and executing of regular studies. Good hygienic standard at the various meat sources (abattoir, open market and cold room) should be maintained. It is highly recommended to eat meat after proper cooking.

V. CONCLUSION

The result of this study revealed that fresh meats were contaminated with bacteria and fungi. The presence of pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli* among others, encountered in fresh meat from conventional beef is alarming. The presence of these organisms in meat foods should receive particular attention, because their presence indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication.

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