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ANTIBIOGRAM OF MEATBORNE PATHOGENS ISOLATED FROM INTERMEDIATE MOISTURE GOAT MEAT

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ABSTRACT

Antibiotic resistance food borne pathogens in recent times has become a major problem in healthcare. In this study, antibiotic resistant bacteria namely*; Brucella Melitensis, Klebsiella Pneumonia, Micrococcus varians, Staphylococcus aureus, Pseudomonas fragi, Enterobacter amnigenus, Salmonella arizonae, Bacillus cereus, Escherichia blattea* and *Leminorella richardii* were isolated from intermediate moisture goat meat using standard procedures. Antibiotic sensitivity tests showed significant resistance towards ciprofloxacin, septrin, ceftriazone, penicillin, gentamycin and erythromycin. The total viable bacterial counts plated on three (3) different culture media were Nutrient agar ($1.25x10^7$ cfu/g), MacConkey agar ($8.0x10^7$ cfu/g) and Mannitol Salt agar ($4.0x10^7$ cfu/g) respectively. All bacteria isolated from dried goat meat with the exception of *Leminorella richardii, Bacillus cereus* and *Enterobacter amnigenus* were resistant to the antibiotic ceftriazone. *Klebsiella pneumonia* and *Micrococcus varians* were found to be resistant to ciprofloxacin. All isolates were resistant to septrin except *Staphylococcus aureus, Leminorella richardii* and *Enterobacter amnigenus*. *Brucella melitensis, Klebsiella pneumonia, Pseudomonas fragi, Escherichia blattea, Bacillus cereus* and *Enterococcus amnigenus* showed resistance to penicillin. All bacterial isolates were resistant to erythromycin except *Leminorella richardii*. Resistance of microorganisms to antibiotics has led to a considerable market loss in the Pharmaceutical and clinical industries. The use of other antimicrobials of biological origin could be screened and used against a wide range of antibiotic resistant bacteria. The use of antibiotics in animal feeds should be discouraged, while good hygiene and infection control is recommended.

KEYWORDS: Antibiotics, Meat, Resistance, Pathogens, Infection.

INTRODUCTION

Antibiotic resistant bacteria are bacteria that are not controlled or killed by antibiotics. They are able to survive and even multiply in the presence of antibiotics. The use of antibiotics is a leading treatment method for bacterial infectious diseases (McGeer, 1998). It is widely accepted that antibiotic resistant pathogens make clinical treatment more difficult (Takafuji, 1977). Antibiotic resistance is a growing problem among humans and wildlife in terrestrial or aquatic environments. In this regard, the spread and contamination of the environment especially through "hot spots" like hospital waste-water and untreated urban wastewater is a growing and serious public health problem (Levy, 2002; Marti et al., 2014). Bacteria often develop resistance due to gradual exposure to the therapeutic concentrations of pharmaceutical antibiotics. Resistance of bacteria to antibiotics arises through one of the following mechanisms; natural resistant genes through horizontal gene transfer in bacterial plasmids, synthesis of microbial enzymes which deactivates the drug active ingredient pumps as well as adaptive resistance in the abuse and misuse of drugs (Levy, 2006)

The transfer of antibiotic resistant bacteria to humans is mostly through the consumption of food particularly those of animal origin due to the frequent use of antibiotics in animal feeds (Huda et al; 2010). This exposes a gradual concentration of these antibiotics on meat borne microorganisms thus promoting resistance. When these meat or meat products are eaten or improperly processed, they often transfer resistance against pharmaceutical antibiotics (David *et al*, 2001).

MATERIALS AND METHODS

Sample collection: Fresh goat meat was purchased at Goldie market, Calabar, Cross River State and transported to Microbiology laboratory University of Calabar for further analysis. Ten (10) grams of the meat containing 100% moisture was cut off using a sterile stainless knife, oven dried at 60^0C for 30 mins to 2g. Eighty percent (80%) of moisture was removed from the

meat to get an intermediate meat sample with 20% moisture and kept at room temperature for 3 days.

Total heterotrophic bacterial count: A two (2) gram weight of dried goat meat was homogenized in 8 mls of buttered peptone water using a sterile electric blender. A serial dilution factor of 10^5 was pour-plated in 15 mls of Nutrient agar, Mannitol Salt Agar and MacConkey Agar respectively in petri dishes and incubated at 37^0C for 24 hours. Viable isolates were purified by sub-culturing, stocked in MacCartney bottles and stored at 40^0C for further characterization.

Standardization of bacterial isolates: Standard stock of the bacterial isolates for disc sensitivity test was prepared by suspending a loopful of each microorganism in about 10 mls nutrient broth. After incubation at 37^0C for 8 hours, the turbidity was adjusted to be visually comparable with 0.5 McFarland standard bacteria giving inoculm size $1x10^8$ cfu/ml.

Characterization of bacteria: This is a preliminary method in the identification of bacterial isolates. It identifies bacteria based on their morphological and structural differences such as pigmentation, shape, size, arrangement, edge, margin, texture, appearance etc. Gram staining as well as other biochemical tests such as catalase, coagulate motility, indole, citrate, oxidize and triple sugar iron agar test were also carried out to characterize and identify the bacterial isolates.

Antibiotic Disc Susceptibility Testing: The plates were inoculated by dipping a swab stick into the suspension of Mueller Hinton agar and streaking across the medium (Harper, 1999). Each bacterial isolates were propagated on 10 mls nutrient broth in test tubes for 8 hours at 37^0C to produce inoculum size of $1x10^8$ cfu/ml and the suspension was made in sterile normal saline and adjusted equivalent to barium sulphate standard. A 0.01ml of prepared standard inoculum was cross streaked on Mueller Hinton agar and incubated at 37^0C for 24 hours. A Whitman filter paper nos. 3 impregnated with antibiotics namely: penicillin (10µg) erythromycin (15µg), septrin, ceftriazone (30µg), ciprofloxancin (30µg) and gentamycin (10µg) were placed evenly on each of the seeded plates and incubated overnight. The formation of zones of inhibition and no zones of inhibition were indicative of bacterial growth susceptibility and interpreted using the Kirby Bauer's chart as "Resistant", "intermediate" or "sensitive".

RESULTS

Table 1: Enumeration of total plate counts of bacterial isolates from goat meat sample Enumeration of total bacterial counts in 3 different culture media.

Culture Media	Colony	Dilution factor	Total viable count (cfu/ml)
Nutrient agar	125	10^{-5}	1.25×10^7
MacConkey agar	80	10^{-5}	8.0×10^7
Mannitol salt agar	40	10^{-5}	4.0×10^7

Cfu/mL^{-1} is given as: No. of colony x plating factor x dilution factor$^-$

Isolate	Gram stain	Pigmentation	Shape	Arrangement	Margin	Elevation	Texture code
$Dgmb_1$	Positive	Creamy	Cocci	Clusters	Continuous	Convex	Slimy
$Dgmb_2$	Negative	No pigment	Rod	Singly	Continuous	Convex	Slimy
$Dgmb_3$	Negative	Translucent	Rod	Chains	Continuous	Convex	Slimy
$Dgmb_4$	Negative	Creamy	Rod	Singly	Not continuous	Flat	Slimy
$Dgmb_5$	Positive	No pigment	Rod	Singly	Irregular	Flat	Slimy
$Dgmb_6$	Negative	Creamy	Rod	Singly	Irregular	Flat	Dry
$Dgmb_7$	Positive	Whitish-yellow	Cocci	Singly	Irregular	Concave	Slimy
$Dgmb_8$	Positive	Creamy	Cocci	Singly	Continuous	Flat	Slimy
$Dgmb_9$	Negative	Yellow	Rod	Singly	Continuous	Flat	Slimy
$Dgmb_{10}$	Positive	Cream	Cocci	Singly	Continuous	Flat	Slimy
$Dgmb_{11}$	Negative	Cream	Rod	Singly	Irregular	Flat	Slimy
$Dgmb_{12}$	Negative	Cream	Rod	Singly	Irregular	Concave	Dry
$Dgmb_{13}$	Positive	Creamy	Cocci	Clusters	Continuous	Convex	Slimy

Key: Dgmb – Dried goat meat bacteria.

Table 3: Biochemical tests This table shows the biochemical characteristics of isolates.

Isolate code	Glucose	Sucrose	lactose	H_2S	Gas	Motility	Indole	Ornithine	Citrate	Coagulase	Catalase	MR	VP	Oxidase	Bacteria suspected
Dgmb1	+	+	+	-	-	-	-	+	-	+	+	-	+	-	*Staphylococcus aureus*
Dgmb2	+	+	+	-	+	-	-	+	d	-	+	+	-	-	*Escherichia blattea*
Dgmb3	+	-	-	+	d	-	-	+	-	-	+	-	+	-	*Leminorella richardii*
Dgmb4	+	-	-	+	-	-	-	+	-	-	+	-	+	+	*Brucella melitensis*
Dgmb5	+	-	-	-	+	+	-	+	-	-	+	-	+	-	*Bacillus cereus*
Dgmb6	+	+	+	-	+	-	-	+	+	-	+	-	+	-	*Klebsiella pneumoniae*
Dgmb7	+	-	-	-	+	-	-	+	-	-	+	-	+	-	*Micrococcus varians*
Dgmb8	+	+	+	-	-	-	-	+	-	+	+	-	+	-	*Staphylococcus aureus*
Dgmb9	+	+	+	-	-	+	-	+	d	+	-	+	-	+	*Pseudomonas fragi*
Dgmb10	+	-	-	-	+	-	+	+	-	-	+	-	+	-	*Micrococcus varians*
Dgmb11	+	+	+	-	+	+	-	+	+		+	-	+		*Enterobacter amnigenus*
Dgmb12	+	-	-	+	+	d	-	+	+	-	+	+	-	-	*Salmonella arizonae*
Dgmb13	+	+	+	-	-	-	-	+	-	+	+	-	+	-	*Staphylococcus aureus*

Key: Dgmb - Dried goat meat bacteria
\+ - Positive
\- Negative
d – delayed to produce

Table 4: Antibiotic Disc Susceptibility test Antibiotics/Maximum zone of inhibition (mm).

Test organisms	GM(10)	CTX(30)	CRO(10)	STX	P(10)	E(15)
S. aureus	21	12	13	17	14	9
B. melitensis	15	9	12	11	16	11
K. peneumoniae	16	12	2	6	9	6
P. fragi	10	13	18	5	10	4
M. varians	21	8	10	6	15	11
L. richardii	14	18	20	15	12	13
E. blattea	18	11	12	10	9	10
B. cereus	16	17	14	5	8	12
E. amnigenus	19	16	13	18	21	11
S. arizonae	14	12	15	8	11	9

Table 4 shows the Antibiogram of Antibiotics susceptibility test of the bacterial isolates
KEY:
GM (10) ---- Gentamycin
CTX (30) ---- Ceftriazone
CRO (10) ---- Ciprofloxacin
STX ---- Septrin
P (10) ---- Penicillin
E (15) ---- Erythromycin

Table 5: Standard Kirby Bauer Chart for interpretation of Zone Size Diameter (mm) of zones of inhibition on medium Mueller- Hinton.

Antimicrobial agents	Sensitive	Intermediate	Resistant
Gentamycin	≥ 13	14 -17	≥12
Ciprofloxacin	≥ 14	12 -13	≥11
Ceftriazone	≥ 18	15 -17	≥14
Septrin	≥ 16	12 -15	≥12
Penicillin	≥ 22	12- 21	≥11
Erythromycin	≥ 18	14 -17	≥13

Table 5 shows zone – size interpretation chart for the Kirby – Bauer method (Devised from the National Committee for Clinical Laboratory Standards, Subcommittee on Antimicrobials susceptibility Testing, 1975).

DISCUSSION

In this study, a total of 11 isolates identified as *Escherichia blattea*, *Staphylococcus aureus*, *Leminorella richardi Brucella melitensis*, *Bacillus cereus*, *Klebsiella pneumonia*, *Micrococcus varians*, *Pseudomonas* fragi, *Enterobacter amnigenus*, and *Salmonella arizonae* were isolated from dried goat meat at 20% moisture content. Table 1 shows the total viable bacterial counts from dried goat meat which indicates a high level contamination of meat by enteric and *coliform* bacteria as well as *Staphylococcus aureus*. The presence of these organisms in goat meat depicts a deplorable state of hygiene, poor environmental conditions and sanitary practices employed by meat handlers and processors during slaughtering and packaging of meat and meat products. This is in agreement with previous reports by Talaro and Talaro, (2006), Lowry, (2003) and Okonkwo *et al*., (2009). Contamination of meat during slaughtering may result to the transfer of antibiotic resistant bacteria to the meat. Studies have it that handling of raw meat by meat buyers especially in Calabar could also lead to contamination of meat with resistant microorganisms.

The emergence of antimicrobial resistant bacteria is associated with the use of antibiotics in animal feed as growth promoter, treatment of infectious diseases with it, treatment of a batch of animals when at least one of them is diagnosed as ill and preventive treatment against disease (CDCP, 2016). There are also global concerns over the use of antibiotics for growth promotion because of the potentials of some drugs to enter the human food chain irrespective of withdrawal measures and testing to prevent antibiotics residues in food. This increases antibiotics resistance in animals, which has been linked to antibiotic resistant infection in human though not proven (CDCP, 2016).

Tables 4 and 5 show that *Staphylococcus aureus* is resistant to the antibiotics ceftriazone and erythromycin. *Brucella melitensis* showed resistance to ceftriazone, septrin, penicillin and erythromycin. *Klebsiella pneumoniae* was found to be resistant to ceftriazone, ciprofloxacin, septrin, penicillin and erythromycin. *Pseudomonas fragi* was resistant to gentamycin, ceftriazone, septrin, penicillin and erythromycin. *Micrococcus varians* showed resistance to ceftriazone, ciprofloxacin, septrin and erythromycin. *Leminorella richardii* did not show resistance to any of the test antibiotics. *Escherichia blattea* was found to be resistant to ceftriazone, septrin, penicillin and erythromycin. *Enterobacter amnigenus* was found to be resistant to penicillin and erythromycin only. Finally, *Salmonella arizone* showed resistance to septrin and erythromycin only. Multi resistance is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. This study showed that the isolates *Brucella melitensis*, *Klebsiella pneumoniae*, *Pseudomonas fragi*, *Micrococcus varians*, *Escherichia blattea* and *Staphylococcus aureus* are multidrug resistant organisms. Some of these isolates are among a group of gram positive and gram negative bacteria recently dubbed as the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) which have shown resistance to multiple antibiotics in the pharmaceutical industries as reported in an article by Boucher et al. (2009).

CONCLUSION

Results from this study confirms that foods of animal origin are reservoir for antibiotics resistant bacteria Good hygiene practices along the food chain to prevent the transmission of these pathogens to humans and a meticulous use of antibiotics in animal feed are essential. Direct contact with livestock should be avoided as studies have shown that this can spread antibiotic resistant bacteria from animals to humans. With resistance to antibiotics becoming more common, there is greater need for alternative antimicrobial treatment. There is a call therefore for new antibiotic therapies namely the use of spices with very broad spectrum antimicrobial activities.

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