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Plasmid Curing Of Bacteria Isolates In Raw Beef (Cow) From Different Abattoirs And Markets In Edo North

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Abstract: ***Plasmid curing of bacteria isolates in raw beef (cow) from different abattoirs and markets in Edo north was carried out. The result shows a significant relationship between personnel and practices at slaughter, dressing and the level of beef carcass contamination; the personnel playing a significant role in general carcass contamination and in the spread of pathogens such as E. coli, Salmonella, Enterococcus Staphylococcus, Pseudomonas and Shigella species. Raw beef carcasses have been indicated to contain high numbers (approximately10^5 cfu/g) of pathogenic bacteria (Small et al., 2002). In a similar study, Avery et al., (2002) had isolated E. coli and Salmonella from cattle hides at slaughter. Of the thirty isolates, tested for antibiotic resistance, E. coli and Staphylococcus aureus from hand swab of personnel in Auchi abattoir were resistant to all antibiotics used. Resistance to amoxicillin was 96.7%, 90.0% were resistant to tetracycline, 88.0% were resistant to Augumentin, 66.7% were resistant to Erythromycin, and 53.3% were resistant to Gentamycin. Isolates recorded the highest sensitivity to Ciprofloxacin (86.7%) followed by Sparfloxacin (80%), Ofloxacin (76.7%) and Graxone (53.3%) before plasmid curing. Multi-drug resistances were observed in all bacteria species isolated. Post-curing results showed one hundred percent sensitivity to Ciprofloxacin, Sparfloxacin and Ofloxacin.***

Keywords: Plasmid curing, Raw beef and Abattoir

I. INTRODUCTION

Beef serves as a significant source of dietary proteins, fats, vitamin c, riboflavin and niacin to man. These are required for normal body growth and development (Okonkwo *et al.*, 2008). It is one of the most perishable of all foods and has the ability to support the growth of several types of bacteria (Lawrie,1991).Cattle are major reservoirs of *E.coli* which results in infection in human consuming raw or undercooked beef and beef products (Dean – Nystrom *et al*.,1999) .

Most often antibiotics are used for control and treatment of bacterial diseases in food animals like cows and when these antibiotics are administered to livestock animals over a long period particularly at low levels, certain species of bacteria become resistant to these antibiotics (Adwan *et al*., 1998) and these resistance can reach humans through the food chain (Van Looveren *et al.*, 2001). Antibiotic resistance by bacteria could be chromosomally or plasmid borne.

Plasmids are extra chromosomal DNA strands that are replicated and inherited by offspring this will result in a fully resistant colony (Brooks, *et al*., 2002). The elimination of plasmid (curing) from a bacterial culture is the best method to expand the relationship between a genetic trait and carriage of a specific plasmid by the culture (Ghosh *et. al*., 2000).The aim of this research is to evaluate and cure plasmids of bacteria isolates in raw beef from different abattoirs in Edo North.

II. MATERIALS AND METHODS

COLLECTION OF SAMPLES

Samples were collected in duplicate from three different abattoirs (Auchi, Jattu and Igarra) in Edo- North. A total of 42

samples of raw beef and swabs from working tools were collected. The raw beef samples were collected in sterile, wide mouthed flask previously plugged with cotton wool wrapped with aluminum and transported to the laboratory. Sterile swab sticks were used to swab butchers' hands, the bowls, knives and tables used for evisceration and taken to the laboratory for bacteriological analysis.

BACTERIOLOGICAL ANALYSIS

This was done both quantitatively and qualitatively. Samples were diluted serially using peptone water as diluent and the pour plate method was used. Identification of the bacterial isolates was based on cultural, morphological, and biochemical characteristics following standard methods (Cheesbrough, 2000). The disc diffusion assay was used. Colonies from agar slants were streaked onto nutrient agar to obtain pure cultures. From the pure cultures, sterile wire loop was used to inoculate Mueller Hilton agar; using sterile forceps, antibiotic discs were evenly distributed on the inoculated plates. Within 30 minutes of applying the discs, the plates were inverted and incubated at 37^{o}c for 18 hours. After an overnight incubation, zones of inhibition were measured. Cultures were tested for sensitivity to 10 antibiotics: (Gentamycin, Amoxycillin, Cotrimoxazole, Ciprofloxacin, Sparfloxacin, Ofloxacin, Augmentin, 9+++Tetracycline, Graxone, and Erythromycin).

III. PLASMID CURING EXPERIMENT

The different bacteria strains that proved resistance to the different antibiotics used were subjected to plasmid curing. Ethidium bromide was used to cure the plasmid of the various bacteria isolates. During the curing experiment, representative strains with resistance pattern were subjected to ethidium bromide mediated plasmid elimination at different concentrations of 50ug/ml, 75ug/ml, and 100ug/ml. An aliquot (0.1g) of ethidium bromide powder was dissolved in 9.9ml of distilled water to 10mg/ml or 10,000ug/ml tenfold dilutions was carried out by adding 1ml of the 10,000ug/ml ethidium bromide to 9ml of peptone water to obtain a concentration of 100ug/ml each. A 1 in 100 dilution was then made on broth culture of the bacterial isolates with resistance pattern by adding 0.1ml of the broth culture to 9.9ml of sterile nutrient broth which was incubated overnight. A sterile pipette was used to add a drop each of the different bacterial isolate to the different concentrations of ethidium bromide in peptone broth and the highest concentration permitting growth was used for the curing. The strains were sub-cultured every 24 h in peptone broth containing a sub lethal concentration of the curing agent. At intervals of 3 days, the cultures were serially diluted, and then plated onto blood agar (Oxoid). After 48hrs of incubation at 37^{o}C, the emergent colonies were duplicated onto fresh blood agar and blood agar containing Gentamicin, Amoxicillin, Cotrimoxazole, Ciprofloxacin, Sparfloxacin, Ofloxacin, Augumentin, Tetracycline, Graxone, and Erythromycin.

Colonies that failed to grow on the blood agar antibiotic plates after incubation were considered to have been cured.

The results are shown in table 1,2,3 and 4. The results obtained from the analysis of meat products from the three abattoirs and markets in Edo-North were contaminated with microorganisms of public health importance. The high bacteria count in the beef products may be a consequence of the low level of hygiene maintained during the processing and sales of the product. This includes the handlers, quality of water used and the utensils used during processing and evisceration. The detection of *E. coli, Salmonella, Shigella, Enterococcus, Staphylococcus aureus and Pseudomonas* as the case may be, indicates poor hygienic practices among handlers of the beef products.

IV. RESULTS

The results of this study revealed that microbial contaminants of beef carcass resulted from the air environment of the abattoirs, the slaughter slabs and knives, the hides and hooves, the gastro intestinal tract of the animals, the wash water due to unhygienic practices and this was in accordance with the reports of Hudson *et al*., (1996).

Sample	**Location**		
	Jattu	Igarra	Auchi
A	9.5×10^{7}	1.3×10^{5}	1.9×10^{5}
B	7.4×10^{5}	1.2×10^{5}	1.2×10^{6}
C	1.3×10^{6}	1.5×10^{7}	3.3×10^{6}
D	1.1×10^{7}	8.6×10^{6}	7.4×10^{6}
E	1.5×10^{7}	1.5×10^{6}	6.6×10^{6}
F	7.9×10^{8}	5.1×10^{8}	6.2×10^{8}
G	7.3×10^{8}	4.2×10^{8}	9.4×10^{6}
H	6.0×10^{7}	3.9×10^{8}	8.2×10^{5}

Key: A =Washed Beef, B=Unwashed Beef, C=Table Swab, D=Bowl Swab,
E=Knife swab, F=Beef from Market, G = Hand Swab 1, H = Hand Swab 2

Table 1: Mean Plate Count (Cfu/g) Of Beef Samples and Swabs from Working Tools in Three (Jattu, Igarra and Auchi) Abattoirs in Edo North

Organism	**Sample Code**							
	JW	**JU**	**JT**	**JB**	**JK**	**JH1**	**JH2**	**JM**
E. coli	+	+	+	-	-	-	-	-
Staph.aureus	-	+	+	-	-	+	-	+
Enterococcus feacalis	+	-	+	+	+	-	-	+
Pseudomonas spp	+	-	-	+	-	-	-	+
Salmonella spp	+	-	-	+	-	-	-	-
Shigella spp	-	-	-	-	-	-	-	+

KEY:
JW = Washed Beef samples from Jattu; JU= unwashed Beef samples from Jattu, JT=Table swab from Jattu ,JB= Bowl sample from Jattu, JK=Knife swab from Jattu, JH1 and JH2= Hand swabs from personnel at Jattu; JM= Beef samples from Jattu market. + =Positive; -=negative.

Table 3a: Bacterial Occurrence in Different Samples From Jattu Abattoir and Market in Edo-North.

Organism	Sample Code							
	AW	AU	AT	AB	AK	AH1	AH2	AM
E. coli	+	+	-	+	-	-	-	-
Staph.aureus	+	-	+	-	-	-	+	-
Enterococcus feacalis	+	-	-	+	-	-	-	-
Pseudomonas spp	+	+	-	+	-	-	-	+
Salmonella spp	-	-	-	-	+	-	-	-
Shigella spp	+	+	-	-	-	-	-	+

Key:
AW = Washed Beef samples from Auchi; AU= unwashed Beef samples from Auchi, AT=Table swab from Auchi, AB = Bowl sample from Auchi, AK=Knife swab from Auchi,
AH1 and AH2= Hand swabs from personnel at Auchi; AM= Beef samples from Auchi market; +=positive,-=negative

Table 3b: Bacterial Occurrence in Different Samples from Auchi Abattoir and Market

Organism	Sample Code							
	IW	IU	IT	IB	IK	IH1	IH2	IM
E. coli	-	-	-	+	+	-	-	-
Staph.aureus	-	-	-	-	-	+	+	+
Enterococcus feacalis	-	-	-	-	-	-	-	-
Pseudomonas spp	-	-	-	-	-	-	-	+
Salmonella spp	-	+	+	-	-	-	-	-
Shigella spp	+	-	-	-	-	-	-	-

Key:
IW = Washed Beef samples from Igarra; IU= unwashed Beef samples from Igarra, IT=Table swab from Igarra ; IB= Bowl sample from Igarra, IK=Knife swab from Igarra;IH1 and IH2= Hand swabs from personnel at Igarra; IM= Beef samples from Igarra market. +=positive,-=negative

Table 3c: Bacterial Occurrence in different Samples from Igarra Abattoir and Market

Antibiotics	No of resistant isolates (procuring)	No (%) of resistant isolates(post curing)	No(%) of isolates cured of plasmid
Gentamycin	16	10(62.5%)	6(37.5%)
Amoxicillin	29	28(96.6%)	1(3.4%)
Cotrimoxazole	30	27(96.7%)	3(3.3%)
Ciprofloxacin	4	0(0%)	4(100%)
Ofloxacin	7	0(0%)	7(100%)
Augmentin	26	20(76.9%)	6(23.1%)
Tetracycline	27	27(100%)	0(0%)
Sparfloxacin	6	0(0%)	6(100%)
Graxone	14	5(35.7%)	9(64.3%)
Erythromycin	20	7(35.0%)	13(65%)
Total	179	124(69.3%)	55(30.7)

Table 4: Plasmid Curing Analysis Of The Isolates From Different Abattoirs And Markets In Edo North

V. DISCUSSION

The results of this study revealed that microbial contaminants of beef carcass resulted from the air environment of the abattoirs, the slaughter slabs and knives, the hides and hooves, the gastro intestinal tract of the animals, the wash water due to unhygienic practices and this was in accordance with the reports of Hudson *et. al*.,(1996). The high bacteria count in the beef products may be a consequence of the low level of hygiene maintained during the processing and sales of the product. This includes the handlers, quality of water used and the utensils used during processing and evisceration. The detection of *E. coli, Salmonella, Shigella, Enterococcus, Staphylococcus aureus and Pseudomonas* as the case may be, indicates poor hygienic practices among handlers of the beef products.

There is a significant relationship between personnel and practices at slaughter, dressing and the level of beef carcass contamination; the personnel playing a significant role in general carcass contamination and in the spread of pathogens such as *E. coli, Salmonella, Enterococcus Staphylococcus, Pseudomonas and Shigella* species. Raw beef carcasses have been indicated to contain high numbers (approximately10^5 cfu/g) of pathogenic bacteria (Small *et al*., 2002). In a similar study, Avery *et al., (2002)* had isolated *E. coli* and *Salmonella* from cattle hides at slaughter.

Of the thirty isolates, tested for antibiotic resistance, *E. coli and Staphylococcus aureus* from hand swab of personnel in Auchi abattoir were resistant to all antibiotics used. Resistance to amoxicillin was 96.7%, 90.0% were resistant to tetracycline, 88.0% were resistant to Augumentin, 66.7% were resistant to Erythromycin, and 53.3% were resistant to Gentamycin. Isolates recorded the highest sensitivity to Ciprofloxacin (86.7%) followed by Sparfloxacin (80%), Ofloxacin (76.7%) and Graxone (53.3%) before plasmid curing. Multi-drug resistances were observed in all bacteria species isolated. Post-curing results showed one hundred percent sensitivity to Ciprofloxacin, Sparfloxacin and Ofloxacin.

VI. CONCLUSION

In conclusion, these data indicate that cows are reservoirs of antibiotic resistant *E. coil, Pseudomonas, Salmonella, Shigella, Enterococcus* and *Staphylococcus* species.

There is potential for these antibiotic resistant bacteria to be transferred to humans through contaminated beef. Multi-drug resistance of food borne pathogens is certainly a public health concern and reinforces the need for more prudent use of antibiotics by farmers, veterinarians and physicians.

VII. RECOMMENDATION

I hereby recommend the removal of antibiotics as growth promoters in livestock so as to reduce the risk of the emergence of resistance to antibiotics. Competent sanitary/health inspectors should be stationed at the abattoirs to see to the day to day sanitary conditions of the abattoirs.

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