International Scholars Journals

International Journal of Public Health and Epidemiology ISSN 2326-7291 Vol. 8 (8), pp. 001-005, August, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria

Iroha I. R.[1]*, Ugbo E. C.[1], Ilang D. C.[1], Oji A. E.[1] and Ayogu T. E.[2]

[1]Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, P. M. B. 053, Abakaliki Ebonyi State, Nigeria.

[2]Department of Food Technology, Institute of Management and technology, Enugu, P. M. B. 01079, Enugu State, Nigeria.

Accepted 24 May, 2019

Frequency of contamination in retail meat sold in Abakaliki, Ebonyi State was examined in the present study. Three hundred raw meat samples including beef (n = 100), chicken (n = 100), chevron (n = 100) were collected from Abakaliki abattoir and were analyzed for microbiological contamination using standard Microbiological methods. Antimicrobial susceptibility of isolated microbes was determined using the Kirby and Bauer method of disc diffusion. Out of the 300 samples, 79 (29.3%) were contaminated with bacteria species including *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi, Shigella dysenteriae* and *Staphylococcus aureus*. Of these, *E. coli* had the highest occurrence (8%), followed by *K. pneumoniae* (5.3%), *S. typhi* (5%), *S. dysenteriae* 2.6%, *P. aeruginosa* 2.0%, *B. cereus* 2.0% and *S. aureus* (1.3%). The antibiotic susceptibility studies showed an alarming level of resistance to all the tested antibiotics reflecting multi-drug resistant strains. Our data confirms the circulation of antibiotic resistant pathogens in raw meat sold in Abakaliki abattoir and market, which could possibly play a role in the spread of antimicrobial resistance amongst food-borne bacteria.

Key words: Meat, abattoir, contamination, antibiotic resistance.

INTRODUCTION

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs (Fratamico et al., 2005). Changes in eating habits, mass catering complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (Hedberg et al., 1992). Contaminated raw meat is one of the main sources of food-borne illness (Bhandare et al., 2007; Podpecan et al., 2007). Meat is the main edible part of domestic mammals; however, recent definition includes species, as well as fish, shellfish, poultry and exotic species such as frogs and allegation (Nakai and Moddler, 2000). Similarly, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood (Hammer, 1987). Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation.

A number of studies have reported outbreak of infections due to consumption of contaminated food and poor hygiene and in most of the cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections. However, a few community-based reports provide evidence of several outbreak caused by *Salmonella*, *Shigella*, *E. coli* and *Listeria* spps in different parts of the world (Zweifer et al., 2008). Moreover, antibiotic resistance levels are also elevated among food-borne pathogens such as in *Salmonella* and *Shigella* (Duffy et al., 1999). It is not inevitable to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections but the presence of such bacteria in food items and their related environment could

*Corresponding author. E-mail: ifynero@yahoo.com.

play a role on the spread of antimicrobial resistance amongst food borne pathogens (Farzana et al., 2009). There are a lot of human health consequences in consuming contaminated foods ranging from protracted illness to death and patients with impaired immunity are at greater risk. Therefore, this study was conducted to investigate the microbial quality of raw meat sold in Abakaliki market/abattoir and to evaluate antibiotic resistance profile of the isolated bacteria.

MATERIALS AND METHODS

Meat sample collections

Meat of market/Abattoir in Abakaliki Ebonyi State of Nigeria was used for this study. A total of 300 raw meat samples including beef (n = 100), chicken (n = 100) and chevron (n = 100) were randomly collected during the periods from December 2009 to January 2010 and from April to May 2010. These periods were chosen because of the large quantity of animals slaughtered in the abattoir then the meat brought to the market for sale as it is a festivity period marking the Christmas and Easter celebration by Christians all over the world. The samples were collected within 8 h post-slaughter and during early afternoon in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings. Collection was dependent on the co-operation of the shop owners. Butchers working in these outlets lack knowledge regarding the importance of disinfecting and sanitizing; consequently they clean their shops once in 24 h with detergent and water. No sanitizers' medium was used before sampling.

Meat sample preparation

Twenty-five grams of collected meat samples were weighed and transferred to sterile flasks containing 100 ml of phosphate buffer saline (PBS). Samples were homogenized using a meat grinder under aseptic conditions and was stored for further analysis.

Microbiological analysis

Diluted meat samples in PBS were inoculated onto nutrient agar, mannitol salt agar, (Merck, Darmstadt, Germany) and 6.5% NaCl Mueller Hinton agar and were incubated at 37ºC in a CO_2 enriched environment for isolation and identification of Gram-positive organisms. MacConkey agar and Eosin methylene blue (EMB) agar were inoculated for the isolation of Gram-negative organism, incubated aerobically at 37ºC for 18 to 24 h. For the detection of *Salmonella*, we used Selenite F broth medium for pre-enrichment for isolation of *Salmonella* spp. (DIFCO, Michigan USA) and incubated for 18 h at 37ºC.

Bacteria identification

Identification of bacteria was carried out using standard microbiological/biochemical methods (Brown, 2005). For gram-negative organisms, the identification battery included gram-staining, oxidase, citrate, urea hydrolysis, sulphide indole motility (SIM).

Triple sugar iron test (TSI); briefly TSI agar was prepared in test tubes as a slant with phenol red and 1% lactose, 1% sucrose 0.1% glucose, sodium thiosulphate and ferrous sulphate were all added and incubated at 37ºC for 24 h after which it was observed for change in colour with acid/gas production.

For identification of gram-positive organisms, the following tests was carried out: Gram staining, catalase, tube coagulase, DNase and characteristic pigment production.

Antibiotic susceptibility studies

Susceptibility patterns of the isolated organisms were tested against a wide range of antibiotics namely nitrofurantoin (100 mg), ciprofloxacin (5 mg), tetracycline (30 mg), norfloxacin (10 mg), amoxicillin (30 mg), ofloxacin (5 mg), chloramphenicol (10 mg), cefotaxime (30 mg), ampicillin (25 mg), gentamicin (10 mg), erythromycin (10 mg) clindamycin (10 mg), cephalexin (30 mg), cotrimoxazole (25 mg), ceftriaxone (30 mg) and augmentin (30 mg) using Kirby and Bauer disc diffusion methods of determining susceptibility (Bauer et al., 1966). No control strain was used.

RESULTS

Throughput at Abakaliki abattoir ranged from 10 to 15 cattle/day, 100 to 150 chicken/day, 80 to 100 chevron/day, 60 to 80 goats/day. Out of the examined 300 samples 46 of beef, 13 of chicken and 20 of chevron were of microbial contamination yielding a percentage of 29.3% were contaminated (Table 1). The frequency of the isolated bacteria from different meat samples was summarized in Table 2 as follows; 2.0% of *B. cereus* was isolated from all the meat sampled while 2 and 4 isolates were from beef and chevron, respectively. 8% of *E. coli* was isolated where 19 isolates were from beef, 2 from chicken and 3 from chevron. 5.3% of *K. pneumoniae* was isolated, where 9 isolates were from beef, 3 from chicken and 4 from chevron, 2% of *P. aeruginosa* were isolated where 6 isolates were from beef, 5.3% of *S. typhi* was isolated where 4 isolates were from beef, 6 from chicken and 5 from chevron. 2.6% of *S. dysenteriae* was isolated where 4 isolates were from beef, 2 each from chevron and chicken, 1.3% of *S. aureus* was isolated where 2 isolates each were from beef and chevron. Using the Clinical Laboratory Standard Institute (CLSI) guidelines for determining susceptibility, antibiotic resistant studies revealed that all the isolates were highly resistant to all the antibiotics tested (Tables 3, 4, 5 and 6).

DISCUSSION

The present study evaluated the microbial quality of raw meat sold in Abakaliki abattoir. Our findings showed that out of 300 meat samples analyzed for microbial quality, 76(29.3%) were contaminated with different kinds of micro-organism namely *B. cereus* (2.0%), *E. coli* (8%), *K. pneumoniae* (5.3%), *P. aeruginosa* (2.0%), *S. typhi* (5.0%), *S. dysenteriae* (2.6%) and *S. aureus* (1.3%). Micro-organism with the highest rate of occurrence was *E. coli* (8.0%) while the least one was *S. aureus* (1.3%). As observed in the course of this study, the method of slaughtering of animals is responsible for the microbial

Table 1. Percentage of contaminated meat products.

Sources of meat sample	Number examined	Percentage contaminated
Beef	100	46
Chicken	100	13
Chevron	100	20

Table 2. Frequency distribution of the isolated bacteria from the examined meat samples.

Bacteria isolates	Beef meat	Chicken meat	Chevron meat	Percentage of occurrence
Bacillus cereus	2	-	4	2.0
Escherichia coli	19	2	3	8.0
Klebsiella pneumoniae	9	3	4	5.3
Pseudomonas aeruginosa	6	-	-	2.0
Salmonella typhi	4	6	5	5.0
Shigella dysenteriae	4	2	2	2.6
Staphylococcus aureus	2	-	2	1.3

Table 3. Distribution of antibiotic resistance among gram-negative bacteria isolates from beef meat. Inhibition zone diameter (mm) of antibiotics used.

Bacteria isolates	Antibiotics used												
	N	CIP	NB	AX	OF	C	CF	AM	GN	CX	CO	FX	AU
E. coli	2	2	2	3	NI	2	10	2	NI	1	3	NI	NI
K. pneumoniae	5	3	3	10	1	8	8	8	NI	5	6	1	1
Sal. typhi	NI	NI	NI	1	NI	1	16	1	NI	1	2	NI	NI
Shi. dysenteria	NI	NI	NI	NI	NI	NI	12	1	NI	1	1	NI	NI

Keys: N = nitrofurantoin, CIP = ciprofloxacin, NB = norfloxacin, AX = amoxicillin OF = ofloxacin, C = Chloramphenicol, CF = cefotaxime, AM = ampicillin, GN = gentamicin, CX = cephalexin, CO = cotrimoxazole, FX = ceftriaxone, AU = augumentin, NI = no inhibition.

Table 4. Distribution of antibiotic resistance among gram-negative bacteria isolates from chicken meat. Inhibition zone diameter (mm) of antibiotics used.

Bacteria isolates	Antibiotics used												
	N	CIP	NB	AX	OF	C	CF	AM	GN	CX	CO	FX	AU
E. coli	3	5	1	6	NI	4	16	7	NI	1	1	1	1
K. pneumoniae	NI	2	NI	2	NI	1	10	2	NI	NI	1	NI	NI
Sal. typhi	3	2	1	3	NI	2	11	4	NI	2	3	1	1
Sh. dysenteria	NI	NI	NI	NI	NI	NI	11	1	NI	NI	1	1	1

Keys: The same as above.

contamination. Traditional method of butchering using knives and cutting lines appears more capable of minimizing fecal contamination than modern mechanized systems which are manned by a team of operators. This was inferred because meat samples collected from the local abattoir were less contaminated (data not shown) than those collected from where modern equipment with more personnel were used in meat processing. Also it was observed that *Salmonella* was most prevalent in poultry (chicken). Such findings have been reported by other investigators with contamination ranging from 13.7% in Switzerland to 26.6% in Tokyo and 66% in Thailand (Baumgartner et al., 1992; Ishizaki et al., 1993; Jerngklinchan et al., 1994).

The presence of bacteria in meat has been widely reported from different parts of the world (Holds et al., 2007; Kinsella et al., 2008). Some groups recognized the presence of bacteria especially gram-negative organisms as an indicator of open air meat spoilage while others argued this assertion and considered the presence of a

Table 5. Distribution of antibiotic resistance among gram-negative bacteria isolates from chevron meat. Inhibition zone diameter (mm) of antibiotics used.

Bacteria isolates	Antibiotics used												
	N	CIP	NB	AX	OF	C	CF	AM	GN	CX	CO	FX	AU
E. coli	NI	NI	NI	NI	NI	NI	14	1	NI	1	3	NI	1
K. pneumoniae	1	2	1	2	NI	1	11	2	NI	3	3	NI	1
Sal. typhi	NI	2	NI	2	NI	NI	14	1	NI	NI	1	NI	1
Sh. dysenteria	NI	NI	NI	NI	NI	NI	13	NI	NI	NI	NI	NI	NI

Keys: The same as above.

Table 6. Distribution of antibiotic resistance of *Staphylococcus aureus* isolated from beef, chicken and chevron meat. Inhibition zone diameter (mm) of antibiotics used.

Bacteria isolates	Antibiotics used							
	AX	TE	OF	AM	GN	E	CD	CO
Staph. aureus (beef meat sample)	6	4	5	9	3	8	10	6
Staphy. aureus (chicken meat sample)	NI	NI	10	6	NI	14	7	12
Staphyl. aureus (chevron meat sample)	10	8	13	11	9	8	8	10
B. cereus (beef meat sample)	1	NI	NI	NI	NI	NI	NI	NI
B. cereus (chicken meat sample)	3	NI	1	4	NI	NI	NI	1
B. cereus (chevron meat sample)	1	NI	NI	NI	NI	NI	NI	1

Keys: CIP = ciprofloxacin, AX = amoxicillin, AM =ampicillin, GN = gentamicin, CX = cephalexin, CO = cotrimoxazole, CD = clindamycin, E = erythromycin, NI = no inhibition.

high number of background organisms as a pathogen-reduction strategy due to the organisms antagonistic effect against pathogenic bacteria and thus safe for meat quality (Eribo and Jay, 1985). Our result indicated the predominance of gram-negative organisms such as *Salmonella*, *Shigella* and *E. coli* as reported by other groups (Zakpaa et al., 2009). The presence of zoonotic bacteria such as *Brucella* and *Listeria* in meat although not found in our study indicates poor ante-mortem inspection of the animals as well as unhygienic meat processing (Lacerda et al., 1997; Barros et al., 2007). To find the prevalence of drug resistance bacteria, assays for susceptibility profiles were performed. High level resistance of bacteria isolates to various classes of antibiotics was observed.

In recent years, the occurrence of antibiotic resistant strains of a number of pathogenic bacteria especially *Salmonella* in foods has caused great concern in relation to public health (Hollingsworth and Kaplon, 1997). While the use of antibiotics has been proven to be an effective means for the prevention and control of bacteria infection, their indiscriminate use can have adverse consequences by promoting the selection and prevalence of drug resistant microbial populations (Braude, 1978; Threlfall et al., 1997). The problem may be due to the natural resistance of species to certain antibiotics (Allison and Gilbert, 1995), 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. This is believed to be largely responsible for the emergency of drug resistance bacteria (Dupont and Steele, 1987). Piddock (1996) suggested 3 possible ways in which the use of antibiotics could pose a risk to human health and these include; (a) antibiotic resistant pathogens in animal are selected, food products then become contaminated during slaughter and /or food preparation, the food is then ingested causing infection which requires antibiotic therapy and therapy is then compromised due to resistant strains; (b) resistant non-pathogenic bacteria are selected in animals transferred to humans via consumption of contaminated food products and resistant genes are subsequently transferred to other bacteria in the gut; (c) antibiotics which may remain as residues in animal products such as meat and milk can also lead to the selection of resistant bacteria in the consumer of the food products.

The susceptibility results of bacteria isolated from meat samples showed that they are highly resistance to all the antibiotics tested. 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 enterobactericeae family, this group of organisms are always resistant to various classes of antibiotics. They are known to harbours series of antibiotic resistance

genes which can be transferred horizontally to other bacteria spps. Their resistance to the cephalosporins may be due to the production of beta lactamase enzymes, this enzymes are known to inactivate anti-biotics 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.

Some strains of *S. aureus* showed some level of inter-mediate susceptibility to erythromycin, even at that it still posses some public health problems because when this antibiotics are mis-used this organism will develop resistant against it. *B. cereus* was completely resistant to all the antibiotics tested. Antibiotic usage in growing of animal and for treating bacteria infections in animals could be responsible for the observed resistance and this has a range of human health consequences ranging from protracted illness and even death as vulnerable patients are those with impaired immunity.

This study presented the degree of contamination status of raw meat sold in Abakaliki market/abattoir and as well demonstrated the role of this meat as a reservoir of antibiotic resistant bacteria that can be transferred to humans, thereby constituting a public health problem. We hereby suggest the application of stringent hygiene practices along the food chain and prudent use of antibiotics in animal husbandry which are essential for the control of further emergency of antibiotic resistance.

REFERENCES

Allison DG, Gilbert P (1995). Modification by surface association of antimicrobial susceptibility of bacterial populations. J. Ind. Microbiol. Biotechnol., 15: 311-317.

Barros MAF, Nero LA, Silvia LC, d'Ovidio I. Monteiro FA, Tamanini R, Fagnani R, Hofer E, beloti V (2007). *Listeria monocytogenes*: occurrence in beef and identification of the main contamination points in processing plants. Meat, Sci., 76: 591-596.

Bauer AW, Kirby WMM Sherris JC, Turch M (1966). Antibiotic testing by standardized single disk method. Am. J. Clin. Path., 45: 493-496.

Baumgartner A, Heurnann P, Schmid H, Liniger M, Simmen A (1992). *Salmonella* contamination of poultry carcasses and human salmonellosis. Archv. Fur Leben., 43: 123-124.

Bhandare SG, Sherikarv AT, Paturkar AM, Waskar VS, Zende RJ (2007). A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food. Contr., 18: 854-868.

Braude R (1978). Antibiotic in animal feed in Great Britain. J. An. Sci., 46: 1425-1436.

Brown EA (2005). Benson's Microbiological applications laboratory manual in general microbiology, 9th ed. McGraw-Hill Companies, Inc., New York, pp. 230-280.

Duffy G, Cloak OM, O'Sullivan MG, Guillet A, Sheridan JJ, Blair IS, McDowell DA (1999). The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. Food. Microbiol., 16: 623-631.

Dupont HL, Steele JH (1987). Use of antimicrobial agents in animal feeds: implications for human health. Rev. Infect. Dis., 9: 447-460.

Eribo BE, Jay JM (1985). Incidence of *Acinetobacter* spp and other Gram-negative bacteria in fresh and spoiled ground beef. App. Env. Microbiol., 49: 256-257.

Farzana K, Akhter S, Jabeen F (2009). Prevalence and antibiotic resistance of bacteria in two ethnic milk based products. Pak. J. Bot., 41: 935-943.

Fratamico PM, Bhunia AK, Smith JL (2005). Foodborne pathogens in Microbiology and Molecular Biology, Caister Academic press, Wymondham Norfolk, UK. Pp. 270-275.

Hammer GF (1997). Meat processing: ripened products Fleischwirtschaft, 67: 71-71.

Hedberg CW, Levine WC, White KE, Carlson RH, Winsor DK, Cameron DN, MacDonald KL, Osterholm MT (1992). An international food-borne outbreak of *Shigellosis* associated with a commercial airline. JAMA 268: 3208-3212.

Holds G, Pointon A, Lorimer M, Kiermeier A, Raven G, sumner J. (2007). Microbial profiles of carcasses and minced meat from Kangaroos processed in South Australia Int. J. Food. Microbiol. 123: 88-92.

Hollingsworth J, Kaplon B.(1997). Federal agencies collaborate to control dangerous new *Salmonella* strain. JAMA, 210: 1712-1716.

Ishizaki N, Kaneko S, Itoh T, Jinbo K, Kataoka J, Kukturbo Y (1993). The detection and sero-typing of *Salmonella* in commercial meat from 1989-1992 in Tokyo. Ann Rep. Met. Res. Lab. Pub. Health,, 44: 101-104

Jerngklinchan J, Koowatananukul C, Daengprom K, Saitanu K (1994). Occurrence of *Salmonellae* in raw broilers and their products in Thailand. J. Food. Protect., 57: 808-810.

Kinsella KJ, Prendergast DM, McCann MS, Blair IS, McDowell DA, Sheridan JJ (2008). The survival of *Salmonella enteric serovar Typhimurium* DT 104 and total viable counts on beef surfaces at different relative humidities and temperatures J. App. Microbiol. 106: 171-180.

Lacerda LM, AIves LMC, Mathias LA, Rodrigues ALB, AImeida FM (1997). Brucelose em trabalhadores de metadourous do municipio de Sao Luis, MA Hig AIiment. 14: 62-65.

Nakai S Modler WH (2000). Food protein, 1st ed. Wiley-VCH, Inc., New York, U.S.A. pp. 128-133.

Piddock L (1996). Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic resistant bacteria that infect man and compromise antimicrobial therapy. J. Antimicrob. Chemother. 38:1-3.

Podpecan B, Pengov A, Vadnjal S (2007). The source of contamination of ground meat for production of meat products with bacteria *Staphylococccus aureus*. Slov. Vet. Res., 44: 24-30.

Threlfall EJ, Ward LR, Rowe B (1997). Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella typhimurium* ST104 in England and Wales. Euro-Surveillance Rep., 2: 11-16.

Zakpaa HD, Imbeah MC, Mak-Mensah EE (2009).Microbial characterization of fermented meat products on some selected markets in the Kumasi metropolis, Ghana. Afr. J. Food. Sci. 3(11): 340-346.

Zweifer C, Fischer R, Stephan R (2008). Microbiological contamination of pig and cattle carcasses in different small-scale Swiss abattoir, Meat. Sci., 78: 225-231.