

Original Paper

Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in Retail Raw Chicken, Pork, Beef and Goat meat in Osogbo, Nigeria

Adesiji Yemisi O^{1*}, Alli Oyeboode T², Adekanle Margaret A² and Jolayemi Justina B²

Department of ¹Medical Microbiology and Parasitology, ²Biomedical Science, College of Health Sciences, Ladoko Akintola University of Technology, Ogbomoso, Nigeria

ABSTRACT

Three hundred (300) samples of fresh raw chicken, beef, goat and pork meat were screened for *Arcobacter* species by selective cultural procedures and for *Escherichia coli*, *Salmonella* species and *Staphylococcus aureus* enriched in peptone water and then streaked onto appropriate bacteriological agar. From the 300 samples analysed, *S. aureus* {138 (46%)} was the most frequently isolated organism, followed by *E. coli* {78 (26%)}, *Arcobacter* spp. {57(19%)} and *Salmonella* spp {6(2%)}. In this study, varying level of resistance of *Escherichia coli* 66(84.6%), *Salmonella* 6(100%) and *Arcobacter* 57(100%) to amoxicillin was observed. The susceptibility pattern indicates that the bacterial isolates exhibited a varying level of resistance to two or more antimicrobial agents with maximum resistance to amoxicillin. The detection of these organisms in meat may constitute a serious public health concern. Hence, there is a need for the implementation of Hazard Analysis Critical Control Point System monitoring of critical contamination points used in meat production to ensure food safety in Nigeria.

Keywords: Abattoir-hygiene, Antibiotic resistance, Foodborne-pathogens, Nigeria

Received 06 February 2010/ Accepted 11 April 2011

INTRODUCTION

It is well documented that contamination of meat with pathogens constitutes a major public health concern (Cohen *et al.*, 2007). In Nigeria, processing procedures and monitoring of critical points in the meat production are not fully developed. Abattoir has also become a source of infection and pollution, attracting domestic and wild carnivores, and rodents due to inadequate slaughtering and disposal facilities (Adeyemo, 2002). Apart from incorrect processing procedures, marketing practices of meat consumed by most populace is a major area of concern (Okoli *et al.*, 2006a).

In most parts of Nigeria, fresh meat is usually hawked on trays or displayed on tables in open market without hygienic precautions and often kept at ambient temperatures. Also, transport facilities are often inadequate and unhygienic as urban food

distribution chains are frequently long and involve different intermediaries, which render controls difficult (Olugasa *et al.*, 2000). Previous studies have documented contamination of raw meat by bacterial pathogens (Uche and Agbo, 1985; Ameh and Amadusa, 2006). Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases in Nigeria (FAO/WHO, 2002).

Food contamination with antibiotic-resistant bacteria can also be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (Van *et al.*, 2007). This increase is attributed to the selection pressure

*Corresponding author: Tel: +234 8032948270; E-mail: drmsadesiji@yahoo.com

created by using antimicrobials in food producing animals, in addition to unregulated use of antibiotics by humans in developing countries (Van den Bogaard *et al.*, 2000). The present study was undertaken to describe the prevalence and evaluate antibiotic resistance profile of *Arcobacter*, *E. coli*, *S. aureus* and *Salmonella* from chicken, pork, beef and goat meat in Osogbo-city, Nigeria. These bacterial pathogens belong to a group of prioritized, extended list of food-and water-borne zoonoses (Cardoen *et al.*, 2009) making it necessary for food safety authorities to focus on them as most relevant hazards in the food chain.

MATERIAL AND METHODS

Sample Collection

Three hundred (300) samples of meat, consisting of beef (n = 75 samples), chicken/poultry (n = 75 samples), pork (n = 75 samples), and goat (n = 75 samples), were purchased from various abattoirs and markets around Osogbo city between February and June, 2009. Meat samples were purchased in units of about 100g in polythene bags. The samples were put in sterile plastic containers and transferred to the laboratory ice-cooled within 2 hours of purchase.

Isolation Procedure

For *Arcobacter*, an enrichment and selective media plating were performed. Briefly, one gram (1g) of meat sample was inoculated directly into 9ml of an *Arcobacter* enrichment broth containing 24 g/L of *Arcobacter* base broth (Oxoid) supplemented with CAT: cepoferazone (12mg), amphotericin B (10mg) and teicoplanin (8mg) and incubated (37°C in air for 48 hours). 100µl aliquot of the enriched broth was transferred to *Arcobacter* selective agar (L13-Oxoid, UK) supplemented with CAT. After a thorough manual homogenisation, the sample was serially diluted before transferring to test tubes. To determine total viable counts, 1ml of 10⁻⁵ and 10⁻⁷ dilutions were plated on *Arcobacter* selective agar plates in triplicates and incubated for 24 hours microaerobically. The culture plates were later examined for the presence of bacterial colonies with morphological features characteristic of *Arcobacter* sp (Vanadamme *et al.*, 1999). For other bacterial pathogens, a gram of the raw meat sample was inoculated in 9ml of peptone water in a capped specimen bottle shaken vigorously and incubated overnight. After a ten-fold serial dilution, 1ml of each of 10⁻⁵ and 10⁻⁷ dilutions were plated on nutrient, blood and MacConkey agar plates in triplicates to determine the total viable counts. One millilitre of peptone rinse from each bottle was then

poured aseptically into three flasks for *Salmonella*, *E. coli* and *Staphylococcus*. Aliquots from each bottle were plated on nutrient, MacConkey and blood agar. The isolates obtained were identified based on established conventional cultural, morphological and biochemical characterizations (Murray *et al.*, 2005).

Statistical Analysis

All data were analyzed using the general linear model procedures of Analysis of Variance (ANOVA).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was performed using disc diffusion according to Bauer *et al.* (1966). The following antibiotics were tested: Gentamycin (10µg), Cotrimoxazole (25µg), Ofloxacin (5µg), Amoxycillin (25µg), Ciprofloxacin (10µg), Tetracycline (30µg), Pefloxacin (5µg), Pefloxacin (10µg), Ampiclox (30µg), Zinnacef (20µg), Amoxacillin (30µg), Rocephin (30µg) Streptomycin (30µg), Erythromycin (10µg), Vancomycin (30µg), Oxacillin (10µg). A suspension of the bacteria in 1ml sterile normal saline (0.98%) was made to correspond to 0.5 McFarland standard. For *Salmonella*, *Staphylococcus* and *E. coli*, a sterile pipette was used to inoculate 100µl of bacteria suspension onto Mueller–Hinton agar (Oxoid: CM337), For *Arcobacter*, the suspension was supplemented with 5% (v/v) defibrinated sheep blood and 7% w/w yeast extract. Each antibiotic disc was placed onto the agar with the aid of a sterile forceps and incubated at 37°C for 24 hours in air and microaerophilically for *Arcobacter*. The sensitivity plates were read and interpreted as recommended by NCCLS (2005) (now Clinical and Laboratory Standards Institute, CLSI).

RESULTS AND DISCUSSION

This study describes the prevalence of foodborne pathogens in retail meat in Osogbo. Overall, 138(46%) of 300 meat samples were contaminated with *S. aureus* species. Also, out of 75 of each sample of chicken, goat and pork, 60(80%), 9(12%) and 48(64%) were positive for *S. aureus* respectively. This is comparable to the result obtained in Akure, Nigeria where *S. aureus* was isolated at higher frequency than *Bacillus subtilis*, *Corynebacterium* sp., *Escherichia coli*, *Salmonella* sp. and *Lactobacillus salivarius* (Onibi and Osho, 2006). Conversely, *Escherichia coli*, *Proteus* and *Pseudomonas* were the most frequent isolates in a study conducted in Jos, Nigeria with 16%, 14% and 7% respectively while *S. aureus* was the highest contaminants (Ameh and Amadusa, 2007).

Table 1: Occurrence of Bacterial Isolates among the Various Meat Samples

<i>Meat Sample</i>	<i>Arcobacter species</i>	<i>Escherichia coli</i>	<i>Salmonella species</i>	<i>Staphylococcus aureus</i>
Chicken (n=75)	24 (32 %)	12 (16 %)	0 (0 %)	60 (80 %)
Beef (n=75)	12 (16 %)	36 (48 %)	0 (0 %)	21 (28 %)
Goat (n=75)	0 (0 %)	0 (0 %)	0 (0 %)	9 (12 %)
Pork (n=75)	21 (28%)	30 (40 %)	6 (8 %)	48 (64 %)
TOTAL (N=300)	57 (19%)	78 (26%)	6 (2%)	138 (46%)

Legend: n = Sample size for each meat carcass; N= Total sample size

Table 2: Viable Counts of Bacterial Isolates from Meat Samples

Organisms	Chicken (cfu/g)	Beef (cfu/g)	Goat (cfu/g)	Pork(cfu/g)	P-value
<i>Arcobacter spp</i>	3 x 10 ⁶	2 x 10 ⁴	2 x 10 ²	3 x 10 ⁵	0.004
<i>E. coli</i>	3 x 10 ⁴	3 x 10 ⁵	2 x 10 ²	4 x 10 ⁵	0.002
<i>Staphylococcus aureus</i>	5 x 10 ⁸	4 x 10 ⁶	3 x 10 ⁴	3 x 10 ⁶	0.001
<i>Salmonella spp</i>	-	-	-	2 x 10 ³	0.006

The high isolation rate of *S.aureus* in this study indicates poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilisation of utensils and working surfaces (Plaatjies *et al.*, 2004). Enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world in which red meat and poultry products have been implicated (Normanno *et al.*, 2005).

Escherichia coli had the highest occurrence in beef (48%) (Table 1). The high level of this microbial contamination obtained in this study could be an indication of faecal spillage of the gut contents of the cow carcasses (Table 2). Almost all the abattoirs where samples were purchased from were located near flowing streams, from which the faecal content are usually disposed as well as for meat processing. It is however remarkable that *Arcobacter spp.* and other organisms were not detected in goat meat sampled in this study except *S. aureus*. The absence of these pathogens besides *S.aureus* in the goat meat may possibly be due to the hygienic processing and clean environment of the government abattoir where the goat meat samples were purchased.

Arcobacter sp. showed highest occurrence in chicken (32%) followed by pork (28%), thus corroborating a report that *Arcobacter* is commonly isolated with high frequency in chicken carcass than from red meats (Houf *et al.*, 2005).

Salmonella was not detected in any of the meat samples except in pork with a prevalence rate of 8% (Table 1). The low prevalence of *Salmonella spp.* in this study could be due to the fact that pre-enrichment step and selective media required for optimal isolation of *Salmonella* was not used in this study because of inadequate funds. Nevertheless, the health hazard from *Salmonella* must not be underestimated. In Belgium, *Salmonella* serovar Typhimurium was responsible for 49.4% of reported human cases of gastrointestinal diseases (Collard *et al.*, 2008) and it was the predominant serovar isolated from pork during monitoring in the European Union (European Food Safety Authority, 2004) suggesting that consumption of pork is a major risk factor for human salmonellosis. It therefore seems appropriate that regular quantitative risk assessment of pork meat should be adopted to ensure its safety for human consumption.

The mean viable bacterial count in meat samples are shown in table 2. *Arcobacter* ranged from 2 x 10² to 3 x 10⁶ *E. coli*, 2 x 10⁴ to 4 x 10⁵, *Staphylococcus aureus* 3 x 10⁴ - 5 x 10⁸ and *Salmonella* 2 x 10³ (Pork samples only). Based on the Public Health Laboratory Service recommendation, the detection limit exceeded acceptable range of 100 cfu/g for *Salmonella* and 100000cfu/g for enterobacteriaceae (FSIS, 1999). It is apparent that these heavy microbial loads are indications of low hygienic abattoir procedures.

The antimicrobial susceptibility results revealed that most *S. aureus* isolates were susceptible to gentamycin, pefloxacin, zinnacef, streptomycin, erythromycin, vancomycin and oxacillin but resistant to ampiclox, rocephin and amoxicillin. All the fifty-seven *Arcobacter* isolates were resistant to cotrimoxazole, vancomycin, oxacillin and amoxicillin. Over eighty percent of the organism was resistant to gentamicin {48(84.2%)} while {48(84%)} were susceptible to tetracycline, pefloxacin, ofloxacin and 39 (68.4%) to ciprofloxacin. This finding is similar to a previous report where almost all the strains tested showed resistance to vancomycin (100%) and methicillin (97.5%) and all were susceptible to ampicillin, tetracycline, streptomycin and kanamycin (Kabeya *et al.*, 2004). While all 78 *Escherichia coli* strains were sensitive to the antibiotics tested, 66(84.6%) were resistant to amoxicillin. The six (6) isolated *Salmonella species* exhibited one hundred percent susceptibility to cotrimoxazole, ofloxacin, ciprofloxacin tetracycline and pefloxacin but were all resistant to amoxicillin.

This pattern of resistance may be attributed to frequent use of amoxicillin, as first line drug for patients presenting with diarrhoeal illness (Adeleye *et al.*, 2008) and also its use in food animals for therapy, prophylaxis and growth promoter in this environment (Okoli *et al.*, 2006b). Moreover, the high prevalence of resistant *Salmonella* in retail meats reflects a reservoir of resistance in animals that can be transmitted to humans. Therefore, efforts are needed to reduce the prevalence of resistant *Salmonella* in food, including the adoption of guidelines for the prudent use of antimicrobial agents in animals used for food, enforcement of food-safety regulations and strict hygienic procedures in slaughterhouses.

The emergence of resistant bacteria and resistance genes following the use of antimicrobial agents in food animals is relatively well documented and it appears that all antimicrobial agents may select for resistance (White *et al.*, 2001). In conclusion, this study offers base-line information that could serve as a basis for further study on the impact on food-borne pathogens on human health in the study area. Speciation of the bacterial pathogens, studies on serotype distribution patterns, genotype profiles of antimicrobial resistant *Salmonella* and lack of corresponding culture data from humans to support the hypothesis that food supply is a major source of antimicrobial resistant *Salmonella* present limitations in interpretation of our data.

REFERENCES

- Adeleye A, Smith S, Akanmu S, Bamiro S, Sobande O, Igbinosum E and Inem V (2008). Chromosomally-mediated Antibiotic Resistance in Non-typhoidal *Salmonellae* Isolated from HIV Patients in Lagos. *West Indian Med.* **57**(5): 519-20
- Adeyemo OK (2002). Unhygienic Operation of a City Abattoir in Southwestern Nigeria: Environmental Implication. *Afr J Environ Assessment and Management.* **4**: 23-28
- Ameh IG and Amadusa JE (2006). Bacterial Isolates from Raw Beef Retailed at Rukuba Market, Jos, Nigeria. *Animal Prod Res Adv.* **4**: 2-4
- Bauer AW, Kirby WMM Sherris JC and Turch M (1966). Antibiotic testing by standardized single disk method. *Am J Clin Path.* **45**: 493-496
- Cardoen S, Van Huffel X, Berkvens D, Quoilin S, Ducoffre G, Saegerman C, Speybroeck N, Imberechts H, Herman L, Ducatelle R and Dierick K (2009). Evidence-Based Semiquantitative Methodology for Prioritization of Foodborne Zoonoses. *Foodborne Pathogens Dis.* **6**(9): 1083-1096
- Cohen N, Ennaji H, Bouchrif B, Hassar M and Karib H (2007). Comparative Study of Microbiology Quality of Raw Poultry Meat at Various Seasons and For Different Slaughtering Process in Casablanca (Morroco). *J Appl Poult Res.* **16**: 502-508
- Collard JM, Bertrand S, Dierick K, Godard C, Wildemaue G, Vermeersch K, Duculot J, Van Immerseel F, Pasmans F, Imberechts H and Quinet C (2008). Drastic Decrease in *Salmonella* Enteritidis Isolated from Humans in Belgium in 2005, Shift in Phage Types and Influence on Foodborne Outbreaks. *Epidemiol Infect.* **136**:771-781
- European Food Safety Authority (2004). Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. *EFSA J.* **310**:1-278
- FAO/WHO (2002). Global Forum of Food Safety Regulators. *Improving Efficiency and Transparency in Food Safety Systems Sharing Experiences*, Marrakesh, Morocco:
www.fao.org/docrep/meeting/004/Y3680E/Y3680E02.htm
- FSIS (1999). FSIS Action Plan for Addressing Food Pathogens U.S. Department of Agriculture, Food

Safety and Inspection Service, Washington, DC

Houf K, On SL, Coenye T, Mast J, Van Hoof J and Vandamme P (2005). *Arcobacter cibarius* sp. nov., isolated from broiler carcasses. *Int. J. Syst. Evol. Microbiol.* **55**: 713–717

Kabeya H, Maruyama S, Morita Y, Ohsuga T, Ozawa S, Kobayashi Y, Abe M, Katsube Y and Mikami T (2004). Prevalence of *Arcobacter* Species in Retail Meats and Antimicrobial Susceptibility of the Isolates in Japan. *Int J Food Microbiol.* **90**:303–308

Murray PR, Baron EJ, Pfaller MA, Tenover FC and Tenover RH (2005). In Nachamkin: *Campylobacter* and *Arcobacter*. In Manual of Clinical Microbiology 7th ed. Washington DC ASM Press. Pp: 716-726

National Committee for Clinical Laboratory Standards (NCCLS) (2005). Performance Standards for Antimicrobial Disk Susceptibility (Approved Standards, M2-A4) Villanoa, 2005; PA National Committee For Clinical Laboratory Standards

Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Decastelli L, Mioni R, Scuota S, Bolzoni G, Di Giannatale E, Salinetti AP, La Salandra G, Bartoli M, Zuccon F, Pirino T, Sias S, Parisi A, Quaglia NC and Celano GV (2005). Coagulase-positive Staphylococci and *Staphylococcus aureus* in Food Products Marketed in Italy. *Internatn J Food Microbiol.* **98**: 73-79

Oboegbulam SI, Oguike JU, Gugnani HC (1995) Microbiological Studies on Cases Diagnosed as Typhoid/Enteric Fever in South-east Nigeria. *J Commun Dis.* **27**:97

Okoli CG, Okoli IC, Okorundu UV and Opara MN (2006a). Environmental and Public Health Issues of Animal Food Products Delivery System in Imo State, Nigeria. *J Health Allied Sci.* **5**: 2-2

Okoli IC, Ndujihe GE, Ogbuewu IP (2006b). Frequency of Isolation of *Salmonella* From Commercial Poultry Feeds And Their Anti-Microbial Resistance Profiles, Imo State, Nigeria *Online J Health Allied Scs.* **4**:2:3

Olugasa BO, Cadmus SIB and Atsanda NN (2000). Actualization of Strategies for Beef Quality Control in Reply to Southwestern Nigeria In Tielon MJM and Voets MTH (Eds.) In *Proceedings of the XTH*

International Congress on Animal Hygiene. Maastricht, Netherlands, ISAH. **1**: 67-71

Onibi GE and Osho BI (2007). Oxidative Stability and Bacteriological Assessment of Meat from Broiler Chickens Fed Diets Containing *Hibiscus sabdariffa* Calyces. *Afr J Biotechnol.* **6** (23): 2721-2726

Uche UE and Agbo JAC (1985). Bacterial Isolates from Nsukka Market. *Int J Zoonosis.* **12**: 105-110

Vandamme P, Vancanneyt M, Pot B, Mels L, Hoste B, Dewettinck D, Vlaes L, van den Borre C, Higgins R, Homme J, Kersters K, Butzler JP and Goossens H (1999). Polyphasic Taxonomic Study of the Emended Genus *Arcobacter* with *Arcobacter* comb. nov. and *Arcobacter skirrowii* sp. nov., an Aerotolerant Bacterium Isolated from Veterinary Specimens. *Int J Syst Bacteriol.* **42**: 344–356

Van den Bogaard AE and Stobberingh EE (2000). Epidemiology of Resistance to Antibiotics: Links between Animals and Humans. *Int J Antimicrob Agents.* **14**:327–335

Van TH, Moutafis G, Tran LT and Coloe PJ (2007). Antibiotic Resistance in Food-Borne Bacterial Contaminants in Vietnam. *Appl Environ Microbiol.* **73**(24): 7906–7911

White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD and Meng J (2001). The Isolation of Antibiotic-Resistant *Salmonella* from Retail Ground Meats. *N Engl J Med.* **345**:1147-1154