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Original Article

Bacterial Contamination Associated with Retail Chicken Carcasses in Osogbo, Nigeria.

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Abstract

Background: Worldwide, food borne illness is often associated with consumption of meat and poultry products sold at retail markets. A study on the bacteriological status of chicken carcass in Osogbo, Nigeria, was carried out to determine the prevalence of *Arcobacter* species, *Escherichia coli* and *Staphylococcus aureus* in chicken carcasses.

Methodology: A total of 100 samples of chicken carcasses were collected from two major processing points in Osogbo, Nigeria. The samples were analysed for the presence of bacterial contaminants using standard microbiological isolation and identification procedures, with antimicrobial susceptibility test performed using the disk diffusion method.

Results: Of hundred chicken carcasses sampled, 38% were positive for *Arcobacter* species and *E. coli* while 60% accounted for *S. aureus* isolates. Ninety percent of *Arcobacter* spp isolates were susceptible to ciprofloxacin, 85% to gentamicin, and pefloxacin, 70% to chloramphenicol and 90% were resistant to amoxicillin, 85% to augumentin and 80% to streptomycin. Hundred percent of *E. coli* isolates were susceptible to ciprofloxacin, pefloxacin, 95% to gentamicin and 100% were resistant to streptomycin, 85 % resistant to amoxicillin, augumentin, while 100% of S. *aureus* isolates were susceptible to trimethoprim sulphamethoxazole, 90% susceptible to gentamicin, 80% to streptomycin and 100% of the S.*aureus* isolates were resistant to ampliclox.

Conclusion: The bacteriological status of chicken carcass revealed high contamination with *Arcobacter, E coli* and *S. aureus* with varying degree of antibiotic resistance therefore, improvement in meat processing procedures and strict hygiene measures towards reduction of these pathogens in food products should be encouraged.

Keywords: Arcobacter, Escherichia coli, Staphylococcus aureus, Chicken carcass, Food safety, Nigeria.

Introduction

Some microorganisms such as *Salmonella*, *Listeria* and *Campylobacter* cause food borne diseases in human, which some control and food safety measures are not able to prevent.[1] In recent years, food borne infections and intoxications have assumed significance as a health hazard.[2] Food production occurs in several stages, each of which provides potential opportunities for bacterial contamination. Poultry processing plants (PPPS) are favorable environments for the survival and transmission of various commensal spoilage and potentially pathogenic bacteria throughout the human food chain.[3] Epidemiological reports suggest that poultry meat is one of the major causes of human food poisoning,[4] with humans often infected through consumption of contaminated foods of animal origin.[1] *Staphylococcus aureus* is a very common organism capable of producing several enterotoxins (SEs) that cause intoxication symptoms of varying intensity in humans when ingested through food

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and also in the evaluation of safety and hygienic quality of chicken meat.[5] The presence of *Escherichia coli* on the chicken carcass usually indicates a direct or indirect faecal contamination of meat.[6] The genus *Arcobacter* has become increasingly important in recent years because its members have been considered potential emerging food and water borne pathogens.[7] This genus is an atypical group within the epsilon subdivision of the proteobacteria because of its wide diversity of habitats and hosts. In animals, arcobacters have been implicated in abortions, mastitis, and gastrointestinal disorders but have also been recovered from asymptomatic animals.[8] The recent increase in isolation of *Arcobacter* from clinical, food and animals sources, has led to it been classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods.[9]

Arcobacter have been detected in chicken meat much more than other bacterial organism.[10] In developing countries, food borne illness causes human sufferings and loss of productivity and adds significantly to the cost of food production and health care.[11] In Nigeria, unhygienic sanitary conditions of our abattoirs is a matter of concern.[12] Since microbiological examination of meat is an important aspect of meat inspection and meat hygiene for food security,[13] as chicken is one of the meats consumed in Osogbo and there is a dearth of information on bacterial contamination of raw chicken meat. The aim of this study was to report the prevalence of *Staphylococcus aureus*, *Escherichia coli* and *Arcobacter* species in chicken carcass sold at two major processing units in Osogbo.

Materials and Method

Sample collection

A total of 100 samples (neck skin of chicken carcasses being the site with probable highest concentration of Arcobacter) [10] were purchased from major market and supermarket in Osogbo, Nigeria for a six month period. Clean and sterile universal bottle was used to collect the neck skin of chicken carcasses used.

Method of isolation

Arcobacter

One gram (1g) of the neck skin was inoculated directly into 9 ml of an *Arcobacter* enrichment broth containing 24 g/L of *Arcobacter* base broth (Oxoid) supplemented with cepoferazone (12 mg), amphotericin B (10 mg) and teicoplanin (8 mg) -CAT for enrichment and incubate at 37 °C in air 48 hours. And later grown on *Arcobacter* selective agar plate (containing 24 g/l *Arcobacter* broth, 12g/l Agar Technical No.3 [L13-Oxoid] for 24 hours in a micro aerophilic atmosphere for 48°C. Plate cultures were later examined for presence of bacterial colonies with morphological features similar to those already described for *Arcobacter* spp.[14]

Escherichia coli

One gram of neck skin of chicken carcass was inoculated in 9 ml of peptone water and homogenized properly. A loopful of the enrichment was streaked on MacConkey agar plate using a sterile wire loop and incubated aerobically at 37 ºC for 24 hours. Lactose fermenting colonies that appeared pink were later plated on Eosin Methylene Blue Agar, those that appeared as metallic sheen with further biochemical tests were confirmed positive for *Escherichia coli* .[15]

Staphylococcus aureus

One gram of neck skin of chicken carcass was inoculated in 9 ml of peptone water, the sample was homogenized in a sterile blender for 2 min. The homogenate were transferred into a sterile wide mouth, screw capped jar and incubated for 6hours at room temperature. After this pre-enrichment step, 1 ml from this homogenate were transferred into enrichment broth consisting of 10 g tryptone/L, 75 g sodium chloride (NaCl)/L, 10 g mannitol / L, and 2.5 g of yeast extract/ L. After 24hours incubation at 37°C, 100 µ L of broth were introduced into enrichment broth MHB (Mueller Hinton Broth) +6.5%NaCl and homogenized. The suspension was incubated for 16–20hours at 37 °C. One ml of the enriched broth followed by incubation for 16–20 hours at 37 °C were plated on the surface of Mannitol salt agar. The plates were examined for typical staphylococcal colonies after incubation. For confirmation, typical colonies per plate were selected and

sub-cultured on Blood agar and MRSA ID (bioMérieux) agar plates and later confirmed as *S. aureus* by colony morphology, Gram stain appearance, catalase, coagulase reactions and were also confirmed using API biomerix (France).

Antimicrobial Susceptibility testing

The following antibiotics discs were used for Gram negative organisms: amoxicillin (30µg). augumentin (10µg), chloramphenicol (30µg), ciprofloxacin (10µg), gentamicin (10µg), ofloxacine (10µg), pefloxacin (30µg), Trimethoprim sulphamethoxazole (30µg), sparfloxacin (10µg) and streptomycin (30µg) while ampiclox (30 g), erythromycin (10µg), pefloxacin (10µg), gentamicin (10 g), ciprofloxacin (10µg), streptomycin (30µg) and Trimethoprim sulphamethoxazole (30µg) were used for Gram positive organism in antimicrobial susceptibility testing.

Antimicrobial susceptibility test was performed using the disk diffusion method and isolates categorized as susceptible and resistant were based upon interpretative criteria developed by the Clinical and Laboratory Standards Institute.[16]

Results

A total of hundred samples of chicken carcasses were collected from two major processing points in Osogbo. Twenty five chicken meat samples were from fresh market and 75 frozen chicken meat samples from processing farm. Results of isolation of bacteria from chicken carcasses in Osogbo are summarized in Table 1. Frozen chicken samples from location A was obtained from a processing farm while fresh chicken samples from location B was from the traditional market. Out of 100 necks skin samples studied, 38 (38 %) tested positive for *Arcobacter*, 38 (38 %) for *Escherichia coli* and 60 (60%) for *Staphylococcus aureus*. Antimicrobial susceptibility testing was also performed on the isolates and the result is summarized in table 2. Ninety percent of *Arcobacter* spp isolates were susceptible to ciprofloxacin, 85% to gentamicin, and pefloxacin, 70% to chloramphenicol and 90% were resistant to amoxicillin, 85% to augumentin and 80% to streptomycin. Hundred percent of *E. coli* isolates were susceptible to ciprofloxacin, pefloxacin, 95% to gentamicin and 100% were resistant to streptomycin, 85 % resistant to amoxicillin, augumentin, while 100% of S. aureus isolates were susceptible to

Table 1 : Occurrence rates of bacterial isolates in chicken from fresh market and processing unit

Sample isolates	Fresh Chicken market n^1=25	% occurrence	Chicken Processing unit n^2=75	% occurrence	f-value	Sig.
Arcobacter spp.	13	52	25	33.3		P<0.05
E.coli	14	56	24	32	9.87	P=0.0000
S. aureus	21	84	39	53		P=0.003

Table 2 : Antibiotics susceptibility of isolates from chicken carcasses

Class of Antibiotics	Arcobacter		E. coli		S. aureus	
	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
Amoxicillin	2 (10)	18 (90)	3 (15)	17 (85)	2 (10)	18 (90)
Augmentin	3 (15)	17 (85)	3 (15)	17 (85)		
Chloramphenicol	14 (70)	6 (30)	10 (50)	10 (50)		
Ofloxain	16 (80)	4 (20)	20 100)	0 (0)		
Sparfloxacin	17 (85)	3 (15)	16 (80)	4 (20)		
Pefloxin	17 (85)	3 (15)	20 100)	0 (0)	20 (100)	0 (0)
Gentamicin	17 (85)	3 (15)	19 (95)	1 (5)	18 (90)	2 (10)
Streptomycin	4 (20)	16 (80)	0 (0)	20 (100)	18 (90)	2 (10)
Trimethoprim/sulphamethoxazole	5 (25)	15 (75)	10 (50)	10 (50)	16 (80)	4 (20)
Ciprofloxacin	18 (90)	2 (10)	20 (100)	0 (0)	20 (100)	0 (0)
Ampiclox					0 (0)	20 (100)
Erythromycin					18 (90)	2 (10)

INDEX :
AM- Amoxicillin (30 µg),
AU -Augumentin (10 µg),
CH - Chloramphenicol (30 µg),
OFX - Ofloxacin (10 µg),
SP - Sparfloxacin (10 µg),
PEF - Pefloxacin (30 µg),
CN Gentamicin (10 µg),
S - Streptomycin (30 µg),
SXT - Trimethoprim /sulphamethoxazole (30 µg),
CPX - Ciprofloxacin (10 µg),
APX - Ampliclox (30 µg),
E - Erythromycin (10 µg),
S - Sensitive
R -Resistant.

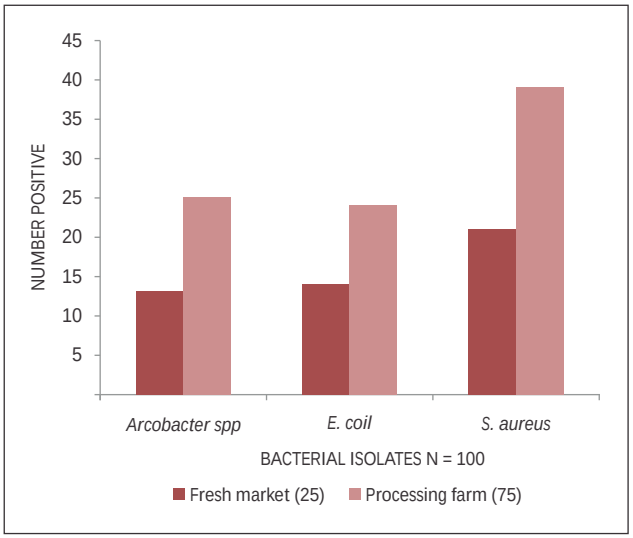

Figure 1 : Prevalence of bacterial isolates from study location

trimethoprim sulphamethoxazole, 90% susceptible to gentamicin, 80% to streptomycin and 100% of the *S aureus* isolates were resistant to ampiclox. Figure 1 is a chart showing the prevalence of bacterial isolates from the two study locations.

Discussion

It is well documented that contamination of food with pathogens is a major public health concern worldwide.[17] As a result of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw poultry products are reported to be responsible for a significant number of cases of human food poisoning.[18]

Poultry meats passed for human consumption in Osogbo are from two sources fresh markets and supermarkets. Fresh markets are traditional open air markets where chickens are sold by individual vendors or farmers, and often sold and stored at ambient temperatures. These markets naturally have multiple sources of potential contamination (rodents, insects, sewage). Processing plant consist of automated machines for carcass defeathering and cutting meats in pieces where they are packaged and distributed to various supermarkets in town which typically offer controlled temperature environments and more hygienic conditions for marketing.

In this study, 75 of 100 samples were obtained from frozen chicken of which 25(33.3 %), 24 (32%), 39(52%) were positive for *Arcobacter* spp, *E. coli* and *S. aureus* respectively. Twenty five of 100 samples were also obtained from fresh chicken carcass processed in the traditional way in which 13(52 %) of *Arcobacter* spp, 14 (56%), E. coli and 21 (84 %) of *S. aureus* was isolated. This study also document a more significant T- test ($P< 0.05$) bacteria isolation rate from fresh chicken compare with the frozen samples. Moreover, it has been shown that fresh meat samples often yield more bacteria than frozen samples,[19] it is also believed that bacteria are killed during freezing and thawing of meat,[20] therefore, culturing frozen meat may dramatically reduce or change the microorganisms isolated in the laboratory.

In this study 38 % of the chicken carcasses were positive for *Arcobacter* species when compared to studies from other countries, it was found that the prevalence rate of *Arcobacter* spp in retail meat varies widely in different countries: 23 % in Japan,[21] 40 % in Mexico[22] and 100 % in Thailand.[23] The variation in isolation rates were attributed to different isolation protocols, sample size, hygienic practices and geographical location where the study was carried out. It is also possible that the low prevalence rate (38 %) obtained from this study could be as a result of fewer antimicrobial supplement and impoverized incubating condition (candle jar) used, compared to other studies where more antimicrobial supplements and adequate microaerobic culture atmosphere for primary isolation were used.[24] The rate of microbial contamination of chicken carcass with E. coli in this study was 38 %. Raw poultry meats are commonly contaminated with *E. coli*; this is particularly true of chicken products. Detection of *E. coli* in food sample is often as a result of faecal contamination.[11]

The reported prevalence of *S. aureus* in retail meats varies widely in different countries. In the present study, 60 % of the chicken carcass samples were positive for *S. aureus* being the most occurring isolates. This could be a reflection of the fact that *S. aureus* can be introduced into chicken from several sources such as the skin of handlers,[25] through the use of unsanitary procedure and equipment.

Other accompanying flora in the course of this experiment

includes *Pseudomonas* spp., *Proteus* spp. which can be opportunistic pathogens of humans. Out of the 38 isolates of *Arcobacter* isolated from chicken samples 20 isolates were subjected to susceptibility testing. Among the 20 isolate for *Arcobacter*, 18 (90%) were found to be susceptible to ciprofloxacin which was in line with the work carried out in Belgium[6] where both *A. butzleri* and *A. cryaerophilus* were found to be susceptible to ciprofloxacin, high resistance was found to chloramphenicol, trimethoprim/sulphamethoxazole, amoxicillin, augumentin and streptomycin. The isolate was also found to be susceptible to gentamicin, sparfloxacin and ofloxacin. These antimicrobial susceptibility result obtained from this study is comparable to those of Atabay*et al*[26], who find out that all 39 *Arcobacter* broiler isolates were susceptible to aminoglycoside (gentamicin).

The low resistance rate to fluoroquinolones observed in this study had been reported in a previous study in which its use was suggested to be used for the treatment of severe *Arcobacter* enteritis.[24]

A study carried out in Imo state, Nigeria, to screen for anti-microbial resistance profile of *E. coli* isolates from rural and urban chicken carcass showed that the anti-microbial resistance of the isolates against ampicillin and chloramphenicol (72–92 % respectively) were very high.[27] The organisms were highly sensitive to other antibiotics, especially gentamicin and ciprofloxacin. This sensitivity profile is comparable with what was obtained in this study (Table 2). However, most*E.coli* isolates in this study were resistant to augumentin and amoxicillin which are the first line drugs often prescribed in patient presenting with gastrointestinal disturbance in Nigeria. The present study also revealed that *S. aureus* isolates were susceptible to pefloxacin, erythromycin, the presence of resistant strain should not be ruled out as two isolates were resistant to erythromycin and all isolates were resistant to ampliclox.

In conclusion, the bacteriological status of chicken carcass revealed contamination with *Arcobacter*, *E. coli* and *S. aureus* with varying degree of antibiotic resistance.

In order to reduce the risk represented by zoonotic agents to the consumer health, it is essential to reduce contamination of carcasses during the slaughtering processes. Therefore the maintenance of slaughter hygiene and marketing conditions to retain keeping quality is consequently of central importance in meat production.

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