



Incidence and Antimicrobial Susceptibility of Coliforms in Broiler Products at the North West Province of South Africa

ABSTRACT

This study was conducted to evaluate the incidence and antibiotic resistance of coliforms in raw and ready-to-eat broiler products in the North West Province of South Africa. A total of 120 raw and 60 ready-to-eat samples obtained from butcheries and supermarkets were screened for the presence of coliforms. Coliform species were identified by correlating cultural characteristics on MacConkey and Blood agars and biochemical reactions on Enteropluri-test®/API 20NE. Identified isolates were then tested for antibiotic resistance by disk diffusion method using ampicillin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), trimethoprim-sulphamethoxazole (25 µg), cefotaxime (30 µg), meropenem (10 µg), gentamicin (10 µg) and erythromycin (15 µg). The results showed that 75% (n = 90/total raw) of raw and 25% (n = 15/total RTE) of RTE products were contaminated with various genera of coliforms. A high frequency of resistance was manifested to ampicillin (93.6%) and erythromycin (97.9%), whereas resistance to other antibiotics tested was moderate to low (27.7% to 6.4%). However, *Yersinia pseudotuberculosis*

(from raw chicken) and *Stenotrophomonas maltophilia* (from polony) were both resistant to seven out of the eight antibiotics tested. The results indicate that consumption of poultry products can increase consumer exposure to antibiotic resistant organisms. This is the first report on the incidence, in RTE foods in the NWP of South Africa, of *Stenotrophomonas maltophilia*, a bacterium that easily transfers antibiotic-resistance genes to other bacterial species/genera in the same family.

INTRODUCTION

Chicken meat contains essential micro- and macronutrients, making it high in nutritive value (32). It thus constitutes a part of balanced diet, with a good consumption rate among South Africans (34). However, poultry meat is often contaminated with pathogens such as pathogenic *E. coli* O157:H7 (35), a situation due to the high nutritive value of the meat, which creates an ideal culture medium for the growth of many organisms (2). The fact that many of the contaminating organisms are associated with foodborne illnesses in humans makes their potential

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presence in meat products a matter of public health concern (11). For instance, a report from the World Health Organization showed an annual estimate of 1.8 million deaths resulting from diarrheal diseases caused by microbial pathogens (36). Recent changes in eating habits, mass catering services, and poor hygienic practices have been identified as contributing factors to this plight, particularly in developing countries (17).

Sources of poultry meat contamination with pathogenic microbes include the contents of the intestinal tract of slaughtered birds, fecal material on feet and feathers, meat handlers, product packing and marketing (2, 32). Globally, the most challenging contaminants of raw and processed meat products are members of the family *Enterobacteriaceae*, particularly *Salmonella*, *Escherichia*, *Proteus* and *Klebsiella* (2). Most genera of *Enterobacteriaceae*, which also include *Enterobacter*, *Serratia*, *Stenotrophomonas* and *Yersinia*, are overt and opportunistic pathogens, many of which have been isolated from hospital settings, where they are strongly associated with nosocomial infections (24, 30). The disease burden caused by the pathogens is further increased by high levels of antibiotic resistance, leading to complications in the treatment of clinical cases.

In addition to causing diseases in humans, species of *Proteus*, *Salmonella*, *Yersinia* and *Citrobacter* have been incriminated in spoilage of refrigerated meat and poultry products (21). In the U.S., for example, reports have shown annual wastage of approximately 3.5 billion kg of meat products at the retail and consumer levels, due mainly to microbial spoilage (22).

The burden of disease and losses from product waste as a result of microbial contamination calls for proper monitoring of the pathogens in meat and poultry products, and for the development of effective control strategies. A few studies have been conducted in South Africa to assess microbial contamination of ready-to-eat (RTE) street-vended foods (5, 26). However, there is inadequate information on microbial contamination of raw and RTE poultry products that are consumed with or without further processing. Therefore, the present study was conducted to evaluate the incidence and antibiotic resistance of coliforms in raw and RTE broiler products obtained from the North West Province (NWP) of South Africa.

MATERIALS AND METHODS

Sampling

A cross-sectional study was carried out in the Northwest Province of South Africa, where 180 samples of both raw and RTE chicken products were obtained from various retail outlets. The outlets sampled consisted of supermarkets (n = 120) and butcheries (n = 60). Samples obtained included whole broiler carcasses (n = 45), raw chicken portions (n = 75) and RTE products such as polonies and viennas (n = 60). The sampling was done during the summer months, when

conditions are expected to favor growth of many bacteria. Samples were purchased under refrigeration conditions, held in separate sterile plastic containers at 4°C and transported immediately to the laboratory for analysis. All samples purchased were within the expiration date for consumption.

Bacterial culture and isolation

To evaluate the coliform contamination of the samples, 25 g of each sample was aseptically removed and homogenized with 225 ml of buffered peptone water (BPW) in a stomacher bag (Nasco, USA) (29). Each bag was sealed and incubated at 37°C for 18 hours. Following incubation, contents of each bag were homogenized, and 1 ml and 0.1 ml portions of the liquid were transferred to 10 ml each of Luria broth (LB) (Merck, SA). The broths were incubated at 37°C for 24 hours. A loopful of each broth sample was then streaked on MacConkey agar (MA) (Merck, SA) and Blood agar (BA) (Merck, SA). Plates were incubated at 37°C for 24 hours. Colonies obtained were further purified on MA. Gram staining and an oxidase test were performed on all obtained isolates. Gram-negative and oxidase-negative isolates were subjected to testing with Entero-pluritest®/API 20NE (Davies diagnostics, South Africa), performed according to manufacturer's instructions. The various coliform species were identified by correlating colony morphology, Gram stain results, oxidase reactions and biochemical characteristics.

Antimicrobial susceptibility testing

Isolates identified by biochemical characterization were tested for antimicrobial susceptibility by the Kirby-Bauer disc-diffusion method, as previously described (25), using a panel of eight antibiotics. This method conforms to the recommended standard of the Clinical and Laboratory Standards Institute (2001). *E. coli* ATCC 25922 was used as a control strain for the susceptibility testing. The antibiotics consisted of ampicillin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), trimethoprim-sulphamethoxazole (25 µg), cefotaxime (30 µg), meropenem (10 µg), gentamicin (10 µg) and erythromycin (15 µg).

RESULTS

Coliform bacterial species in raw broiler products

Coliforms were identified in 90 (75%) raw broiler products analyzed. A total of eight genera of coliforms belonging to the family *Enterobacteriaceae* were isolated from raw products. These included four species of *Enterobacter* (n = 34), three species of *Klebsiella* (n = 13), two species of *Citrobacter* (n = 6) and one species each of *Serratia* (n = 14), *Hafnia* (n = 9), *Escherichia* (n = 11), *Yersinia* (n = 1), and *Proteus* (n = 2) (Table 1). In some instances, multiple isolates were identified in one sample, with two genera/species, although this finding usually involved fewer pathogenic strains.

Coliform bacterial species in RTE chicken products

Coliforms were isolated from 15 (25%) of RTE products, and three genera of coliforms were identified, viz: *Stenotrophomonas* (n = 1), *Serratia* (n = 11) and *Klebsiella* (n = 3) (Table 2).

Antibiotic resistance patterns

The percentages of resistant coliform isolates to individual antibiotics are shown in Fig. 1. All isolates were resistant to ampicillin, with the exception of *E. sakazakii*, *K. ozonae*, and one strain of *E. coli*. Similarly, resistance to erythromycin was found in all isolates, except one strain of *E. cloacae*.

TABLE 1. Incidence of coliform bacterial species in raw broiler products

Coliform species	No. of positive samples	Percentage positive samples (%)
<i>Enterobacter cloacae</i>	24	26.7
<i>Enterobacter sakazakii</i>	4	4.4
<i>Enterobacter aerogenes</i>	4	4.4
<i>Enterobacter amnigenus 2</i>	2	2.2
<i>Serratia liquefaciens</i>	14	15.6
<i>Klebsiella pneumoniae</i>	11	12.2
<i>Klebsiella ornitholytica</i>	2	2.2
<i>Hafnia alvei</i>	9	10
<i>Citrobacter freundii</i>	4	4.4
<i>Citrobacter braakii</i>	2	2.2
<i>Escherichia coli</i>	11	12.2
<i>Yersinia pseudotuberculosis</i>	1	1.1
<i>Proteus mirabilis</i>	2	2.2
Total	90	100

TABLE 2. Incidence of coliform bacterial species in RTE poultry products

Coliform species	No. of positive samples	Percentage positive samples (%)
<i>Serratia liquefaciens</i>	11	73.3
<i>Klebsiella pneumoniae</i>	1	6.7
<i>Klebsiella ozonae</i>	2	13.3
<i>Stenotrophomonas maltophilia</i>	1	6.7
Total	15	100

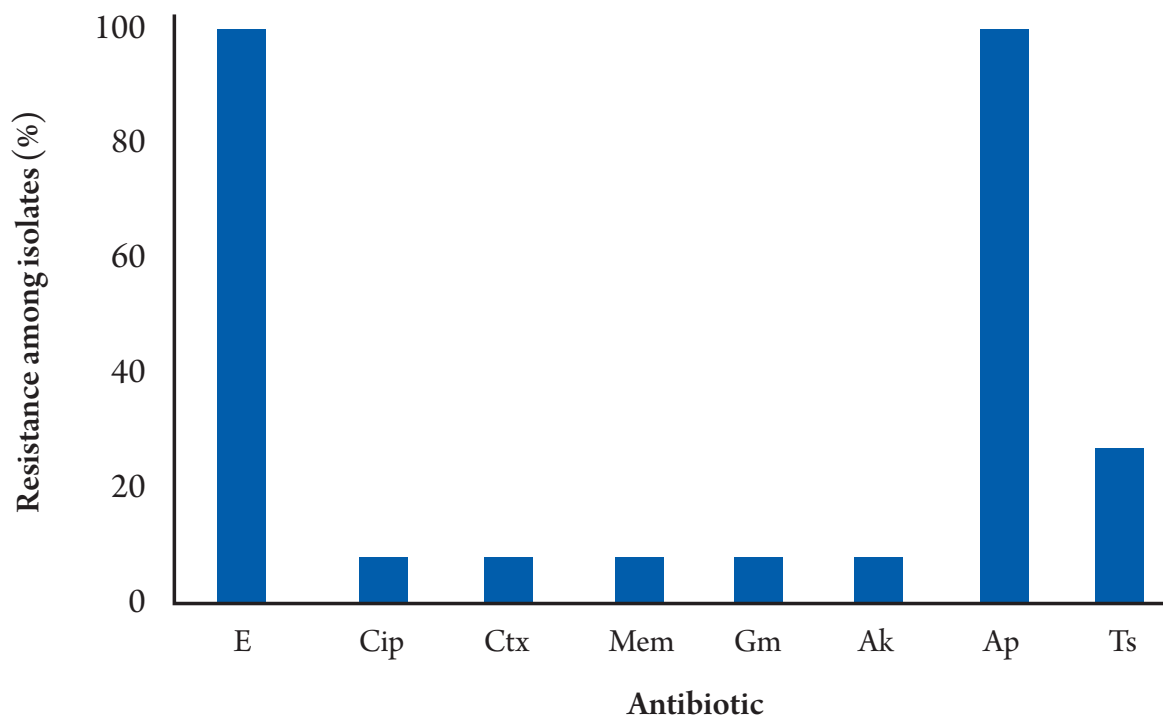


Figure 1. Percentage resistance of coliform isolates to individual antibiotics.

Cip = ciprofloxacin; Ctx = cefotaxime; Mem = meropenem; Gm = gentamycin;

Ak = amikacin; Ap = ampicillin; Ts = trimethoprim-sulphamethoxazole; E = erythromycin

Furthermore, *Y. pseudotuberculosis* and *S. maltophilia* were both resistant to seven out of the eight antibiotics tested, and were susceptible to only ciprofloxacin and trimethoprim-sulphamethoxazole, respectively. In addition, strains of *E. cloacae* showed four antibiotic resistance patterns, whereas *K. pneumoniae* showed three patterns. *S. liquefaciens*, *H. alvei* and *E. coli* had two resistance patterns each (Table 3).

DISCUSSION

Coliforms are ubiquitous in nature, being found in soil and water environments. However, intestinal tracts of warm-blooded animals remain the primary reservoirs; thus, coliforms are usually present in large numbers in the feces of such animals (18). Species such as *E. coli* are almost exclusively of fecal origin. Hence, their presence in foods can be an indication of fecal contamination. The recovery rate and variety of coliforms observed in this study may be a reflection of either poor hygienic conditions in the processing environment or post-processing contamination by personnel involved in packaging or handling. Javadi and Safarmashaei (20) also reported 100% incidence of coliforms in broiler meat marketed in Iran. An average of 50% in the incidence of coliforms, belonging mostly to genera identified in this study, has also been reported in vegetables (8). Saikia and Joshi (32) similarly noted a high incidence of *Enterobacter*

aerogenes (100%), *Escherichia coli* (98%), *Klebsiella pneumoniae* (98%), and *Proteus* (49%) and *Citrobacter* (52%) species in raw chicken products in Northeast India.

Furthermore, analysis of microbial contamination of raw meat products in Abakaliki, Nigeria revealed *Escherichia coli* and *Klebsiella pneumoniae* as the most frequent pathogens identified in the products (19). Members of the genus *Klebsiella* have also been recovered from free-range chicken samples in the Western Cape Province of South Africa (10). Thus, the results of the current study, together with reports from previous researchers, incriminate *Klebsiella* and *E. coli* as the most frequent contaminants of raw broiler portions. The incidence of *Enterobacter cloacae* (26.7%) (Table 1) observed in the current study is higher than the 9% incidence reported in street foods in Malaysia (16). In addition, *E. cloacae* and *E. sakazakii* have been isolated in infant formula (13.3 – 20%) and crushed wheat (33.3%), respectively (33). Although only a few of the genera of bacteria identified in the current study have been recognized as common pathogens in humans and animals, all can become pathogenic under special circumstances.

For instance, strains of *E. coli* are commonly associated with enterovirulent urinary tract infections and meningitis (15). Similarly, *Klebsiella pneumoniae*, a nosocomial pathogen associated primarily with respiratory and urinary

TABLE 3. Antibiotic resistance patterns of coliform species isolated from raw and RTE broiler products

Coliform species	Resistance patterns
<i>Enterobacter cloacae</i>	Cip-Ap-Ts-E; Ap-Ts-E; Mem-Ap-Ts; Ap-E
<i>Enterobacter sakazakii</i>	Cip-Ts-E
<i>Enterobacter aerogenes</i>	Gm-Ak-Ap-Ts-E
<i>Enterobacter amnigenus 2</i>	Ap-E
<i>Serratia liquefaciens</i>	Ap-Ts-E; Ap-E
<i>Klebsiella pneumoniae</i>	Ctx-Mem-Ap-Ts-E; Ap-Ts-E; Ap-E
<i>Klebsiella ozonae</i>	E
<i>Klebsiella ornitholytica</i>	Ap-E
<i>Hafnia alvei</i>	Ap-Ts-E; Ap-E
<i>Citrobacter freundii</i>	Ap-E
<i>Citrobacter braakii</i>	Ap-E
<i>Stenotrophomonas maltophilia</i>	Cip-Mem-Ctx-Gm-Ak-Ap-E
<i>Escherichia coli</i>	Gm-E; Ap-E
<i>Yersinia pseudotuberculosis</i>	Ctx-Mem-Gm-Ak-Ap-Ts-E
<i>Proteus mirabilis</i>	Ap-Ts-E

Cip = ciprofloxacin; Ctx = cefotaxime; Mem = meropenem; Gm = gentamycin; Ak = amikacin; Ap = ampicillin; TS = trimethoprim-sulphamethoxazole; E = erythromycin

tract infections, was reported as an enteroinvasive pathogen in 1998 (31). *Yersinia pseudotuberculosis* has also been identified as a cause of gastroenteritis and lymphadenitis in humans, with a fairly high case fatality rate (23) in the United States. *Enterobacter* species are important nosocomial pathogens, and are capable of causing various community-acquired infections (17). Although the pathogenic roles of *Serratia* and *Hafnia* species are yet to be defined, they are opportunistic pathogens that have also been incriminated in nosocomial infections (14, 28). Therefore, the observations in this study are of serious public health significance. This finding is important, given that when undercooked contaminated products are consumed, immunocompromised persons are the most often affected, usually with high fatality rates (6). In addition to the public health significance, *Proteus* and *Yersinia* are generally associated with spoilage of meat products (21), leading to product waste. In the case of RTE products, the recovery rate of coliforms observed in this

study is lower than the incidence of 42% and 75% reported for similar products in Korea and Taiwan, respectively (4, 9). Similarly, the incidence of *Stenotrophomonas maltophilia* (6.7%) in the current study is lower than the 78% reported by Qureshi et al. (27) in RTE salads in the United Kingdom. *Stenotrophomonas maltophilia* has been incriminated in cases of meningitis and pacemaker endocarditis (30).

Multiple antibiotic resistance was noted among coliforms isolated in the current study. Members of the *Enterobacteriaceae* family are commonly associated with production of extended spectrum β -lactamases (ESBLs), which enables their resistance to β -lactam antibiotics (7, 13). This attribute may be responsible for the observations of the current study. Multiple antibiotic resistance was similarly reported in large proportions of *Klebsiella* species and *E. coli* isolated from slaughtered chickens in Italy (37). Furthermore, *Stenotrophomonas maltophilia* was

resistant to all the antibiotics tested, except trimethoprim-sulphamethoxazole, which is in agreement with the report of Qureshi et al. (27). However, a contrary observation was reported by Rostoff et al. (30) in a patient who died of pacemaker endocarditis caused by *Stenotrophomonas maltophilia*. The source of the isolates may be the reason for the difference in observations. The resistance of the organism to β -lactams, aminoglycosides, and ciprofloxacin is in accordance with the observations of Micozzi et al. (24), who reported a hematology patient with *S. maltophilia* bacteremia. The antibiotic resistance exhibited by the coliform isolates in this study poses a serious public health threat, as these findings could lead to complications in the treatment of clinical infections caused by the organisms.

CONCLUSIONS

The isolation of various genera of *Enterobacteriaceae* from both raw and RTE products (polonies) in the present study suggests a lack of strict hygiene control measures in the food chain, considering the fact that members of this bacterial family are commonly found in the environment and in the intestinal tracts of animals. Although a few of the organisms isolated are associated with nosocomial infections, many of them are capable of causing serious and even life-threatening illnesses. This is compounded by the resistance to multiple antibiotics expressed by the majority of the isolates, which poses a serious public health threat. Of particular significance is the isolation of multidrug-resistant *Stenotrophomonas maltophilia* from chicken polony (RTE), the report of which is the first report on isolation of the organism from food in the NWP of South Africa. Polonies, which are cooked sausages that have been finely blended to the consistency of a fine paste, are prepared by adding ready mixed ingredients to a specified amount of minced meat. The mixture is later

stuffed in the desired casing, hung for one hour at room temperature, and then smoked at 60 – 70°C, after which they are cooled by being dipped in cold water and finally placed in a refrigerator. The procedure, if effectively carried out, is expected to rid the product of bacterial contamination, thus qualifying it as an RTE. *Stenotrophomonas maltophilia* sometimes resides as flora on the intact skin and in the nasal passages of humans and farm animals (1, 30). Thus, its isolation from polony in the current study suggests product contamination along the food chain.

Stenotrophomonas maltophilia is emerging as an important nosocomial pathogen, particularly in immunocompromised persons. Although infections caused by *S. maltophilia* in healthy individuals are uncommon, its phenotypic display of antibiotic resistance, including new generation antibiotics, makes it a high risk organism (1, 12). The organism has the potential to transfer antibiotic resistance genes to other members of the *Enterobacteriaceae* family, some of which could then cause more serious infections (3). The results of this study indicate that the consumption of raw and RTE (usually consumed without further preparation) poultry products may contribute to the spread of antibiotic resistant organisms. This finding calls for establishment of good hygienic practices in the food production chain and adequate processing of raw products and RTE foods, by such means as following the guidelines provided by the Codex Alimentarius commission (9). These measures may help to curtail the dissemination of antibiotic resistance genes in the community.

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