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Presence of Coliforms in Water, Poultry Mouth and Rectal Swabs from Selected Smallholder Poultry Farming Projects of Capricorn District, South Africa

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ABSTRACT

Background: Limited biosecurity measures in smallholder poultry facilities expose birds to various pathogens as a result, poultry products particularly raw ones are reported to be responsible for cases of human foodborne diseases. The aim of the study was to profile and characterise the zoonotic bacterial pathogens in water, poultry mouth and rectal swabs in a value chain project of Capricorn district, Limpopo Province, South Africa.

Methods: A total of 74 samples comprising of 14 water samples, 60 mouth and rectal swab samples were collected from the farms. The samples were screened for the presence of *Escherichia coli, Salmonella* spp. and *Shigella* spp. through selective cultivation.

Result: The study revealed that the water resources, mouth and rectal swab samples of chickens were contaminated by coliform bacteria. *Escherichia coli* and *Klebsiella spp*., were dominant isolates. *E. coli* strains that were isolated from the water sources, mouth and rectal swabs of the chickens showed strong resistance to gentamycin, neomycin, penicillin, streptomycin, tetracycline, erythromycin, nalidixic acid, ciprofloxacin and ampicillin. *K. pneumoniae* showed resistant to neomycin; penicillin; erythromycin while *K. oxytoca* and *E. absuriae* showed similar antibiotic resistance profile as penicillin, erythromycin, nalidixic acid and ampicillin.

Key words: Antibiotic resistance, *E. coli,* Gentamycin, *K. pneumonia,* Rectal swab.

INTRODUCTION

Poultry production provides by products such as meat and eggs which are regarded as affordable sources of proteins and other important nutrients needed by the human body (Mack *et al.,* 2005). The South African poultry industry continues to dominate the agricultural sector, providing 63.1% of animal protein in 2019 (SAPA, 2020). However, a high incidence of bacterial pathogens in poultry products was reported (Olobatoke *et al.,* 2014; Zhang *et al.,* 2018). Muvhali *et al.* (2017) reported high morbidity and poultry mortalities following outbreaks of *Salmonella enteretidis* in South Africa. The contributing factors to this demise could be feed and water resources utilised by poultry farmers which are often of compromised quality as these are low input production systems with limited biosecurity measures (Mwale and Masika, 2011). Water sources for poultry are generally open and there is usually no treatment at the point of consumption (Walters *et al.,* 2014). Faecal shedding by food-producing animals is the leading source of contamination of water and the environment, whereas intestinal carriage often leads to contamination of carcasses at slaughter (Abraham *et al.,* 2014). These circumstances expose poultry to various contaminating pathogens since contaminated feed and water provides an excellent media for the growth of microorganisms (Asiegbu *et al.,* 2016). The pathogens are transmitted through the food chain to humans and cause foodborne illnesses (Chapman, 2013). *Campylobacter jejuni*, *Escherichia coli, Salmonella* spp, *Listeria monocytogenes* and *Shigella* spp thrive in poor hygienic conditions. These bacterial species are the most commonly isolated pathogens in the poultry industry of South Africa, where they are implicated in high incidences of morbidity and mortality among chickens (Rossaint *et al.,* 2015; Asiegbu *et al.,* 2016). Shonhiwa *et al.* (2017) reported the presence of other pathogens such as *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli O157:H7* from farm products. Contamination of feeds and drinking water with pathogenic microorganisms may lead to low product yield (Nyamongo and Okioma, 2005). Rouger *et al*. (2017) reported that microbiological metabolic activities may lead to spoilage and production of toxins and these pose a serious health risk to the chicken consumers. Furthermore, bacterial pathogens are becoming drug resistant (Ayukekbong *et al.,* 2017). Antibiotic resistance is of great public health concern

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because the antibiotic-resistant bacteria associated with the animals may be pathogenic to humans, easily transmitted to humans via food chains and widely disseminated in the environment via animal wastes. These may cause complicated, untreatable and prolonged infections in humans, leading to higher healthcare cost and sometimes death (Williams-Nguyen *et al.,* 2016 and Ayukekbong *et al.,* 2017). The objective of the study was to identify the bacterial species present in the water supplies, mouths and recta of chickens and determine their antimicrobial resistance profiles. The findings contribute to the literature on the zoonotic risks associated with the consumption of poultry products in the study area which to the best of our knowledge has not been extensively explored. Information on the microbiological quality of feeds and knowledge of common contaminants is important for improved monitoring and designing of quality control measures in the handling of important raw materials in poultry production such as water and feedstock.

MATERIALS AND METHODS

The study was conducted in randomly selected smallholder poultry farms during the period of July to December 2020. The farms were all located in the Capricorn district, Limpopo Province, South Africa (51.4818°N latitude, 7.2162°E longitude) co-ordinates 29°00′4.68″ (Statistics South Africa, 2015). Laboratory consumables used in this study were from SIGMA ALDRICH ® Company, South Africa. Seventy-four (74) samples comprising of water, mouth and rectal swab samples were collected from the farms. Fifty millilitres of water samples were collected from the same bulk tank in duplicates and from the drinkers within each poultry house into 50ml sterile bottles. Mouth and rectal samples were collected from 10% of the randomly selected chickens. Collected samples were transported on ice to the University of Limpopo, Microbiology laboratory for analysis within three hours of collection.

Samples were cultured on the following selective media that were prepared according to the manufacturer's instructions: MacConkey agar (SIGMA-ALDRICH ®) was used for the isolation of Gram-negative enteric bacteria. Water samples were prepared, processed and analysed for bacterial contamination according to the ISO 6222:1999. *Escherichia coli* and other coliform bacteria were analysed according to ISO 9308-1:2000. To process the swab samples, each swab was rolled onto the total agar surface of MacConkey media and incubated according to the manufacturer's instruction. The cotton bud of the swab was then cut and inoculated into the tubes containing the Rappaport Vassiliadis broth. The plates that had evidence of colony growth were observed, selected and counted. Enumeration was performed manually under white light on the media plates that contained total colony counts of 30-300. All the media cultures including the broth culture colonies were sub-cultured and Gram stained to check for purity and cellular characteristics of the isolates. The isolates were sub-cultured on MacConkey media for subsequent identification assay (Szakál, 2003).

The bacterial isolates were identified with MALDI-TOF MS through a modified bio- typing protocol that was provided by the manufacturer (Bruker Daltonics, Maldi Biotyper). A rapid, on-plate method that requires less time and reagents for its performance was adopted in this study (Matsuda *et al.,* 2012; Rodriguez-Sanchez *et al.,* 2014). Antibiotic sensitivity assays for the isolated bacteria were performed using nine different antibiotics of varying strengths presented on discs (MASTIDISCS ™) following the Kirby Bauer disk diffusion method (Bauer *et al.,* 1966). The antibiotics discs used include streptomycin (S_{10}), ampicillin (AP_{25}), penicillin (PG_{10}), tetracycline (T_{30}), nalidixic acid (NA_{30}), erythromycin (E_{15}), neomycin (NE_{30}), ciprofloxacin (CIP_5) and gentamycin (GM_{10}). The results were interpreted as susceptible, intermediate, or resistant according to the zone diameter interpretative standards suggested by the Clinical and Laboratory Standards Institute (CLSI, 2001). The multiple antibiotic resistance (MAR) index of each strain was also detected using the equation provided by Singh *et al.* (2010) as follows:

$$MAR = \frac{\text{Number of antimicrobial drugs to which the bacterium is resistant}}{\text{Total number of antimicrobial drugs used}}$$

Statistical Package for Social Sciences (IBM SPSS, 2019) version 26.0 was used for data analysis. Descriptive statistics such as percentages were computed for bacterial percentage resistance to antibiotics and the average number of colonies counted from water samples.

RESULTS AND DISCUSSION

The current study adopted MALDI-TOF MS technique using the simplified on plate method for microbial analysis of the samples. MALDI-TOF MS results revealed that all sampled farms were contaminated with the targeted microorganisms (*Escherichia Coli* and *Klebsiella spp*) at varying levels (Table 1). *Escherichia coli* was found to be in abundance and positive to 88% of the sampled farms. *Klebsiella spp* tested positive in 38% of the farms. Bacterial contaminations from culture source (mouth, rectum and water) showed similar trend to farm contamination where *E. coli* tested positive to all culture source. Mouth swabs tested highly positive followed by rectum swabs and water resources were the least contaminated. Great variation in resistance to antibiotics tested was observed with respect to the water, poultry mouth and rectal swabs. The highest resistance occurred in rectal samples from farm code RAS with 96% resistance (Table 2) and the lowest resistance was observed in water samples from farm codes RAM and MAU with 44% resistance (Table 3).

Coliform bacteria are often referred to as "indicator organisms" because they indicate the potential presence of disease-causing bacteria in water. The presence of coliform bacteria in water indicates that a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, *etc.*) and the water supply. Our study revealed 18% occurrence of *E. coli* in collected samples.

The detection level of *E. coli* reported in the presented study is higher compared to 13% reported by Strongmberg *et al.* (2017). The current study reported an occurrence of 13.3% of *E. coli* in poultry rectal swabs, however, Nyabundi *et al.* (2017) reported an occurrence rate of 3.6% in poultry rectal swabs. Obeng *et al.* (2012) reported a 10% occurrence rate

Table 1: Bacterial species identified with MALDI-TOF MS from varying samples: Swabs (mouth and rectal) and water collected at different farms.

Organism identity	Culture source						Cellular morphology by gram staining	Colony morphology	
	M	R	W	M	R	W		Media used for culture	
	No of positive samples			Average score value				XLD	Mac conckey
						*SEO			
Escherichia coli	2	1	-	1.871	1.995	-	Negative pink/red rod	Large flat yellow	Pink coloured
						*MAT			
Escherichia coli	2	-	2	2.08	-	1.93	Negative pink/red rod	Red with black centre	Pale transparent
						*RAS			
Escherichia coli	-	1	-	-	1.858	-	Negative pink/red rod	Yellow	Pale transparent
						*RAM			
Escherichia coli	2	-	-	1.978	-	-	Negative pink/red rod	Yellow	Yellow
Klebsiella oxytoca	-	-	1	-	-	1.864	Negative pink/red rod	Red	Pink
Klebsiella pneumoniae	-	-	1	-	-	1.933	Negative pink/red rod	Red	Pink
						*MAU			
Klebsiella oxytoca	1	2	-	1.988	1.8705	-	Negative pink/red rod	Mucoid yellow	Pale transparent
Table1 continues									
Klebsiella pneumoniae	-	1	-	-	2.127	-	Negative pink/red rod	Large flat yellow	Pink coloured
Escherichia coli	-	1	-	-	1.994	-	Negative pink/red rod	Yellow	Pale transparent
Enterobacter asburiae	-	-	1	-	1	1.738	Negative pink/red rod	Yellow	Pale transparent
Enterobacter kobei	-	-	1	-	-	1.728	Negative pink/red rod	Large flat yellow	Pale transparent
						*SEL			
Klebsiella pneumoniae	-	2	-	-	2.109	-	Negative pink/red rod	Red	Pink
						*MAD			
Escherichia coli	-	1	-	-	1.941	-	Negative pink/red rod	Red	Pink

*****Indicates farms under investigation; M, R, W indicates samples from mouth water and rectum.

Key: Sample identity refers to the identity codes assigned to the farmers. Culture source refers to where the samples were collected, water resources and (mouth and rectal swabs). Colonial morphology is the morphology of the colonies identified as suggested by the media manufacture's guidelines. Cellular morphology by Gram staining refers to the morphology of the cells as determined through Gram staining. Identity, these are the results of the organisms obtained through MALDI-TOF MS identification and the score values.

Table 2: Bacterial percentage resistance to antibiotics, phenotype observed among the isolates from poultry swabs (Mouth and rectal).

Farm	Site of collection	Occurrence of bacterial species			No of isolates	AB resistance %	AB phenotype
		E. coli	K.oxytoca	K.pneumoniae			
SEO	Mouth	+	-	-	1	56	PG-S-T-E-AP
	Rectum	+	-	+	1	56	PG-S-T-ECIP
MAT	Mouth	+	-	-	1	56	NE-PG-T-EAP
	Rectum	-	-	-	1	67	PG-S-T-E-NA-AP
MAD	Mouth	-	-	-	1	67	GM-NE-PG-S-T-E-NA-AP
	Rectum	+	-	-	1	89	GM-PG-T-E-NA-AP
RAS	Mouth	+	-	-	1	56	NE-PG-S-T-E-NA-CIP-AP
	Rectum	+	-	-	1	96	NE-PG-T-E-AP
SEO	Mouth	+	-	-	1	56	PG-S-TE-AP
	Rectum	+	-	-	1	56	PG-S-T-E-CIP
Ram	Mouth	+	-	-	1	56	GM-NE-PG-T-E
	Rectum	-	-	-	1	56	PG-S-T-E-AP

*Streptomycin (S); Ampicillin (AP); Penicillin (PG); Tetracycline (T); Nalidixic acid (NA); Erythromycin (E); Neomycin (NE); Ciprofloxacin (CIP) and Gentamycin (GM) *AB- Antibiotic; (+) - Means that the target organism was identified in that particular sample.

* (-) - Means the sample was free from that particular sample.

of *E. coli* isolates from faeces of commercial egg layers. Varying isolation methods, classification methods, geographic locations and management practices are the possible reasons for the differences in frequency of occurrence between these studies. In addition, direct transmission of the bacteria from humans as well as differences in contamination levels of poultry feeds may be used to justify these observations.

Differences in *E. coli* contamination were observed between the farms. These differences may be linked to either environmental factors or the equipment being used in the farms (Voidarou *et al.*, 2011). The type of bacteria and their loads depend on the initial bacterial contamination and proliferation of the bacterial pathogens might be aggravated by poor hygiene (Brightwell *et al.,* 2007). Ribot *et al.* (2006) reported that *E. coli* is distributed among poultry of all ages. *Escherichia coli* in poultry production facilities may be introduced by air or faecal contamination or by any other contaminated substances to the water resources. On the other hand, the presence of *Klebsiella spp* could be attributed to the exposure of the poultry drinkers and harvested rain to the unsanitary environment which allowed such organisms to proliferate and contaminate the water resources (Tzouvelekis *et al.,* 2012; Berendonk *et al.,* 2015).

The results of the present study agree with the reports by Klein (2018), that *E. coli and K. oxytoca* are commonly isolated bacteria in environmental polluted water. Bunkova *et al*. (2010) isolated *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella pneumonia* from poultry carcasses. Agapi *et al.* (2012) on spoilage microbiota associated with the storage of raw meat in different conditions, also isolated *Salmonella* spp and *K. pneumoniae*, which contribute to poultry foodborne outbreaks and poultry carcasses spoilage. These isolates are similar to the isolates obtained in the current study. *K. pneumoniae* has been proposed as a model organism for poultry diseases due to its presence in both the environment and in animal guts and in the development and spread of resistance (Tzouvelekis *et al.,* 2012; Berendonk *et al.,* 2015). The results of this study indicate poor microbiological quality of the drinking water that is supplied to the chickens. The isolates from the mouth and rectal swabs were different from the isolates from the water. The epidemiology and ecology of *Klebsiella* spp and *E. coli O157* suggest faecal contamination of feed or water may be a possible source of exposure of different microorganisms in poultry convectional houses and water resources. In the present study, rectal swabs showed higher contamination levels compared to other samples. This agrees with the report by (Adegunloye, 2006) that poultry faeces promote a significant growth of foodborne pathogens. The authors concluded that this prompt several foodborne diseases outbreak that result in devastating effects of mortalities in broilers. The environment of the poultry house can act as reservoir for pathogens (Gast, 2007). Magwedere *et al.* (2015) isolated several bacterial spp., including *Escherichia* spp and *Klebsiella spp*., in poultry feeds and water resources which are considered to be brought by faecal contamination in feeds and water. High levels of bacteria in drinking water have been shown to negatively impact productivity in poultry (Derouchey *et al.,* 2004).

Amit *et al.* (2017) reported a high resistance of more than 70% of *E. coli* isolates to common antibiotics such as co-trimoxazole, ampicillin, penicillin and tetracycline. The study also reported a low resistance percentage of less than 30% to aminoglycosides such as ampicillin, amikacin and gentamicin. This antimicrobial resistance results affirms similarity to the results obtained in our study and support the reports from previous studies that there is high prevalence of bacterial resistant strains in poultry environments (Furtula *et al.,* 2013 and Laube *et al.,* 2014). Resistant bacteria proliferate and can also be transferred to humans through several routes such as direct contact of handlers, live animals and carcasses at poultry farms and slaughter houses. From the poultry water resources, the highest resistance was reported for *Escherichia coli* with 78 % resistance to neomycin, penicillin, tetracycline, nalidixic acid, ciprofloxacin and ampicillin. Similar findings were reported in literature (Makhol *et al.,* 2011).

Antibiotic resistance is of great public health concern, because the antibiotic resistant bacteria associated with the

Table 3: Bacterial percentage resistance to antibiotics, phenotype observed among the isolates from poultry water resources in the study site.

Farm	Sample ID	Occurrence of bacterial species			No of isolates	AB resistance %	AB phenotype
		E. coli	*K. oxytoca*	*K. pneumoniae*			
MAU	I1	-	-	-	1	44.4	PG-E-NA-AP
	I2	-	-	+	1	44.4	PG-T-E-AP
SEL	I1	+	-	-	1	56	NE-PG-T-E-AP
	I2	-	-	+	1	67	GM-NE-PG-E-NA-AP
MAT	I1	+	-	-	1	78	NE-PG-T-E-NA-CIP-AP
	I2	+	-	-	1	67	PG-S-T-E-NA-AP
RAM	I2	+	-	-	1	44.4	PG-E-NA-AP
	I2	+	-	-	1	56	NE-PG-E-NA-AP

*Streptomycin (S); Ampicillin (AP); Penicillin (PG); Tetracycline (T); Nalidixic acid (NA); Erythromycin (E); Neomycin (NE); Ciprofloxacin (CIP) and Gentamycin (GM) *AB- Antibiotic.

*(+) - Means that the target organism was identified in that sample. (-) – means the sample was free from the...

animals are mostly pathogenic to humans (Economou and Gousia, 2015; Friedman *et al.,* 2016). According to Friedman *et al.* (2016), bacterial resistant strains are easily transmitted to humans via food chains and widely disseminated in the environment *via* animal wastes. The rising level of antibiotic-resistant bacterial pathogens hampers future treatment and prevention of infectious diseases in both animals and humans (Vincent *et al.,* 2016). Marshall *et al.* (2011) also confirmed that the application of antimicrobials in animals, particularly in food animals, may leads to a development of resistant strains of bacteria, which propagates to infect both animals and man. Different resistance patterns of *Escherichia coli* to antibiotics reported in the current study are consistent with previous related studies on antimicrobial resistance (Kariuki and Dougan, 2014; Abbassi *et al.,* 2017). Similar to the current study, studies have reported resistance to Neomycin, penicillin and co-trimoxazole (Nyabundi *et al.,* 2017).

CONCLUSION

The current study revealed the presence of coliform bacteria in water, poultry rectal and mouth swabs confirming that chickens raised in the study area are susceptible to infection by this class of bacteria. This may partially be attributed to their rearing systems, especially the deep litter system which allows enteric microbial contamination within the poultry houses.

Conflict of interest: None.

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