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PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERN OF SELECTED PATHOGENIC BACTERIA ISOLATED FROM POULTRY DROPPINGS IN ZARIA, NIGERIA

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ABSTRACT
Antibiotic use in poultry production is a major risk factor for emergence of resistant bacteria. This study was aimed at assessing the prevalence of selected pathogenic bacteria and antibiotic resistance pattern of some public health significant bacteria (*Salmonella enterica* and *Klebsiella* species) from poultry droppings in Zaria, Nigeria. Fresh chicken droppings (382) were collected between July 2018 and March 2019 from commercial chicken farms and plated on selective and differential media. Bacteria were identified by standard microbiological methods and confirmed using Microgen^{TM} kits. Antibiotic susceptibility testing was carried out by Kirby-Bauer disk diffusion technique using 14 antibiotic discs. The bacterial prevalence were *Escherichia coli* (45.52%), *Proteus* spp. (9.10%), *Enterococcus* spp. (8.25%), *Staphylococcus* spp. (6.83%), *Klebsiella* spp. (4.55%), *Enterobacter* spp. (4.41%), *Bacillus* spp. (4.27%), *Pseudomonas* spp. (3.28%), *Providencia* spp. (3.13%), *Citrobacter* spp. (1.85%), *Shigella* spp. (1.14%), *Serratia* spp. (0.99%), *Yersinia* spp. (0.99%), *Salmonella enterica*. (0.71%) and others (4.98%). Among the 5 strains of *Salmonella enterica* isolated, 4(80%) were resistant to tetracycline and 2(40%) to ampicillin, azithromycin, trimethroprim-sulphamethoxazol and nitrofurantoin. Also, among the 5 strains of *Klebsiella* spp, 4(80%) were resistant to azithromycin, tetracycline and nitrofurantoin, while 3(60%) were to ampicillin and cefotaxim. Also, *Salmonella enterica* 1(20%) and *Klebsiella* spp. 3(60%) had multiple antibiotic resistance indices ≥0.36. Conclusively, this study observed high prevalence of *Salmonella enterica* and *Klebsiella* spp among the studied samples. Isolation of antibiotic resistant bacteria were also noticed among these bacteria, therefore, regulatory authority should enforce the ban on use of some antibiotic in poultry production to maintain public health safety.

Keywords: Prevalence, Antibiotic Resistance, Bacteria, Poultry.

INTRODUCTION

Antibiotic use in poultry production is a major risk factor for the emergence of resistant pathogenic bacteria which is of public health significance (Obeng*et al*., 2012). Also, from public health perspective, there is inherent risk associated with zoonotic transmission of pathogens commonly associated with poultry husbandry and production (Kauber *et al.*, 2016). Sixty-one percent of the 1,415 infectious agents recognized to be pathogenic to humans are zoonotic (CDC, 2015). Zoonoses are infectious diseases directly or indirectly transmitted from animals to humans. Common pathogens of interest include avian influenza, *Salmonella enterica*, *Campylobacter jejuni* and *Esherichia coli* O157, which are the leading causes of diarrhoea caused by food borne illness due to the consumption of, or contact with, poultry products (Scallan *et al.*, 2011). At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multidrug resistant *Escherichia coli* O157:H7, *Salmonella enterica* and *Klebsiella pneumonae*; likewise eggs become contaminated during laying (Bogaard *et al*., 2001). Hence, resistant faecal enteric bacteria from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora.

Therefore, contact with live chickens or its droppings is of public health concern (Behravesh *et al*., 2014; Pabilonia *et al*., 2014). In the United States, from 1990 to 2014, 53 outbreaks of human salmonellosis linked to live poultry have been documented resulting in 2611 known illnesses, 387 known hospitalizations and 5 known deaths (CDC, 2015). However, the prevalence of public health significant bacteria occurring in poultry flocks varies depending on geographical location, husbandry, antibiotic usage in livestock industries and hygiene practices (Pollock *et al*., 2012). The transfer of antimicrobial resistant strains of *Salmonella enterica* and *Klebsiella pneumonia* to the food chain from chickens is of special interest due to the high morbidity associated with them (Ewers *et al*., 2009). Thus, this study was aimed at assessing the prevalence of selected pathogenic bacteria and determination of antibiotic resistance pattern of some public health significant bacteria (*Salmonella enterica* and *Klebsiella* species) from poultry droppings in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

Zaria is a town in Kaduna State, in the North-Western part of Nigeria and is located on latitude 11º04N and longitude 7º43E and about 660M above sea level (National Population Census, 2010; Oladimeji and Ojibo, 2012).

Collection of Samples

Five sampling sites were selected in Zaria Metropolis for this study. They are Samaru , Wusasa, Tudunwada , Zaria City and Sabon Gari. The study design was a cross-sectional study, while the sample size was determined using the formula described by Naing *et al.* (2006). A total of 382 faecal samples were proportionately collected from randomly selected poultry farms in Zaria metropolis, North-Western Nigeria between July 2018 and March 2019.

Processing of Samples

Upon arrival at the laboratory, 1g of each faecal sample was homogenized in 9ml of buffered peptone water (Oxoid, UK). A disinfected blender (using 70% ethanol for disinfection) was used to obtain the homogenate. The homogenate was pre-enriched with peptone water by incubating for 24 hours at 37ºC. A loopful of pre-enriched specimen was cultured after streaking on some selective and differential media (Salmonella Shigella agar, Eosine Methyline Blue agar, MacConkey agar, Manitol salt agar and Cysteine Lactose Electrolyte Deficient agar), the plates were inverted and incubated aerobically at 37ºC for 24 hours after which the plates were examined for growth. Isolates were characterized and identified using standard conventional biochemical tests and confirmed using Microgen^{TM} kits (Cheesbrough, 2006).

Antibiotic Susceptibility Testing of *Salmonella enterica* and *Klebsiella* species

The selected 10 isolates was drawn from the pull of some public health significant bacterial isolates using random probability sampling to select 1 representative strain of *Klebsiella* species from each location (Samaru, Wusasa, Tudunwada, Zaria City and Sabon Gari) out of the total 32 strains of *Klebsiella* species isolates obtained in this study, while all the 5 strains of *Salmonella enterica* were purposively selected for antibiotic susceptibility testing.

The antibiotic susceptibility testing of some public health significant bacteria (*Salmonella enterica* and *Klebsiella* species) mostly implicated in human diseases (Cheesbrough, 2006), were carried out using the Kirby-Bauer disk diffusion technique according to the methods recommended by Clinical Laboratory and Standards Institute (CLSI, 2018) to determine susceptibility or resistance profiles of the isolates, discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared pre-dried Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed (20mm away from each other) on the inoculated Mueller-Hinton agar plates. The antibiotic discs used were Ampicillin (10µg), Cefotaxim (30µg), Ceftazidime (30µg), Amoxicillin-Clavulanic acid (20/10µg), Cefoxitin (30µg), Etarpenem (10µg), Azithromycin (15µg), Aztreonam (30µg), Tetracycline (30µg), Trimethroprim-Sulphamethoxazol (1.25/23.75µg), Chloramphenicol (30µg), Gentamicin (10µg), Ciprofloxacin (5µg) and Nitrofurantoin (300µg) (Oxoid, UK). The reference standard strain used as control in this study was *Escherichia coli* ATCC 25922. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant were made according to CLSI, (2018) manual. The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotic to which the isolate was resistant to, by the total number of antibiotic tested (Olayinka *et al.*, 2004).

RESULTS

Prevalence and Distribution of Public Health Significant Bacteria Isolated from Poultry Droppings in Zaria Metropolis.

Table 1 shows the prevalence and distribution of some public health significant bacteria isolated from poultry (chicken) droppings in Zaria Metropolis, from the result, seven hundred and three (703) strains of bacteria were isolated from three hundred and eighty two (382) samples. Highest number of pathogens were isolates from Zaria City 217 (30.88%) and lowest was isolated in Samaru 85 (12.09%). Out of the total bacterial pathogens isolated and identified from the chicken droppings, *Escherichia coli* 320 (45.52%) was the most prevalent while the least prevalent bacterial pathogens were *Serratia* spp. 7(0.99%), *Yersinia* spp. 7(0.99%) and *Salmonella* spp. 5(0.71%).

Antibiotic Susceptibility Profile of selected Public Health Significant Bacteria Isolated from Poultry Droppings in Zaria Metropolis.

Table 2 showed the results of antibiotic resistance profile of selected public health significant bacteria isolated from poultry droppings in Zaria Metropolis. Fourteen commonly used antibiotics in the study area were selected for the susceptibility testing according to the guidelines by Clinical and Laboratory Standards Institute (CLSI, 2018). Both *Salmonella enterica* and *Klebsiella* spp. isolates had resistance which ranged from 0.0% - 80.0%, in this study. It was also observed that the *Salmonella enterica* 5(100%) and *Klebsiella* spp. 5(100%) isolates were completely susceptible to 2 out of 14 antibiotics tested in this study as shown in Table 2. Table 3 showed the comparison of the multiple antibiotic resistance pattern and indices of *Salmonella enterica* and *Klebsiella* spp. isolated from poultry droppings in Zaria Metropolis. It was observed that *Salmonella enterica* 1(20%) and *Klebsiella* spp. 3(60%) had resistance to 5-11 combinations of antibiotics with corresponding MAR indices ≥0.36.

Table 1: Prevalence and Distribution of Public Health Significant Bacteria Isolated from Poultry Droppings in Zaria Metropolis

Locations	Samples Collected	*E. coli* n(%)	*Proteus* spp. n(%)	*Salmonella* spp. n(%)	*Klebsiella* spp. n(%)	*Enterobacter* spp. n(%)	*Serratia* spp. n(%)	*Yersinia* spp. n(%)	*Citrobacter* spp. n(%)	*Providencia* spp. n(%)	*Shigella* spp. n(%)	*Pseudomonas* spp. n(%)	*Staphylococcus* spp. n(%)	*Bacillus* spp. n(%)	*Enterococcus* spp. n(%)	Others n(%)	Total n(%)
Samaru (A)	82	41 (48.24)	6 (7.06)	0 (0.00)	5 (5.88)	3 (3.53)	0 (0.00)	0 (0.00)	2 (2.35)	2 (2.35)	0 (0.00)	3 (3.53)	7 (8.24)	4 (4.71)	8 (9.41)	4 (4.71)	85 (12.09)
Tudun Wada (B)	41	41 (33.33)	14 (11.38)	2 (1.63)	6 (4.88)	6 (4.88)	2 (1.63)	2 (1.63)	3 (2.44)	4 (3.25)	2 (1.63)	4 (3.25)	11 (8.94)	7 (5.69)	12 (9.76)	7 (5.69)	123 (17.49)
Wusasa (C)	68	47 (43.73)	12 (10.91)	0 (0.00)	5 (4.55)	6 (5.45)	1 (0.91)	1 (0.91)	3 (2.73)	3 (2.73)	1 (0.91)	4 (3.64)	8 (7.27)	4 (3.64)	10 (9.08)	5 (4.55)	110 (15.65)
Zaria City (D)	136	136 (62.67)	15 (6.91)	0 (0.00)	6 (2.76)	7 (3.23)	1 (0.46)	2 (0.92)	2 (0.92)	6 (2.78)	2 (0.92)	5 (2.30)	9 (4.15)	5 (2.30)	13 (5.99)	8 (3.69)	217 (30.88)
Sabon Gari (E)	55	55 (32.74)	17 (10.12)	3 (1.79)	10 (5.95)	9 (5.36)	3 (1.79)	2 (1.19)	3 (1.79)	7 (4.17)	3 (1.79)	7 (4.17)	13 (7.74)	10 (5.95)	15 (8.93)	11 (6.55)	168 (23.89)
Total	382	320 (45.52)	64 (9.10)	5 (0.71)	32 (4.55)	31 (4.41)	7 (0.99)	7 (0.99)	13 (1.85)	22 (3.13)	8 (1.14)	23 (3.28)	48 (6.83)	30 (4.27)	58 (8.25)	35 (4.98)	703 (100)

Key: n = Number of isolates.

Table 2: Antibiotic Susceptibility Profile of Some Public Health Significant Bacteria Isolated from Poultry Droppings Sampled from Zaria Metropolis

Antibiotic (Disc contentμg)	Symbol	*Salmonella enterica* (n=5)			*Klebsiella* spp. (n=5)			Total Resistance n=10 (%)
		R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)
Ampicillin (10)	AMP	2(40)	1(20)	2(40)	3(60)	1(20)	1(20)	5(50)
Cefotaxim (30)	CTX	0(0)	1(20)	4(80)	3(60)	0(0)	2(40)	3(30)
Ceftazidime (30)	CAZ	0(0)	0(0)	5(100)	1(20)	0(0)	4(80)	1(10)
Amoxicillin-Clavulanic acid (20/10)	AMC	0(0)	1(20)	4(80)	0(0)	1(20)	4(80)	0(0)
Cefoxitin (30)	FOX	1(20)	0(0)	4(80)	0(0)	0(0)	5(100)	1(10)
Etarpenem (10)	ETP	0(0)	1(20)	4(80)	0(0)	0(0)	5(100)	0(0)
Azithromycin (15)	AZM	2(40)	0(0)	3(60)	4(80)	0(0)	1(20)	6(60)
Azthreonam (30)	ATM	0(0)	0(0)	5(100)	1(20)	0(0)	4(80)	1(10)
Tetracycline (30)	TE	4(80)	0(0)	1(20)	4(80)	1(20)	0(0)	8(80)
Trimethroprim –Sulphamethoxazol (1.25/23.75)	SXT	2(40)	0(0)	3(60)	2(40)	0(0)	3(60)	4(40)
Chloramphenicol (30)	C	0(0)	1(20)	4(80)	1(20)	1(20)	3(60)	1(10)
Gentamicin (10)	CN	0(0)	1(20)	4(80)	2(40)	0(0)	3(60)	2(20)
Ciprofloxacin (5)	CIP	0(0)	3(60)	2(40)	1(20)	1(20)	3(60)	1(10)
Nitrofurantoin (300)	F	2(40)	0(0)	3(60)	4(80)	0(0)	1(20)	6(60)

Key: R= Resistance, I= Intermediate, S= Susceptible, n= number of isolates.

Table 3: Multiple Antibiotic Resistance Patterns and Indices of Some Public Health Significant Bacteria Isolated from Poultry Droppings Sampled from Zaria Metropolis

		Number of isolates with pattern (%)		
Number of antibiotic combination	Resistance phenotypes combination	*Salmonella enterica* . n=5 (%)	*Klebsiella* spp. n=5 (%)	MARI
3	AMP, AMC*, TE	1(20)	NIL	0.21
4	AMP*, CTX*, TE, CIP*	1(20)	NIL	0.29
	AMP, AZM, SXT, F	1(20)	NIL	0.29
	TE, SXT, CN*, CIP*	1(20)	NIL	0.29
	AZM, TE, CIP*, F	NIL	1(20)	0.29
	AMP*, AZM, TE*, F	NIL	1(20)	0.29
5	AMP, CTX, AMC*, TE, CN	NIL	1(20)	0.36
6	FOX, AZM, TE, C*, CIP*, F	1(20)	NIL	0.43
7	AMP, CTX, AZM, TE, SXT, C*, F.	NIL	1(20)	0.43
11	AMP, CTX, CAZ, AZM, ATM, TE, SXT, C, F, CN, CIP.	NIL	1(20)	0.79

Key: *= Intermediate-Resistance, n = number of isolates, MARI = Multiple Antibiotic ResistanceIndex, AMP =Ampicillin, CTX =Cefotaxim, CAZ =Ceftazidime, AMC=Amoxicillin-Clavulanic acid, FOX =Cefoxitin, ETP =Etarpenem, AZM =Azithromycin, ATM =Aztreonam, TE =Tetracycline, SXT =Sulphamethoxazol-Trimethroprim, C =Chloramphenicol, CN =Gentamicin, CIP =Ciprofloxacin, F =Nitrofurantoin.

DISCUSSION

Prevalence and Distribution of Public Health Significant Bacteria Isolated from Poultry Droppings in Zaria Metropolis

The distribution of bacteria isolated from poultry (chicken) droppings in Zaria Metropolis showed that seven hundred and three (703) strains of bacteria were isolated from three hundred and eighty two (382) samples. Highest number of pathogens were isolates from Zaria City 217 (30.88%) and lowest was recorded for Samaru 85 (12.09%). Out of the total bacterial pathogens isolated and identified from the chicken droppings, *Escherichia coli* 320 (45.52%) was the most prevalent while the least prevalent bacterial pathogens were *Serratia* spp. 7(0.99%), *Yersinia* spp. 7(0.99%) and *Salmonella* spp. 5(0.71%). These pathogens are of public health importance, For instance, *E. coli* is implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis (Sayah *et al.,* 2005). Whereas, *Salmonella enterica* are capable of producing acute and chronic enteritis, diarrhoea, and septicemia in all or most types of birds and other animals (Maciorowski, 2007; Omojowo and Omojasola, 2013). The detection of these organisms in this study agrees with the fact that the bacteria are part of the enteric flora of the poultry birds (Adegunloye, 2006). In this research, bacterial pathogen isolated were mostly enteric bacteria and *Staphylococcus* spp. with *E. coli* having highest prevalence (45.52%) and is in agreement with the report of Omoya *et al*. (2016) who reported *Escherichia coli* (53.50%) prevalence from poultry dung obtained from free-range chicken and nine commercial chicken farms in Akure, Ondo State, Nigeria. However, it was observed from results obtained that there was a variation in the carriage of the microorganisms in poultry farms across the study locations. This could be due to a host of factors such as environmental conditions in which the birds are raised, the nutritional status of the birds, the probiotic and physiological state of the gut of animals which could influence the distribution, and ultimately the recovery rate of microorganisms from the gut of animals (Ajayi and Egbebi, 2011).

Antibiotic Susceptibility Profiles of Some Public Health Significant Bacteria Isolated from Poultry Droppings in Zaria Metropolis.

The results of antibiotic resistance profiles of selected public health significant bacteria isolated from poultry droppings in Zaria Metropolis showed that *Salmonella enterica* and *Klebsiella* spp. isolates had resistance which ranged from 0.0% - 80.0%, in this study. It was also observed that both *Salmonella enterica* 5(100%) and *Klebsiella* spp. 5(100%) isolates were completely susceptible to 2 out of 14 antibiotics tested. This could mean that *Salmonella enterica* and *Klebsiella* spp. harbors antibiotic resistance plasmids since resistance to most antibiotics are plasmid mediated with a wide variety of genetic determinants (Sayah*et al*., 2005). It was also observed that *Salmonella enterica* 1(20%) and *Klebsiella* spp 3(60%) had resistance to 5-11 combinations of antibiotics with corresponding MAR indices ≥0.36. This may also indicate that *Klebsiella* spp 3(60%) harbors larger sizes of resistance plasmids which tends to accommodate multiple antibiotic resistance genes per plasmid. Also, MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used or abused (Olayinka *et al*., 2004).

CONCLUSION

This study observed high prevalence of *Salmonella enterica* and *Klebsiella* species among the studied samples. Isolation of antibiotic resistant bacteria were also noticed among these bacteria. *Salmonella enterica* and *Klebsiella* species had resistance which ranged from 0.0% - 80.0%. It was also observed that *Salmonella enterica* 1(20%) and *Klebsiella* species 3(60%) had multiple antibiotic resistance (MAR) indices ≥0.36. This has very significant implications on human health, because MAR index greater than 0.2 indicates that such bacteria must have originated from an environment where antibiotics are often used or abused which leads to difficulty in treatment and long term therapy. This could increase the cost of treatment and mortality rate. Therefore, the regulatory authority in Nigeria and in the other parts of the World should enforce the ban on the use of some antibiotic in poultry production to maintain public health and safety. Further work is necessary on the molecular basis of antibiotic resistance and the use of alternative therapy in poultry production.

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