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Research Article

Antibiotic Profiling of Bacterial Isolates Obtained from Turkey and Chicken in Selected Farms in Ibadan, Nigeria

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ABSTRACT

In recent times, the prevalence of antimicrobial drug resistance has increased tremendously due to a number of factors including use of human drugs for the treatment of animal diseases, leading to the transfer of antibiotic resistance in terms of antibiotic residues in poultry meat to pathogenic bacteria. This study determined the antibiotic profiles of bacterial isolates in poultry cloacal swabs from selected farms in Ibadan. Fifty and twenty cloacal swabs were collected aseptically from turkey and chicken at Apete and University of Ibadan research farm respectively. The samples were immediately transported to the laboratory for microbiological analysis. Thus, the cloacal swabs were screened using MacConkey agar, blood agar and xylose lysine deoxycholate agar. Isolates were identified using standard microbiological techniques and tested to ten different antibiotic discs according to Kirby-Bauer procedure. Sixty-one and thirteen different isolates were detected from turkey and chicken cloacal swabs respectively. Of the turkey isolates, Pseudomonas had the highest occurrence of 25% while Escherichia coli (46%) had the highest occurrence of the chicken isolates. The Gram-negative isolates showed high resistance to augmentin (69%), streptomycin (69%), sulphamethoxazole (78%) and chloramphenicol (82%). Staphylococcus species which was the only Gram-positive isolate in this study was greatly resistant to gentamicin (83%). Both the turkey and chicken isolates had different antibiotic resistance rates and patterns with a huge percentage (86%) of them being multi-drug resistant. This work observed a higher resistance to many of the commonly used antibiotics in the poultry industry thereby, posing a public health risk since most of these drugs are used for treatment of human infections.

Keywords: *Antibiotic profiling, bacteria isolates, turkey, chicken, selected farms, Ibadan*

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INTRODUCTION

Bacteria are peculiar organisms which are capable of coping with environmental changes by developing protective devices against toxic agents. These organisms' ability to resist and inactivate antimicrobial drugs have become alarming, thereby reducing therapeutic options (Cohen, 1992). Although most studies suggested irrational use of antibiotics as a major factor responsible for drug resistance, other researchers have also stated that widespread distribution of drug-resistant bacteria has huge influence in causing drug resistance (Livermore, 2003). Occurrence of multi-drug resistant bacteria such as *Escherichia coli* and other bacteria species have been noted in cloacal swab samples of poultry animals (Shobrak and Abo-Amer, 2014). These animals serve as vectors responsible for the transfer of resistant bacterial strains to human hosts through consumption of poultry meats and other poultry products (Pan and Yu, 2014). The increasing prevalence of multi-drug resistant bacteria is of serious clinical relevance due to their ability to render antibiotics ineffective in treating infections caused by these organisms (Kalantar and Mansouri, 2010). Poultry farming is a rapid growing industry supplying meat and egg to consumers globally. In modern poultry farming, broilers can attain table size in less than six weeks. This feat was possible through genetic selection, enhanced feed supply and proper health management measures involving usage of antibiotics as therapeutic agents for bacterial diseases in sophisticated farming (Apata, 2009). Bacterial resistance to commonly used antimicrobial agents has been noticed since the addition of antibiotics to poultry feeds. Studies have reported a high increase in antibiotic resistance in the last twenty years in most countries (Kapil,

2004). Also, the regular addition of antibiotics to poultry feeds could lead to the modification of poultry gut flora by forming a selective pressure supporting resistance of bacteria populations which may contaminate the environment and the food chain (Furtula *et al*., 2010). The unceasing usage of antimicrobial agents over a particular time frame has not only led to bacteria resisting a single antibiotic but also multiple antibiotics thereby making some diseases highly challenging to treat (Moustafa and Mourad, 2015).

The high antimicrobial resistance pattern has a massive impact by increasing the incidence of poultry diseases which subsequently affects the economy of the poultry industry. Therefore, this study was designed to isolate and determine the antibiotic profile of bacteria found in turkey and chicken cloacal swabs in selected farms in Ibadan, Oyo state, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 70 samples were collected from 2 poultry farms around University of Ibadan-Apete axis in Ibadan, Oyo State. Fifty cloacal swabs were collected from some selected unhealthy turkey from a farm in Apete, Ibadan while 20 other cloacal swabs were collected from chicken from the Teaching and research farm, University of Ibadan. These samples were collected aseptically using sterile swab sticks. They were subsequently placed in a flask containing ice packs and transported in to the Research Veterinary Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Bacteria Isolation.

All the samples were inoculated into enrichment media using peptone water and incubated at 37^{O}C for 24 hours. A loopful of the overnight peptone broth culture was inoculated onto Xylose Lysine Deoxycholate (XLD) agar, 7% sheep blood agar and MacConkey agar plates and subsequently incubated at 37^{O}C for 24 hours. The plates were observed for bacterial isolates which were phenotypically identified and later subjected to Gram-staining and biochemical test for further identification. The antibiotic sensitivity profile of all the identified isolates were then determined.

Antibiotic sensitivity test

The bacteria isolates were tested for antibiotic susceptibility by the standard disk diffusion method according to Kirby-Bauer and the Clinical and Laboratory Standards Institute (CLSI) guidelines, (CLSI, 2012). Pure colonies of the test isolate were emulsified in sterile normal saline and the turbidity was adjusted to 0.5 McFarland standards. A sterile swab was dipped into the bacterial suspension in normal saline and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. The antimicrobial disks were firmly placed on the inoculated MHA plate. Gram-positive antibiotic discs with different antibiotics such as Pefloaxin, Gentamicin, Ampiclox, Zinnacef, Amoxacillin, Rocephin, Ciprofloxacin, Streptomycin, Sulphamethoxazole and Erythromycin and Gram negative antibiotic discs with different antibiotics such as Chloramphenicol, Sparfloxacin, Augmentin, Tarivid, Sulphamethoxazole, Ciprofloxacin, Pefloxacin and Streptomycin were placed on inoculated Mueller-Hinton agar and incubated for 24 hours at 37^{o}C. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolates and to the diffusion rate of the antibiotics through the agar medium. The zone diameters of each drug were interpreted using the criteria published by CLSI guidelines (CLSI, 2012).

RESULTS

total of 74 (100%) bacterial isolates were obtained from 70 cloacal swab samples collected in this study. Of these isolates, 61 (82%) were found in the 50 cloacal swabs collected from turkey while 13 (18%) were found in the 20 cloacal swabs collected from chicken.

Table 1:
The breakdown of each of the bacteria obtained from the cloaca of Turkey and Chickens.

BACTERIA	Turkey	Chicken	Total
Yersinia spp	3 (5%)	1(8%)	4 (5%)
Citrobacter spp	3 (5%)	2 (15%)	5 (7%)
Enterobacter spp	5 (8%)	0 (0%)	5 (7%)
Staphylococcus spp	7 (11%)	0 (0%)	7 (9%)
Proteus spp	9 (15%)	1(8%)	10 (13%)
Escherichia coli	5 (8%)	6 (46%)	11 (15%)
Pseudomonas spp	15 (25%)	1(8%)	16 (22%)
Salmonella spp	14 (23%)	2 (15%)	16 (22%)

Table 2a
Resistance Rates of isolated Gram-positive bacteria

Antibiotics	Organisms	*Staphylococcus* (N=7)	Total (N=7)
CPX	Sensitivity	6 (86%)	6 (86%)
	Resistance	1 (14%)	1 (14%)
AMX	Sensitivity	6 (86%)	6 (86%)
	Resistance	1 (14%)	1 (14%)
ERY	Sensitivity	2 (29%)	2 (29%)
	Resistance	5 (71%)	5 (71%)
GEN	Sensitivity	1 (14%)	1 (14%)
	Resistance	6 (86%)	6 (86%)
PEF	Sensitivity	2 (29%)	2 (29%)
	Resistance	5 (71%)	5 (71%)
APX	Sensitivity	1 (14%)	1 (14%)
	Resistance	6 (86%)	6 (86%)
STR	Sensitivity	2 (29%)	2 (29%)
	Resistance	5 (71%)	5 (71%)
SXT	Sensitivity	2 (29%)	2 (29%)
	Resistance	5 (71%)	5 (71%)
ZIN	Sensitivity	6 (86%)	6 (86%)
	Resistance	1 (14%)	1 (14%)
ROC	Sensitivity	2 (29%)	2 (29%)
	Resistance	5 (71%)	5 (71%)

Table 2b

Antibiotic Resistance Rates of isolated Gram-negative bacteria

ANTIBIOTICS	ORGANISMS	*Salmonella* N=16	*Proteus* N=10	*E. coli* N=11	*Citrobacter* N=5	*Enterobacter* N=5	*Pseudomonas* N=16	*Yersinia* N=4	Total N=67
CPX	Sensitivity	14 (87%)	7 (70%)	10 (91%)	5 (100%)	4 (87%)	15 (94%)	3 (80%)	58 (87%)
	Resistance	2 (13%)	3 (30%)	1 (9%)	0 (0%)	1 (13%)	1 (6%)	1 (20%)	9 (13%)
AMX	Sensitivity	7 (44%)	1 (10%)	5 (45%)	3 (60%)	3 (60%)	7 (44%)	2 (50%)	28 (42%)
	Resistance	9 (56%)	9 (90%)	6 (55%)	2 (40%)	2 (40%)	9 (56%)	2 (50%)	39 (58%)
AUG	Sensitivity	7 (44%)	1 (10%)	2 (18%)	3 (60%)	1 (13%)	7 (44%)	0 (0%)	21 (31%)
	Resistance	9 (56%)	9 (90%)	9 (82%)	2 (40%)	4 (87%)	9 (56%)	4 (100%)	46 (69%)
GEN	Sensitivity	10 (63%)	3 (30%)	6 (55%)	4 (80%)	2 (40%)	7 (44%)	0 (0%)	32 (48%)
	Resistance	6 (37%)	7 (70%)	5 (45%)	1 (20%)	3 (60%)	9 (56%)	4 (100%)	35 (52%)
PEF	Sensitivity	10 (63%)	6 (60%)	10 (91%)	4 (80%)	4 (87%)	13 (81%)	2 (50%)	47 (70%)
	Resistance	6 (37%)	4 (40%)	1 (9%)	1 (20%)	1 (13%)	3 (19%)	2 (50%)	18 (30%)
OFX	Sensitivity	13 (81%)	6 (60%)	10 (91%)	4 (80%)	1 (13%)	13 (81%)	3 (80%)	50 (75%)
	Resistance	3 (19%)	4 (40%)	1 (9%)	1 (20%)	4 (87%)	3 (19%)	1 (20%)	17 (25%)
STR	Sensitivity	4 (25%)	3 (30%)	1 (9%)	1 (20%)	1 (13%)	9 (56%)	2 (50%)	21 (31%)
	Resistance	12 (75%)	7 (70%)	10 (91%)	4 (80%)	4 (87%)	7 (44%)	2 (50%)	46 (69%)
SXT	Sensitivity	4 (25%)	1 (10%)	2 (18%)	0 (0%)	1 (13%)	4 (25%)	0 (0%)	12 (18%)
	Resistance	12 (75%)	9 (90%)	9 (82%)	5 (100%)	4 (87%)	12 (75%)	4 (100%)	55 (82%)
CHL	Sensitivity	4 (25%)	1 (10%)	4 (36%)	1 (20%)	2 (40%)	3 (19%)	0 (0%)	15 (22%)
	Resistance	12 (75%)	9 (90%)	7 (64%)	4 (80%)	3 (60%)	13 (81%)	4 (100%)	52 (78%)
SPX	Sensitivity	12 (75%)	3 (30%)	9 (82%)	0 (0%)	4 (87%)	12 (75%)	3 (80%)	43 (64%)
	Resistance	4 (25%)	7 (70%)	2 (18%)	5 (100%)	1 (13%)	4 (25%)	1 (20%)	24 (36%)

The bacterial isolates found in the turkey cloacal swabs are *Yersinia* 3 (5%), *Citrobacter* 3 (5%), *Enterobacter* 5 (8%), *Escherichia coli* 5 (8%), *Staphylococcus* 7 (11%), *Proteus* 9 (15%), *Salmonella* 14 (23%), *Pseudomonas* 15 (25%) while *Yersinia* 3 (5%), *Proteus* 9 (15%), *Pseudomonas* 15 (25%), *Citrobacter* 3 (5%), *Salmonella* 14 (23%), *Escherichia coli* 6 (46%) (Table1). The bacterial isolates showed resistance to tested antimicrobials as follows: *Staphylococcus* (n=7) had a resistance of 1 (14%), 1 (14%), 5 (71%), 6 (86%), 5 (71%), 6 (86%), 5 (71%), 5 (71%), 1 (14%), 5 (71%) to ciprofloxacin, amoxicillin, erythromycin,, gentamicin, pefloxacin, ampiclox, streptomycin, sulphamethoxazole, cefuroxime and ceftriazone individually. (Table 2a)

The overall rate of *Yersinia* (n=4) showed: 1 (20%), 2 (50%), 4 (100%), 4 (100%), 2 (50%), 1 (20%), 2 (50%), 4 (100%), 4 (100%), 1 (20%) resistance to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin respectively. (Table 2b)

Citrobacter (n=5) had a resistance of 0 (0%), 2 (43%), 2 (43%), 1 (14%), 1 (14%), 1 (14%), 4 (86%), 5 (100%), 4 (86%), 5 (100%) to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin correspondingly. (Table 2b)

Enterobacter (n=5) exhibited 1(20%), 2 (40%), 4 (80%), 3 (60%), 1 (20%), 4 (80%), 4 (80%), 4 (80%), 3 (60%), 1(20%) resistance to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin respectively. (Table 2b)

Escherichia coli (n=11) displayed 1 (9%), 6 (55%), 9 (82%), 5 (45%), 1 (9%), 1 (9%), 10 (91%), 9 (82%), 7 (64%), 2 (18%) resistance to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin respectively. (Table 2b)

Proteus (n=10) presented a resistance of 3 (30%), 9 (90%), 9 (90%), 7 (70%), 4 (40%), 4 (40%), 7 (70%), 9 (90%), 9 (90%), 7 (70%) to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin correspondingly. (Table 2b).

Table 3a:
Resistance pattern of Gram-positive bacteria isolates

Resistance pattern	*Staphylococcus*
APX, GEN, PEF, ZIN	1
AMX, AUG, CHL, CPX, OFX, SPX, STR, SXT	1
AMX, APX, ERY, GEN, PEF, ROC, STR, SXT, ZIN	3
AMX, AUG, CHL, CPX, GEN, OFX, PEF, STR, SXT	1
AMX, AUG, CHL, CPX, GEN, OFX, PEF, SPX STR, SXT	1

Table 3b:
Resistance pattern of Gram-negative bacteria isolates

Resistance pattern	*Salmonella spp*	*Proteus spp*	*E. coli*	*Citrobacter spp*	*Enterbacter spp*	*Pseudomonas spp*	*Yersinia spp*
AMX	0	1	0	0	0	0	0
CPX	1	1	1	0	0	0	0
AUG, CPX	1	0	0	0	0	0	0
AMX,AUG, CPX	2	2	1	0	1	0	0
AMX, AUG, GEN	3	2	2	4	1	12	2
CHL, STR, SXT	0	0	1	0	2	0	1
AUG, CHL, GEN, SXT	1	0	0	0	0	0	1
AMX, AUG, CHL, GEN, SXT	2	0	0	1	1	0	0
AMX, AUG, CHL, STR, SXT	1	1	0	0	0	0	0
AMX, AUG, CHL, GEN, STR, SXT	2	1	2	0	0	2	0
AMX, AUG, CHL, PEF, SPX, SXT	0	0	0	0	0	1	0
AMX, AUG, CHL, GEN,PEF, STR, SXT	1	0	1	0	0	0	0
AMX, AUG, CHL, GEN, PEF, SPX, STR, SXT	0	0	2	0	0	0	0
AMX, AUG, CHL, GEN, OFX, PEF, STR, SPX, SXT	0	1	0	0	0	1	0
AMX, AUG, CHL, CPX, GEN, OFX, PEF, SPX, STR, SXT	2	1	1	0	0	0	0

KEYS: SXT- Septrin (Trimethoprim-Sulphamethazone), CHL- Chloramphenicol, SPX- Sparfloxacin, CPX- Ciprofloxacin, AMX,- Amoxacillin, AUG- Augmentin (Amoxicillin-Clavulanic Acid), GEN- Gentamicin, PEF- Perfloxacin, OFX- Tarivid (Ofloxacin), STR- Streptomycin, APX- Ampiclox , ZIN- Zinnacef (Cefuroxime), ROC- Rocephin (Ceftriaxone), ERY- Erythromycin.

Salmonella (n=16) showed a resistance of 2 (13%), 9 (56%), 9 (56%), 6 (37%), 6 (37%), 3 (19%), 12 (75%), 12 (75%), 12 (75%), 4 (25%) to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin individually. (Table 2b)

Finally, *Pseudomonas* (n=16) showed a resistance of 1 (9%), 9 (55%), 9 (55%), 9 (55%), 3 (19%), 3 (19%), 7 (45%), 12 (73%), 13 (82%), 4 (27%) to ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, sulphamethoxazole, chloramphenicol and sparfloxacin respectively. (Table 2b).

Antimicrobial susceptibility profile showed that *Staphylococcus species*(The only Gram- positive organisms isolated) were resistant to at least four antimicrobial agents resulting in 5 different resistance patterns (Table 3a). On the other hand, the Gram – negative bacterial isolates were resistant to at least one antimicrobial agents resulting in 15 different resistance patterns (Table 3b).

DISCUSSION

The present study screened cloacal swabs collected from turkey and chicken in selected farms in Ibadan. Pathogenic bacteria in diverse genera were obtained in the study and majority of these bacteria are of the family Enterobacteriaceae while some are of the families Pseudomonadaceae and Staphylococcaceae. The rate of isolation of Escherichia coli from turkey and chicken in the work is 8% and 46% respectively. This is slightly different from an earlier study by Zhao et al. (2001) who reported a rate of 12% and 39% in turkey and chicken respectively. The prevalence of Salmonella species as observed in the present work is 23% and 15% in turkey and chicken respectively. This is lower than a prevalence rate of 71% and 84% stated by Ahmed et al. (2008) in Bangladesh and Ramya et al. (2012) in India respectively.

The differences observed in the prevalence could be due to locations and management practices by farmers. Turkey and chicken convey antibiotic resistant bacteria to which humans are exposed through consumption of poultry meat (Cook et al., 2009; Aslam et al., 2012). Previous surveillance discoveries also showed that other poultry meats carry resistant bacteria (Agunos *et al*., 2012). Antimicrobial resistance burdens arising in public health are mounting duress on veterinarians and poultry producers in order to ensure that antimicrobials are used judiciously from animal and food safety perspectives (CDC, 2012). Cloacal swabs have been found to provide indication of continuous colonization of the intestine by bacteria (Gast *et al*., 2013), thus supporting the discovery in the study as it detected seven various Gram-negative bacteria (Salmonella, Escherichia, Citrobacter, Yersinia, Pseudomonas, Proteus, and Enterobacter) with Staphylococcus as the only Gram-positive bacterium. These are pathogenic bacteria which contaminate poultry meats and cause food poisoning in humans through consumption of poultry meats and other poultry products (Bhandare et al., 2007). These organisms showed high resistance to commonly used antibiotics such as chloramphenicol, streptomycin, sulphamethoxazole, augmentin and other drugs. This agrees with earlier studies by Kim et al., (1994) who observed a similar reaction of bacterial organisms to frequently used antimicrobial agents in both animals and humans. The resistance of these bacteria to antimicrobial agents could be associated with the increased occurrence of these pathogens in animals that are fed antibiotics (Kim et al., 1994). There is also straight indication that the administration of antimicrobial drugs in animal feeds selects for resistant serotypes of bacteria which can be transmitted to humans through the food chain or direct contact (Feinman, 1998). The staphylococcal isolates were susceptible to most of the antibiotics examined in this study with few exceptions. This is in agreement with an initial statement that staphylococcal isolates of turkey are susceptible to commonly used antimicrobial agents (MAPAQ, 2011). However, it is not clear if these in-vitro results connect with field efficacy as staphylococcal diseases with localized lesions (i.e., arthritis, osteomyelitis) are therapeutically challenging and difficult to reach when antimicrobials are administered orally (Dowling and Kruth, 2006). This study isolated and identified a high number of pathogenic bacteria from cloacal swabs showing that these bacteria could serve as sources of contaminants to poultry meats and other poultry products. The work found that poultry birds harbor pathogenic bacteria and serve as vectors for other vertebrate animals. In addition, the study observed a higher resistance to many of the commonly used antibiotics in the poultry industry which is of public health significance since most of these drugs are used for treatment of human infections.

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