STUDYING THE PREVALENCE OF QUINOLONES AND AMINOGLYCOSIDES RESISTANCE PROFILES AND TRANSMISSION OF AAC (6')-IB GENE FROM CHICKEN TO CONTACT HUMAN

Gamal A. Salem[1,3], Suzan K. Murad[2], Fathi B. Alamyel[3], Manal A. Ebowah[4], Marwa A. Mahmoud[4], Abdullah R. Abulifa[5]

1- Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, P.O. Box 44511, Egypt.
2- Department of Microbiology, Faculty of Nursing, Misurata University, Misurata, P.O. Box 2478, Libya
3- Department of Pharmacology and Toxicology, Faculty of Pharmacy, Misurata University, Misurata, P.O. Box 2478, Libya.
4- Faculty of Pharmacy, Misurata University, Misurata, P.O. Box 2478, Libya.
5- Faculty of Chemistry, Faculty of Pharmacy, Misurata University, P.O. Box 2478, Libya.

RNLNL 139/2018

ABSTRACT

The spread of resistant Enterobacteriaceae to antibiotics in chicken farms constitute a reservoir of resistant genes that could be easily transmitted to contact human. So, this study conducted to investigate the prevalence of ciprofloxacin and gentamycin resistance profiles and the frequency of aac (6')-Ib gene in chicken farms in Misurata city and living population in the vicinity of these farms. (135) cloacae swab from chicken and (107) urine samples from human living in these farms were collected. Isolation and identification of different Enterobacteriaceae strains is performed in antibiotic resistance profiles against ciprofloxacin and gentamycin antibiotics. DNA extracted from each sample and further screening for aac (6')-Ib-cr in ciprofloxacin and/or gentamycin resistant isolates by using of using PCR technique. Our results showed that 88.8% and 93.4% of chicken and human isolates confirmed to be Enterobacteriaceae respectively. There were non-significant association between the source of Enterobacteriaceae isolates and quinolones or aminoglycosides resistance profiles. The screening of the genetic determinant of gentamycin and ciprofloxacin resistance aac (6')-Ib-cr, revealed an impressive proximity between the frequency of this gene within the chicken and human isolates (33.6%, 33 %) respectively. In conclusion quinolones and aminoglycosides resistance profiles and the frequency of aac (6')-Ib-cr between chicken and human were nearly comparable, that would suggest the possible transmission of this gene from chicken to human.

KEY WORDS: Antibiotic resistance, Quinolones, Aminoglycosides, Enterobacteriaceae.

INTRODUCTION

Antimicrobial agents are widely used in food-producing animal farms such as chicken for prevention, treatment of animal diseasess and also as growth promoters. The intensive use of antimicrobial agents has major concern due to the possibility of emergence and dissemination of the resistant genes to human through animals[(1)]. The workers in the farms, abattoirs, veterinarians, and their families are directly at high risk of infection with resistant bacteria due to close contact with animals [(2)]. This transmission does not constitute initially a major health threat for the population. However, workers and their families represent a pass channel of resistance genes to the hospital, and community environment. Moreover, the extra spread of these resistant genes to other pathogens is likely to be possible[(3)]. Many studies have highlighted the possible transmission of resistance from poultry to workers[(4)].

Enterobacteriaceae spp. are important human pathogens that because hospital acquired infection such as urinary tract infection (UTI) and gastrointestinal infection[(5)]. The resistant Enterobacteriaceae have a major health concern in both pathogenic and commensal ones[(6)]. Many species of Enterobacteriaceae family cause UTI, which is the second cause of community-onset infection[(7)]. 75-95% of all uncomplicated cystitis and pyelonephritis caused by E. coli which the most common cause of UTI[(8)].

Quinolone antibiotics such as ciprofloxacin, ofloxacin and levofloxacin are used to treat wide range of gram-negative and gram-positive bacterial infections. They are used clinically because of proved bactericidal effect ageist most members of Enterobacteriaceae[(9)].

Aminoglycosides have antimicrobial activity against gram-negative and gram-positive bacteria, therefore aminoglycosides are considered a broad-spectrum antibiotic that are active against Enterobacteriaceae and Pseudomonas[(10)].

The use of fluoroquinolones, aminoglycosides, and third generation cephalosporins in animal farms and to treat human infection is hazardous those antibiotics are already used to treat human infections[(11)].

AAC (6') genes have clinical importance because they can modify a number of aminoglycosides antibiotic, including amikacin, gentamicin, and tobramycin. The aac (6')-I type responsible for resistance to amikacin by acetylation of the drug, while the aac (6')-II type acetylates gentamicin[(12–13)]. A recent mechanism of quinolones resistance is correlated to plasmid mediated quinolone resistant genes (aac(6')-Ib-cr, qnrA, qnrB, qnrS, and qepA)[(14)]. The first gene aac (6')-Ib-cr is a variant of aminoglycoside acetyltransferase which reduces flouroquinolones activity by binding of acetyl group to the drug. AAC (6′)-Ib-cr is common gene that causes resistance to both aminoglycoside and fluoroquinolone antibiotics[(15)].

Correspondence and reprint request:
Gamal A. Salem
Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig
E-mail: gamal_vet_85@yahoo.com

Usually, plasmid mediated quinolone resistance (PMQR) is correlated with extended spectrum beta-lactamases (ESBLs) and/or aminoglycosides resistant genes on the same plasmid, and transfer of multidrug resistant plasmids between Enterobacteriaceae strains would negatively affect the empirical treatment of complicated urinary tract infections[16].

So, this work is conducted to Investigate the prevalence of ciprofloxacin and gentamycin resistance profiles and the frequency of aac(6')-Ib-cr gene in chicken farms in Misurata city and living population in the vicinity of these farms.

METHODS

Bacterial isolates:

In this study, 135 bacterial isolates were collected from chicken cloacae at 20 different chicken farms located in Misurata, sterile cotton swabs were used to collect fecal samples from chicken cloacae then swabs are immediately transferred to sterile collection containers. A 107 Clean-Catch midstream urine samples were collected in a sterile tube (4-5 ml) from people in contact with chicken farms. At the bioresearch and consultancies center, Misurata University, samples were cultured on MaCconkey agar (OXOID, England) then incubated for 18-24hr at 35-37°C. Colonies may appear pink (lactose fermenting) or colorless (lactose non-fermenting). Size and shape of colonies vary with individual species. Suspected colonies are identified using the APIE20: (bioMérieux®, France)[17].

Antibiotic susceptibility testing:

Antibiotic sensitivity test for chicken and human samples is performed by using Mueller-Hinton agar according to Kirby-Bauer Disc Diffusion method following clinical and laboratory standards institute (CLSI) guidelines. The antibiotics included in this study are ciprofloxacin 5µg and gentamycin 10µg (Bioanalyse®, Turkey)[18]. The interpretation and results reporting of the categories of susceptible, intermediate or resistant was also based on the CLSI guidelines[19].

Bacterial DNA extraction:

The isolates are streaked on nutrient agar and incubated for 14-16 hr at 37° C. A single colony was picked up from the media plate and inoculated to 5 ml liquid culture media, then incubated overnight at 37° C. Genomic DNA was then extracted at the bioresearch and consultancies center, Misurata University, using the G-spinTM Total DNA Extraction Kit (INTRON Biotechnology, Korea) according to the manufacturer's recommendation.

Detection of antibiotic-resistance genes:

This procedure was performed in animal health institute, Cairo, Egypt, where Polymerase chain reaction (PCR) identification of aminoglycoside and quinolone resistance genes aac (6')-Ib was performed as described in previous study[20].

The PCR conditions involved an initial denaturation for 3 min at 95 °C followed by 30 cycles of (95 °C for 30 s, specific annealing temperature for 1 min, and extension at 72 °C for 30 s) followed by a final extension at 72 °C for 5 min. Sequences of the resistance-genes primers used in the study and their annealing temperatures are shown in (table 1).

(Table 1) Primers used for detection of aac(6')-Ib gene and RAPD typing, annealing temperatures (Ta), and expected product sizes.

Primers	Sequence (5'-3')	Target gene	T_a	Product size	Reference
Aac (6')-Ib-F aac (6')-Ib-R	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	aac(6')-Ib	54°C	482 bp	18

Statistical analysis:

Data were presented in frequency and percentage. Chi square test using SPSS/21 was done for these data where the level of significance ($p \leq 0.05$).

RESULTS AND DISCUSSION

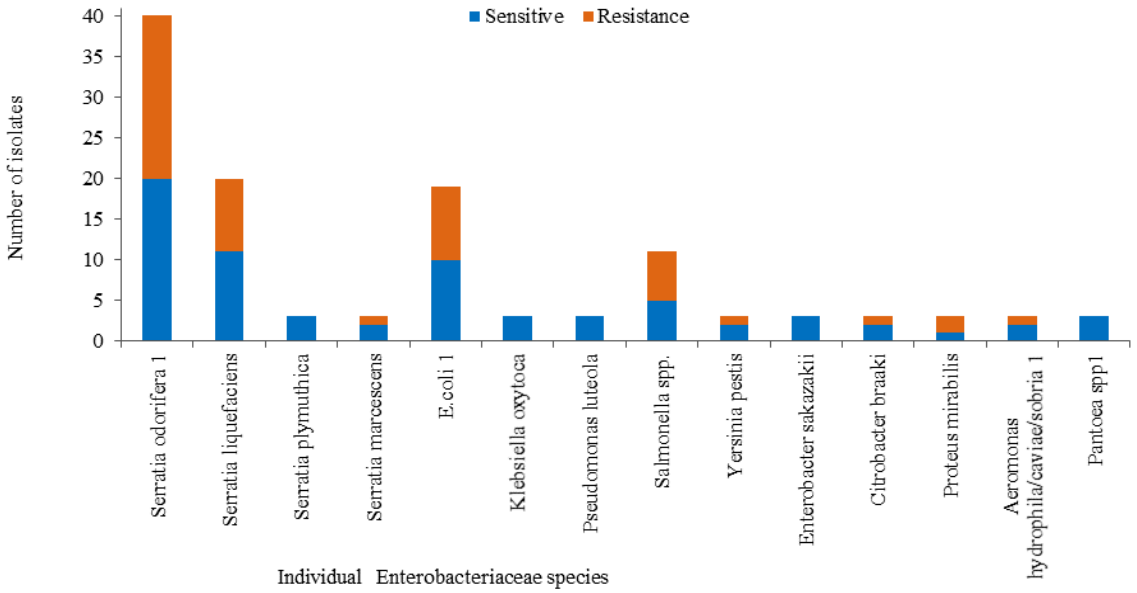
An inevitable side effect of the use of antibiotics is the emergence and dissemination of resistant bacteria. Most studies show that after the introduction of an antibiotic not only increase the level of resistance in pathogenic bacteria, but also in commensal bacteria. Commensal bacteria constitute a reservoir of resistant genes for (potentially) pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens[21]. Antibiotic resistance in bacteria from the family Enterobacteriaceae is an important indicator of the emergence of resistant bacterial strains in the community[22]. Farm and slaughterhouse workers with resistant bacteria through close contact with colonized or infected animals. Although this limited transmission does not initially appear to pose a population-level health threat. Occupational workers and their families provide a conduit for the entry of resistance genes to the community and hospital environments, where further spread into pathogens is possible[23,24]. We performed this study in order to investigate the prevalence of quinolone and aminoglycosides resistance pattern and the frequency of aac (6')-Ib-cr gene in commensal and pathogenic isolates from chicken farms and urine of contact human (either farm workers or living population near to farms). In this study, Enterobacteriaceae species isolates accounts for (88.8%) in chicken and (93.4%) In human isolates.

Different Enterobacteriaceae strains were identified from chicken including; E. coli 19/120 (15.8%), Salmonella spp. 11/120 (9.1%) and Yersinia pestis 3/120 (2.5%) pathogenic bacteria and represent a zoonotic threat to contact human. There were other Enterobacteriaceae isolates from chicken that would be opportunistic pathogen such as Serratia spp. 66/120 (55%), and 3/120 (2.5%) for Pseudomonas luteola, Klebsiel-

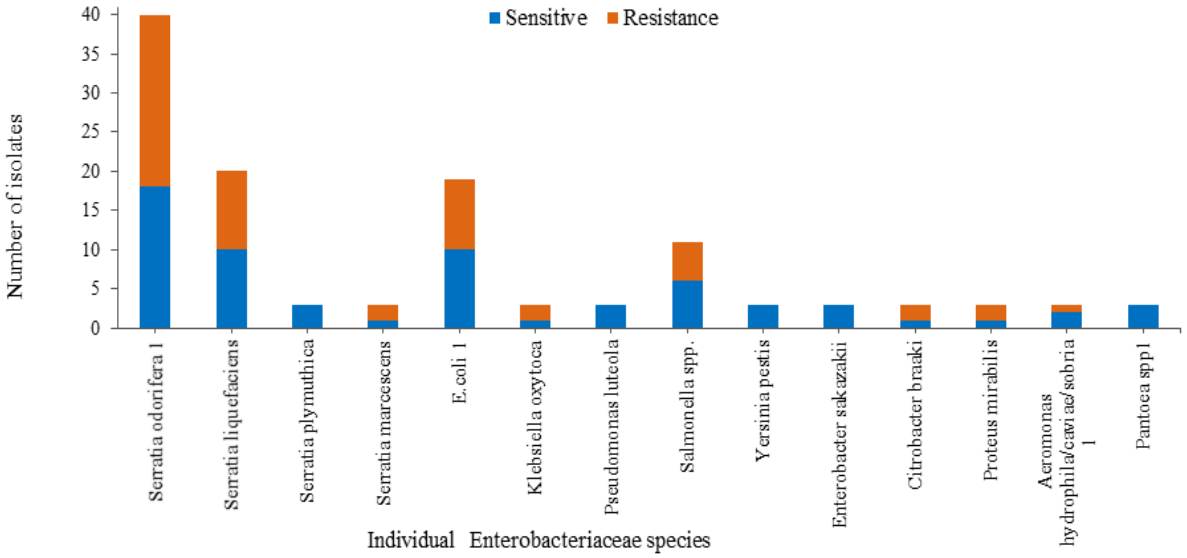
la oxytoca, Citrobacter brakii, Enterobacter sakazakii, Proteus mirabilis and Pantoea spp**.** The frequency of individual Enterobacteriaceae spp. of chicken isolates and their resistance pattern are shown in (figure 1,2) and (table 2).

(Table 2) The frequency and antibiotics (ciprofloxacin & gentamycin) resistance profiles of individual Enterobacteriaceae in chicken and human isolates.

Name of Enterobacteriaceae	Source of Enterobacteriaceae	No. % of Enterobacteriaceae isolates	Ciprofloxacin		Gentamycin	
			R	S	R	S
Serratia odorifera 1	Human	12/100 (12)	5/12 (41.7)	7/12 (58.33)	6/12 (50)	6/12 (50)
	Chicken	40/120 (33.3)	20/40 (50)	20/40 (50)	22/40 (55)	18/40 (45)
Serratia liquefaciens	Human	11/100 (11)	3/11 (27.3)	8/11 (72.7)	6/11 (54.5)	5/11 (45.5)
	Chicken	20/120 (16.6)	9/20 (45)	11/20 (55)	10/20 (50)	10/20 (50)
Serratia plymuthica	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Serratia marcescens	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
E. coli 1	Human	20/100 (20)	8/20 (40)	12/20 (60)	9/20 (45)	11/20 (55)
	Chicken	19/120 (15.8)	9/19 (47.4)	10/19 (52.6)	9/19 (47.4)	10/19 (52.6)
Klebsiella pneumonia	Human	17/100 (17)	7/17 (41.2)	10/17 (58.8)	9/17 (52.9)	8/17 (47.1)
	Chicken	-	-	-	-	-
Klebsiella oxytoca	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	2/3 (66.6)	1/3 (33.3)
Pseudomonas luteola	Human	6/100 (6)	4/6 (66.6)	2/6 (33.3)	2/6 (33.3)	4/6 (66.6)
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Pseudomonas fluorescens/putida	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	0/3 (0)	3/3 (100)
	Chicken	-	-	-	-	-
Salmonella spp.	Human	3/100 (3)	1/3 (33.3)	2/6 (66.6)	2/3 (66.6)	1/3 (33.3)
	Chicken	11/120 (9.1)	6/11 (54.5)	5/11 (45.5)	5/11 (45.5)	6/11 (54.5)
Yersinia pestis	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.66)	0/3 (0)	3/3 (100)
Enterobacter sakazakii	Human	3/100 (3)	1/ (33.3)	2/3 (66.6)	2/3 (66.6)	1/ (33.3)
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Enterobacter cloacae	Human	6/100 (6)	2/6 (33.3)	4/6 (66.6)	2/6 (33.3)	4/6 (66.6)
	Chicken	-	-	-	-	-
Citrobacter braaki	Human	3/100 (3)	2/3 (66.6)	1/3 (33.3)	0/3 (0)	3/3 (100)
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
Proteus mirabilis	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)
	Chicken	3/120 (2.5)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)
Raultella ornithinolytica	Human	7/100 (7)	3/7 (42.3)	4/7 (57.7)	2/7 (28.6)	5/7 (71.4)
	Chicken	-	-	-	-	-
Acintobacter baumannii/calcoaceticus	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	0/3 (0)	3/3 (100)
	Chicken	-	-	-	-	-
Aeromonas hydrophila/caviae/sobria 1	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)
Pantoea spp1	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)



(Figure 1) Isolation, identification and ciprofloxacin resistance profile of chicken isolates.



(Figure 2) Isolation, identification and gentamycin resistance profile of chicken isolates.

Kilonzo-Nthenge et al.[25] studied the occurrence of Enterobacteriaceae in retail meats, Out of 281 bacteria isolates from raw meat samples, (12.1%) were identified as E. coli, Morganella morgana (1.1%), Vibrio parahemolyticus (0.4%), Yersinia enterocolitica (0.4%), Salmonella spp. (5.7%), Proteus mirabilis (1.1%), Enterobacter aerogenes (6.4%), Klebseiella oxytoca (27.4%), Citrobacter freundii (1.7%) Hafnia alvei (11.4%) Serratia ssp. (14.3%) Enterobacter aerogenes (6.4%), Kluvyera spp. (5.6%), and Pantoea spp. (3.6%). The occurrence of Klebseiella oxytoca in retail meats was the highest among all other pathogens. While, Yulistiani et al.[19] investigated the prevalence of antibiotic-resistant Enterobacteriaceae isolated from chicken meat contaminated during evisceration and sold at traditional markets in Surabaya Indonesia. In all 203 isolates; Salmonella spp. (41.7%), E.coli (26.1%), Citrobacter spp. (10.8), Klebsiella spp. (6.4%), Proteus spp. (11.8%), Yersinia spp. (7.3), Enterobacter spp. (3.4%) and Serratia spp. (2.9%) were identified. These results were high consistent with our results except for Serratia spp. In our study the prevalence of Serratia spp. is high in chicken isolates which are divided into four spp.; Serratia odorifera 1 (33.3%), Serratia liquefaciens (16.6%), Serratia plymuthica (2.5%), and Serratia marcescens (2.5%). Serratia marcescens is serious pathogen capable of causing important infections in human and animals such as (UTIs) and pneumonia, which becomes highly prevalent and shows a multidrug resistance profile[26].

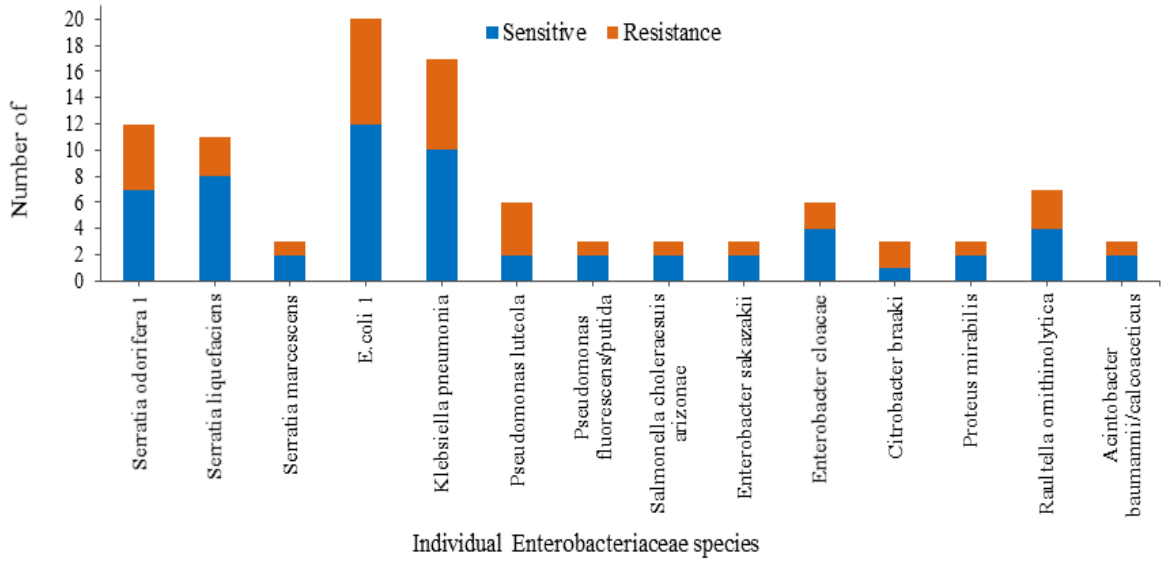
After investigation of chicken isolates, we found that 41.7% of Enterobacteriaceae were resistant to ciprofloxacin and 45.8 % resistant to gentamycin as shown in (table 3).

(Table 3) Antibiotic resistance profile of Enterobacteriaceae family against ciprofloxacin and gentamycin antibiotics in chicken and human isolates.

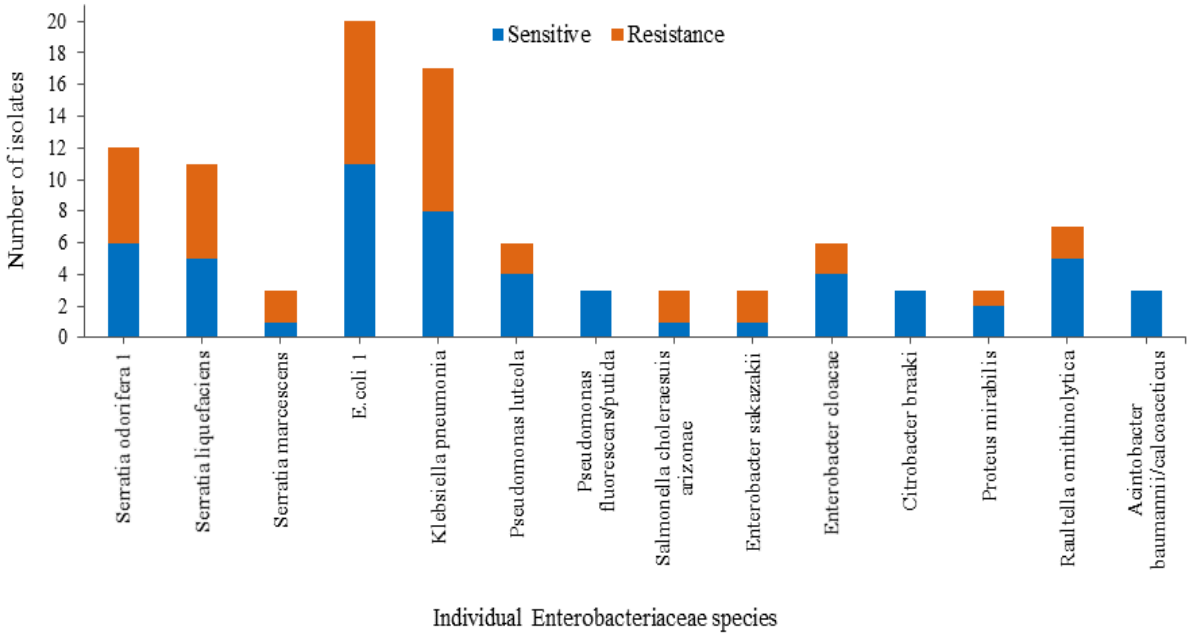
Source of Enterobacteriaceae	Ciprofloxacin		P- value	Gentamycin		P-value
	R	S		R	S	
Human	40/100 (40)	60/100 (60)	0.802	43//100 (43)	57/100 (57)	0.67
Chicken	50/120 (41.7)	70/120 (58.3)		55/100 (45.8)	65/120 (54.2)	

Kilonzo-Nthenge et al.[25] and Yulistiani et al.[22] found lower resistance rates of Enterobacteriaceae against gentamycin (9.6%) and (5.91%) isolated from retail meat respectively. previous studies reported that ciprofloxacin resistance in chicken meat isolates were (12.5%) and (0%) respectively[27,28].

The isolated Enterobacteriaceae strains from urine samples of accompanying human were; E.coli (20%), Klebsiella pneumonia (17%), Serratia odorifera 1 (12%), Serratia liquefaciens (11%), Raultella ornithinolytica (7%), Pseudomonas luteola and Enterobacter cloacae (6%), and others were lower percentage as; Serratia marcescens, Pseudomonas fluorescens/putida, Salmonella spp., Enterobacter sakazakii, Citrobacter braaki, Proteus mirabilis, Acintobacter baumannii/calcoaceticus. The frequency of individual Enterobacteriaceae spp. of human isolates and their resistance pattern shown in (figure 3,4) and (table 2).



(Figure 3) Isolation, identification and ciprofloxacin resistance profile of human isolates.



(Figure 4) Isolation, identification and gentamycin resistance profile of human isolates.

The present study conducted in Misurata city in Libya showed that higher prevalence of E. coli, K. pneumonia, E. cloacae and Serratia marcescens than that conducted in Tripoli city in Libya[29]. While Yang et al.[30] reported that Enterobacteriaceae composed of 88.5% of the total isolates, with exceeding rate of E. coli (63.2%) and K.pneumoniae (12.2%).

Concerning human isolates about 40% and 43% exhibited resistance against ciprofloxacin and gentamycin respectively as presented in (table 3).

Tobgi et al.[31] tested the susceptibility of E. coli to antibiotics. 148 E. coli isolated from urine specimens of outpatients attending Al-Jalla Hospital in Benghazi. They reported that 18% of isolates were resistant to gentamicin. Ghenghesh et al.[32] found 7% resistance to gentamicin of 538 E. coli isolates from patients with UTIs in Tripoli.

M. I. Issack et.al.[33] observed the presence of high rate of resistance to fluoroquinolone (26.4% to ciprofloxacin). In a survey conducted in India between 2010 and 2014, they found an increase resistance rate in Enterobacteriaceae isolated from hospitalized patients against ciprofloxacin (39.2-56.1%)[33,34].

Statistical analysis of the antibiotic resistance profiles against ciprofloxacin and gentamycin in both chicken and human revealed no association ($P > 0.05$) between the source of isolates and the pattern of resistance. In other words, we can say that the rates of resistance against ciprofloxacin and gentamycin in the two species were nearly comparable (table 4). These results convey a proof of the possible transmission of resistant gene between chicken and contact human.

(Table 4) Antibiotic resistance profiles of the common Enterobacteriaceae individual spp. in chicken and human isolates

Name of Enterobacteriaceae	Source of Enterobacteriaceae	Ciprofloxacin			Gentamycin		
		R	S	P-value	R	S	P-value
Serratia species	Human	9/26 (34.6)	17/26 (65.4)	0.343	14/26 (53.8)	12/26 (46.2)	0.840
	Chicken	30/66 (45.4)	36/66 (54.5)		34/66 (51.5)	32/66 (48.5)	
Serratia marcescens	Human	1/3 (33.3)	2/3 (66.6)	1.000	2/3 (66.6)	1/3 (33.3)	1.000
	Chicken	1/3 (33.3)	2/3 (66.6)		2/3 (66.6)	1/3 (33.3)	
E. coli 1	Human	8/20 (40)	12/20 (60)	0.643	9/20 (45)	11/20 (55)	0.882
	Chicken	9/19 (47.4)	10/19 (52.6)		9/19 (47.4)	10/19 (52.6)	
Salmonella species	Human	1/3 (33.3)	2/6 (66.6)	0.515	2/3 (66.6)	1/3 (33.3)	0.515
	Chicken	6/11 (54.5)	5/11 (45.5)		5/11 (45.5)	6/11 (54.5)	
Citrobacter braaki	Human	2/3 (66.6)	1/3 (33.3)	0.414	0/3 (0)	3/3 (100)	0.083
	Chicken	1/3 (33.3)	2/3 (66.6)		2/3 (66.6)	1/3 (33.3)	
Proteus mirabilis	Human	1/3 (33.3)	2/3 (66.6)	0.414	1/3 (33.3)	2/3 (66.6)	0.414
	Chicken	2/3 (66.6)	1/3 (33.3)		2/3 (66.6)	1/3 (33.3)	

The screening of the genetic determinant of gentamycin and ciprofloxacin resistance, aac (6) Ib-cr, revealed an impressive proximity between the frequency of this gene within the chicken and human isolates (28.33%, 33 %) respectively that supposes prodigious evidence for the transmission of resistance (table 5).

(Table 5) Prevalence of aac (6')-Ib-cr gene in chicken and human.

Source of Enterobacteriaceae	aac(6')-Ib-cr (No)	aac(6')-Ib-cr (%)
Chicken	34	28.3
Human	33	33

The aac (6')-Ib-cr is a plasmid-mediated quinolone resistance (PMQR) gene embedded within a gene cassette, most often within an integron. It confers resistance to both quinolone and aminoglycoside[35]. Agabou et al.[36] found that commensal E. coli isolates collected from chickens, their farmers, and patients in the Constantine region (North-east Algeria) were analyzed for plasmid mediated quinolone resistance (PMQR) gene contents types. A high prevalence of resistance to fluoroquinolone (51.4 % to ciprofloxacin) was recorded in avian isolates. Of these, (22.2 %) carried the aac 6')-Ib-cr gene, While seventy pathogenic isolates were resistant to fluoroquinolone, with aac(6')-Ib-cr present in (72.8 %). Park et al.[37] isolated 313 human Enterobacteriaceae isolates from the United States, aac(6')-Ib was present in 50.5% of isolates, and of these, 28% carried the (cr) variant responsible for low-level ciprofloxacin resistance. Yang et al.[30] showed that aac(6')-Ib-cr was present in 17.0% of the E.coli human isolates, and 7.9% of the isolates carried both the qnr and the aac(6')-Ib-cr genes.

Resistance to aminoglycosides may be observed at several levels. High-level resistance is generally associated with chemical modifications of the aminoglycoside by enzymes[38]. Studies of aminoglycoside-resistant organisms from different countries have shown that the resistance mechanisms are different and may be a country/area specific resistance[39]. A surveillance study conducted in European countries on Enterobacteriaceae showed that resistance against gentamicin was between (2-13%) and the found gene responsible for this resistance were aac (3')-IIa, followed by aac (6')-Ib-cr[40]. AAC enzymes are important because they are among the few that confer resistance to aminoglycosides and quinolones Schmitz et al.[43].

Miró et al.[41] studied the characterization of aminoglycoside-modifying enzymes in Enterobacteriaceae clinical Strains and found the most frequent gene was aph (3')-Ia (13.9%) and aac (3)-IIa (12.4%), followed by aac (6')-Ib (4.2%), and among the 14 aac (6')-Ib eight showed the (cr) variant (57.1%)

CONCLUSION

In this study, it is concluded that Enterobacteriaceae isolates from chicken and human showed no difference in their resistance pattern against ciprofloxacine and gentamicin antibiotics. The prevalence of aac (6')-Ib gene within chicken and human provide a possible proof of transmission of this resistance gene between the two species.

REFERENCES

1- Marshall BM, Levy SB. (2011). Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev; 24(4): 718.

2- van den Bogaard AE, Willems R, London N, Top J, Stobberingh EE (2002 Mar). Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother; 49(3): 497-505.

3- Welton LA, Thal LA, Perri MB, Donabedian S, McMahon J, Chow JW, Zervos MJ. (1998). Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. Antimicrob Agents Chemother; 42(3): 705-8.

4- Price LB1, Graham jp, Lackey LG, Roess A, Vailes R, Silbergeld E (2007). Elevated risk of carrying gentamicin-resistant Escherichia coli among U. S poultry workers. Environ Health Perspect; 115(12): 1738-42.

5- Karlowsky JA, Jones ME, Thornsberry C, Friedland IR, Sahm DF (2003). Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. Antimicrob Agents Chemother; 47(5): 1672-80.
6- Brower CH, Mandal S, Hayer S, Sran M, zehra A, patel SJ, Kaur R, Chatterjee L, Mishra S, Das BR, Singh P, Sing R, Gill JPS, Laxminarayan R (2017). The Pervalence of Extended-Spectrum Beta-lactamase-Producing Multidrug-Resistant Escherichia Coli in Poultry Chickens and Variation according to farming practices in Punja, India. workers. Environ Health Perspect; 125(7): 077015.
7- Gupta K, Hooton TM, Naber KG, Wullt BR, Colgan R, Miller LG, et al. (2011). International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis; 1; 52(5): e103-20.
8- Hooton TM (2012). Clinical practice. Uncomplicated Urinary Tract Infection. N Engl J Med; 366(11):1028-37.
9- Andriole VT (2005). The Quinolones: Past, Present, and Future. Clin Infect; 41(Supplement 2): S113-9.
10- Craig WA (2011). Optimizing Aminoglycoside Use. Crit Care Clin;27(1):107-21.
11- Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. (2015). The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect; 6: 22-9.
12- Shaw KJ, Rather PN, Hare RS, Miller GH (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev; 57(1): 138–63.
13- Vakulenko SB, Mobashery S (2003). Versatility of aminoglycosides and prospects for their future. Clin Microbiol Rev; 16(3): 430-50.
14- Paterson DL (2006). Resistance in Gram-Negative Bacteria: Enterobacteriaceae. Am J Med; 119(6): S20–8.
15- Vetting MW, Park CH, Hegde SS, Jacoby GA, Hooper DC, Blanchard JS (2008). Mechanistic and Structural Analysis of Aminoglycoside N -Acetyltransferase AAC (6′)-Ib and Its Bifunctional, Fluoroquinolone-Active AAC (6′)-Ib-cr Variant. Biochemistry; 47(37): 9825-35.
16- Shams E, Firoozeh F, Moniri R, Zibaei M (2015). Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum β-Lactamase-Producing Klebsiella pneumoniae Human Isolates in Iran. J Pathog; 2015: 434391.
17- Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al (2008). Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol; 74(17): 5402-7.
18- Hudzicki J (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.
19- FAO (2001) Performance Standards for Antimicrobial Disk Susceptibility Tests, 12th Edition
20- Doi Y, Arakawa Y (2007). 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis; 45(1): 88-94.
21- van den Bogaard AE, Stobberingh EE (2000). Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents;14(4):327-35.
22- Yulistiani R, Praseptiangga D, Supyani, Sudibya, Raharjo D, Shirakawa T (2017). Prevalence of Antibiotic-resistance Enterobacteriaceae strains Isolated from Chicken Meat at Traditional Markets in Surabaya, Indonesia. IOP Conf Ser Mater Sci Eng;193(1):012007.
23- Welton LA, Thal LA, Perri MB, Donabedian S, McMahon J, Chow JW, et al (1998). Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. Antimicrob Agents Chemother; 42(3): 705-8.
24- Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol; 13(5): 269-84.
25- Kilonzo-Nthenge A, Rotich E, Nahashon SN, (2013). Evaluation of drug-resistant Enterobacteriaceae in retail poultry and beef. Poult Sci; 92(4):1098-107.
26- Mahlen SD (2011). Serratia infections: From military experiments to current practice. Clin Microbiol Rev; 24(4): 755-91.
27- Hawkey PM (1998). The origins and molecular basis of antibiotic resistance. BMJ; 317(7159): 657–60.
28- Hanson R, Kaneene JB, Padungtod P, Hirokawa K, Zeno C (2002). Prevalence of salmonella and E. coli, and their reistance to antimicrobile agents, in farming communities in northern thailand. Southeast Asian J Trop Med Public Health; 33 Suppl 3:120-6.
29- Abujnah AA, Zorgani A, Sabri MAM, El-Mohammady H, Khalek RA, Ghenghesh KS (2015). Multidrug resistance and extended-spectrum β-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med;10(1):26412.
30- Yang H, Chen H, Yang Q, Chen M, Wang H (2008). High prevalence of plasmid-mediated quinolone resistance genes qnr and aac (6')-Ib-cr in clinical isolates of Enterobacteriaceae from nine teaching hospitals in China. Antimicrob Agents Chemother; 52(12): 4268–73.
31- Tobgi R, Taher I, Ali M (2001). Antibiotic susceptibility of uropathogens in Benghazi, Libya. Jamahiriya Med J; 1(2):46-49.
32- Sifaw Ghenghesh K (2003). High Antimicrobial-Resistant Rates of Escherichia coli from Urine Specimens in Tripoli -Libya. Garyounis Med J; 20:89–93.
33- Issack M (2016). Antibiotic resistance among hospitalized patients in Mauritius in 2014. Int J Infect Dis; 45:94.
34- Gay N, Belmonte O, Collard J-M, Halifa M, Issack MI, Mindjae S, et al (2017). Review of Antibiotic Resistance in the Indian Ocean Commission: A Human and Animal Health Issue. Front public Heal; 5:162.
35- Raherison S, Jove T, Gaschet M, Pinault E, Tabesse A, Torres C, et al (2017). Expression of the aac (6′)-Ib-cr gene in class 1 integrons. Antimicrob Agents Chemother; 61(5): e02704-16.
36- Agabou A, Lezzar N, Ouchenane Z, Khemissi S, Satta D, Sotto A, et al (2016). Clonal relationship between human and avian ciprofloxacin-resistant Escherichia coli isolates in North-Eastern Algeria. Eur J Clin Microbiol Infect Dis; 35(2):227-34.

37- Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC (2006). Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother;50(11):3953-5.
38- Montie T, Patamasucon P (1995). Aminoglycosides: The complex problem of antibiotic mechanisms and clinical applications. Eur J Clin Microbiol Infect Dis; 14(2): 85-7.
39- Dornbusch K, Miller GH, Hare RS, Shaw KJ (1990). Resistance to aminoglycoside antibiotics in Gram-negative bacilli and staphylococci isolated from blood. Report from a European collaborative study. J Antimicrob Chemother; 26(1): 131-44.
40- Shaw KJ, Rather PN, Hare RS, Miller G (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev; 57(1): 138-63.
41- Miró E, Grünbaum F, Gómez L, Rivera A, Mirelis B, Coll P, et al (2013). Characterization of Aminoglycoside-Modifying Enzymes in Enterobacteriaceae Clinical Strains and Characterization of the Plasmids Implicated in Their Diffusion. Microb Drug Resist; 19(2): 94-9.