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Short Communication

Isolation and antimicrobial resistance of *Escherichia coli* isolated from farm chickens in Taif, Saudi Arabia

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ABSTRACT

Objectives: Poultry is one of the main sources of food in the world. Antimicrobial-resistant *Escherichia coli* can be transmitted to humans by contact with poultry waste or by contaminated poultry products, contributing to the increasing crisis of antimicrobial resistance. This study aimed to determine the incidence of antimicrobial resistance in *E. coli* isolated from chickens in Taif province, Saudi Arabia, and to identify the genes responsible for any resistance observed.
Methods: A total of 150 cloacal swabs were aseptically obtained from chickens from different farms, from which 180 colonies of *E. coli* were identified using standard microbiology procedures. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method. The genes bla_{SHV}, *aac(3)-IV*, *tet*(A), *tet*(B), *aadA1*, *catA1*, *cmlA*, *ere*(A) and *sul1* were detected by PCR.
Results: Most of the *E. coli* isolates showed resistance to oxacillin (99%), lincomycin (98%) and oxytetracycline (97%). The prevalence of resistance to chloramphenicol (73%), ciprofloxacin (59%) and ampicillin (51%) was lower. Genes conferring resistance to β-lactams (bla_{SHV}) and tetracyclines [*tet*(A) and *tet*(B)] were observed at prevalences of 96% and 95%, respectively, among the *E. coli* isolates. Chloramphenicol (*catA1* and *cmlA*) and erythromycin [*ere*(A)] resistance genes showed prevalences of 72% and 15%, respectively, whereas gentamicin [*aac(3)-IV*], streptomycin (*aadA1*) and sulfonamide (*sul1*) resistance genes were detected in 20%, 20% and 10% of the studied isolates, respectively.
Conclusion: A significant prevalence of antimicrobial resistance genes was observed among *E. coli* isolates from farm chickens, supporting strict regulatory procedures for the use of antimicrobial agents.

1. Introduction

Poultry is an increasing source of food in the world. However, it is also one of the most consumed foodstuffs commonly associated with outbreaks of foodborne disease. Pathogenic micro-organisms can be transferred to humans by contact with poultry waste or by contaminated poultry foodstuffs. The avian gut has been considered as a reservoir of *Escherichia coli* that could potentially be transmitted from birds to humans [1]. *Escherichia coli* is a Gram-negative bacterium that generally acts as a natural commensal in the digestive tracts of humans, animals and birds, but some strains are significant intestinal and extraintestinal pathogens [2].

Pathogenic *E. coli* from animals, birds and humans can cause a variety of diseases, ranging from self-limiting gastrointestinal infections to bacteraemia. Antimicrobial agents have been used for various veterinary and agricultural purposes, including animal husbandry and poultry production where poultry feed is supplemented with antibiotics [3]. Moreover, antibiotics are widely utilised to control infectious illnesses and as growth promoters in poultry production. Application of antimicrobials and their misuse is considered to be the most important selecting influence for the spread of antimicrobial resistance in bacteria both in human and veterinary medicine [4]. Indeed, antimicrobial resistance developed in pathogens colonising animals can cause the emergence and distribution of resistant *E. coli* that are subsequently transmitted to humans by contact with infected animals or derived products [5]. During carcass processing, resistant bacteria from the poultry gastrointestinal tract can contaminate the meat product. Even wild migrating and resident birds can act as carriers and transmitters of multidrug-resistant (MDR) *E. coli* and *Escherichia vulneris* [1].

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Recently, the levels of antimicrobial resistance reported in bacteria have increased due to the high use of antibiotics in veterinary medicine, partly mediated by the spread of resistance-conferring plasmids between and within bacterial species [6].

MDR but non-pathogenic *E. coli* in the gastrointestinal tract could be a significant reservoir of resistance genes [7]. Therefore, the aim of this work was to isolate *E. coli* strains from chickens in different farms of Taif (Makkah Province, Saudi Arabia) in order to evaluate their resistance patterns to selected antimicrobial agents and to identify the genes conferring any resistance detected.

2. Materials and methods

2.1. Sample collection

Sterile swab sticks moistened with sterile normal saline were inserted into the cloacae of 150 chickens from different farms in Taif and were placed in sterile vials. Following sample collection, the samples were transported immediately to the laboratory in an insulating foam box with ice and were stored at 4 °C until use.

2.2. Isolation and identification of Escherichia coli

Cloacal swabs were inoculated on MacConkey agar plates (Oxoid Ltd., Basingstoke, UK) and were incubated at 37 °C for 18–24 h. Then, 300 bacterial colonies (2 colonies per chicken) were picked from the MacConkey agar as smooth pink colonies. Only 180 colonies were analysed further. Suspected colonies of *E. coli* were grown on nutrient agar plates (Oxoid Ltd.) after a series of subculturing on MacConkey agar. The isolates were characterised by Gram staining, triple sugar iron agar and lysine iron agar, and for oxidative/fermentative degradation of glucose, citrate utilisation, urease production, indole test, tryptophan degradation, glucose degradation (methyl red test) and motility. The *E. coli* isolates were stored in tryptic soy broth (Merck, Darmstadt, Germany) with 15% glycerol at −20 °C.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) [8] on Mueller–Hinton agar plates using single antimicrobial disks (Bio-Rad Laboratories, Hemel Hempstead, UK). The following antimicrobials were used: cefaclor; oxacillin; ampicillin; chloramphenicol; cefalexin; neomycin; colistin; ciprofloxacin; oxytetracycline; norfloxacin; lincomycin; gentamicin; amoxicillin; enrofloxacin; piperacillin; amikacin; cefalotin; cefuroxime; cefoxitin; ceftazidime; ceftriaxone; cefepime; aztreonam; amoxicillin/clavulanic acid (AMC); piperacillin/tazobactam (TZP); trimethoprim/sulfamethoxazole (SXT); and levofloxacin. Plates were incubated at 37 °C for 24 h and the inhibition zone diameter was measured with a meter rule and was recorded.

2.4. DNA extraction of Escherichia coli isolates

Escherichia coli isolates were subcultured overnight in Luria–Bertani broth and genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Southampton, UK) according to the manufacturer's instructions.

2.5. Primers and PCR assay for specific genes

The incidence of genes related to resistance to β-lactams (bla_{SHV}), gentamicin [*aac(3)-IV*], streptomycin (*aadA1*), tetracyclines [*tet*(A) and *tet*(B)], chloramphenicol (*catA1* and *cmlA*), erythromycin [*ere*(A)] and sulfonamides (*sul1*) was determined by basic PCR. The set of primers used for each gene is shown in Table 1.

The primers were designed using the Primer-BLAST website according to Ye et al. [9].

PCR reactions were performed in a total volume of 25 μL using GoTaq® Green Master Mix (Promega), including 12.5 μL of GoTaq® Green Master Mix (2×), 2.5 μL of upstream primers (10 μM), 2.5 μL of downstream primers (10 μM), 2.5 μL of nuclease-free water and 5 μL (40–260 ng/μL) of DNA. Amplification reactions were carried out using a DNA thermocycler (Fisher Scientific UK, Loughborough, UK) as follows: 3 min at 95 °C; 35 cycles each consisting of 1 min at 94 °C, 90 s at the annealing temperature (Table 1) and 1 min at 72 °C; followed by a final extension step of 10 min at 72 °C. PCR amplification was performed in duplicate. Amplified samples were analysed by electrophoresis in 1.5% agarose gel and were stained with ethidium bromide. A molecular weight marker with 100-bp increments (100-bp DNA ladder) was used as a size standard.

3. Results

3.1. Isolation and identification of Escherichia coli

A total of 300 bacterial isolates (2 colonies per chicken) were selected from MacConkey agar as smooth pink colonies. According

Table 1
Primers used in this study.

Antimicrobial class/agent	Resistance gene	Primer sequence (5′ → 3′)	PCR product size (bp)	Melting temperature (°C)	Annealing temperature (°C)
β-Lactams	$bla_{SHV-199}$	F-CTATCGCCAGCAGGATCTGG	543	60.04	55
		R-ATTTGCTGATTTCGCTCGGC		59.90	
Gentamicin	*aac(3)-IVa*	F-ATGTCATCAGCGGTGGAGTG	454	60.11	55
		R-GGAGAAGTACCTGCCCATCG		59.89	
Streptomycin	*aadA1*	F-TCGCCTTTCACGTAGTGGAC	816	60.04	55
		R-CAACGATGTTACGCAGCAGG		59.90	
Tetracyclines	*tet*(A)	F-CCTCAATTTCCTGACGGGCT	712	60.04	55
		R-GGCAGAGCAGGGAAAGGAAT		60.03	
	tet(B)	F-ACCACCTCAGCTTCTCAACG	586	59.97	55
		R-GTAAAGCGATCCCACCACCA		60.04	
Chloramphenicol	*catA1*	F-GAAAGACGGTGAGCTGGTGA	473	59.97	55
		R-TAGCACCAGGCGTTTAAGGG		60.04	
	cmlA5	F-GTGACATTTACGCAGGTCGC	532	59.91	55
		R-TGCGAAGCCCATATTTCGGT		60.11	
Erythromycin	*ere*(A)	F-CGATTCAGGCATCCCGGTTA	897	59.89	55
		R-CCATGGGGGCATCTGTCAAT		60.11	
Sulfonamides	*sul1*	F-ACTGCAGGCTGGTGGTTATG	271	60.32	55
		R-ACCGAGACCAATAGCGGAAG		59.54	

to morphological and biochemical characterisation, 180 (60%) of the bacterial isolates from chickens were *E. coli*.

3.2. Antimicrobial susceptibility testing

The *E. coli* isolates were screened for antimicrobial susceptibility (Table 2). Among the 180 *E. coli* isolates, high rates of resistance were observed to oxacillin (99%), lincomycin (98%) and oxytetracycline (97%). The following levels of resistance to other antimicrobials were observed: chloramphenicol, 73%; ciprofloxacin, 59%; ampicillin, 51%; amoxicillin, 43%; piperacillin, 28%; gentamicin, 21%; neomycin, 21%; and cefalexin, 14%. However, much lower resistance was observed to SXT (11%), cefaclor, norfloxacin, enrofloxacin (each 10%), amikacin, cefalotin (each 2%), colistin, cefuroxime, cefoxitin, ceftazidime, ceftriaxone, cefepime, aztreonam, AMC, TZP and levofloxacin (each 1%). Moreover, 178 (99%) of the 180 *E. coli* were MDR (resistant to at least three different classes of antimicrobials in the panel of drugs examined).

3.3. Antimicrobial resistance genes

The distribution of resistance genes among phenotypically resistant *E. coli* isolated from chickens is shown in Table 3. The resistance genes bla_{SHV} for β-lactams, *aac(3)-IV* for gentamicin, *tet*(A) and *tet*(B) for tetracyclines, *aadA1* for streptomycin, *catA1* and *cmlA* for chloramphenicol, *ere*(A) for erythromycin and *sul1* for sulfonamides were investigated. Among the 180 *E. coli* isolates, 96% gave positive amplicons for the bla_{SHV} gene, followed by *tet*(A) and *tet*(B) (95%). Moreover, 72% of the *E. coli* isolates carried *catA1* and *cmlA*. The resistance genes *aac(3)-IV* (20%), *aadA1* (20%), *ere*(A) (15%) and *sul1* (10%) were also observed among the *E. coli* isolates.

4. Discussion

This study revealed a high percentage (99%) of MDR isolates among *E. coli* isolated from farm chickens compared with MDR *E. coli* recovered from chicken farms (85.3%) in Tien Giang Province, South Vietnam [10]. The percentage of tetracycline resistance among *E. coli* in the current study was higher (97%) than tetracycline-resistant *E. coli* in Vietnam (93.4%). The current study showed that 99% and 98% of *E. coli* were resistant to oxacillin and lincomycin, respectively. Moreover, this study revealed that 99% of *E. coli* isolates recovered from chickens were MDR. This high prevalence of multidrug resistance among *E. coli* in these samples might due to the use of antimicrobials for veterinary purposes both for prescription reasons to control bacterial invasion and as growth promoters to increase poultry chicken production. A previous study reported a high prevalence of antimicrobial-resistant *E. coli* O157:H7 isolates from poultry farms in Eastern Ethiopia [11]. Recent results revealed that the antimicrobial susceptibility of 174 *E. coli* isolates collected from healthy poultry, bovines and ovines showed high incidences of resistance to tetracyclines, streptomycin, amoxicillin and SXT [12].

Resistance of *E. coli* isolates of poultry origin to some conventional antimicrobials has been reported both within and outside Nigeria [13,14]. Antimicrobial-resistant *E. coli* may persist in the intestinal tract of poultry birds for a long period of time with or without the use of antimicrobials, and they can serve as route via which they can be transmitted to the human population or through consumption. Continuous usage of antimicrobials outside the health system, especially for veterinary and livestock purposes, still continues. In order not to return to the pre-antibiotic era when there were no conventional antimicrobials as we now have to treat many bacterial-related diseases, it is very important that urgent and consolidated efforts are put in place and sustained in order to abate the problem of antimicrobial resistance.

However, some strains of *E. coli* have been implicated in a number of resistant infections in humans, and this includes *E. coli* isolates harbouring multidrug resistance genes such as extended-spectrum β-lactamase (ESBL) enzymes, amongst others [15]. The multidrug resistance detected in this study might be mediated by mobile genetic elements such as resistance genes, as seen in the case of other studies. In the present study, there was a high percentage of *E. coli* harbouring bla_{SHV} (96%). A recent study reported that the most prevalent β-lactamase genes of *E. coli* isolated from environmental, human and food samples in Spain were $bla_{CTX\text{-}M\text{-}14}$ (26%) and $bla_{CTX\text{-}M\text{-}1}$ (21.4%), followed by $bla_{SHV\text{-}12}$, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}42}$ [16]. The current study reported that the *aadA1* and *aac(3)-IV* genes were present in 20% of *E. coli*. AAD aminoglycoside adenylyltransferases can confer resistance to gentamicin, tobramycin or streptomycin among Gram-negative bacteria [17].

The *sul1* gene was observed in 10% of *E. coli* in the present study. In 2007 in Vietnam, dissemination of *sul* genes in three environments (swine farms, shrimp ponds and a city canal) was investigated and the incidence generally followed *sul1* > *sul2* > *sul3* [18], in agreement with the frequency of the *sul* gene in the present study conferring resistance to sulfamethoxazole. The *tet*(A) and *tet*(B) genes were detected in 95% of *E. coli* isolates in the current study. Previous results stated that the *tet*(A) resistance gene was prevalent in 86% of *E. coli* [19].

Table 2
Prevalence of antimicrobial resistance among 180 *Escherichia coli* isolates.

Antimicrobial agent	Percentage of isolates
Cefaclor	10
Oxacillin	99
Ampicillin	51
Chloramphenicol	73
Cefalexin	14
Neomycin	21
Colistin	1
Ciprofloxacin	59
Oxytetracycline	97
Norfloxacin	10
Lincomycin	98
Gentamicin	21
Amoxicillin	43
Enrofloxacin	10
Piperacillin	28
Amikacin	2
Cefalotin	2
Cefuroxime	1
Cefoxitin	1
Ceftazidime	1
Ceftriaxone	1
Cefepime	1
Aztreonam	1
Amoxicillin/clavulanic acid	1
Piperacillin/tazobactam	1
Trimethoprim/sulfamethoxazole	11
Levofloxacin	1
Multidrug-resistant	99

Table 3
Prevalence of antimicrobial resistance genes among 180 *Escherichia coli* isolates.

Antimicrobial class/agent	Resistance gene	Percentage of isolates
β-Lactams	bla_{SHV}	96
Gentamicin	*aac(3)-IV*	20
Streptomycin	*aadA1*	20
Tetracyclines	*tet*(A), *tet*(B)	95
Chloramphenicol	*catA1*, *cmlA*	72
Erythromycin	*ere*(A)	15
Sulfonamides	*sul1*	10

Use of antimicrobials in veterinary medicine and as animal growth-promoting agents during the past decade has produced enormous pressure for selection of antimicrobial resistance among bacterial pathogens worldwide. Therefore, reducing the use of and careful application of antimicrobials in animal farming and clinical practices should be applied.

5. Conclusion

These results confirm the faecal carriage of antimicrobial-resistant *E. coli* in poultry chickens raised in Taif, Saudi Arabia, making it the first study to be conducted on the matter. Also, chickens were serving as reservoirs of highly diverse and abundant antimicrobial resistance genes. These resistance genes pose a potential health threat to the public and animal farming in the country.

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Competing interests

None declared.

Ethical approval

Not required.

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