

Detection of tetracycline resistance determinant *tetA* gene and antimicrobial resistance pattern in *Escherichia coli* isolates recovered from healthy layer chickens

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Abstract

Aim: The aim was to study the occurrence of tetracycline resistance determinant *tetA* gene, and antibacterial resistance pattern in commensal *Escherichia coli* recovered from healthy non-clinical layer chickens.

Materials and Methods: Twenty-four cloacal swabs were obtained from 15 flocks in five different layer farms located at around Namakkal, which is a place of high-intensity layer chicken rearing in south India. Identification of *E. coli* was carried out by performing cultural and biochemical tests. Antimicrobial resistance test was carried out using disc diffusion method. The polymerase chain reaction employing *tetAC* forward and *tetAC* reverse primers were carried out to detect *tetA* gene conferring resistance to tetracycline.

Result: All the collected cloacal swabs yielded *E. coli*. Twenty-one isolates (88%) were resistant to tetracycline antibiotic in disc diffusion method. All the isolates showed resistance to more than six antibiotics, which implied existence of multidrug resistant microbiota in intestine of poultry. Only seven (29%) isolates showed the presence of *tetA* gene indicating the involvement of either other one or more efflux gene(s), namely *tetB*, *tetC* and *tetD* or ribosomal protection encoded by *tetM*, *tetO*, *tetQ* and *tetS* genes than *tetA* gene.

Conclusion: Based on the presence of *tetA* gene among tetracycline-resistant bacteria in healthy non-clinical food-producing animals such as layer chickens, it can be significant in human medicine as *tetA* gene could easily be spread to other bacteria. This kind of phenomenon can be extrapolated in transfer of resistance for other antibacterial essential for treating bacterial infections in human. We conclude that preventing the spread of antimicrobial resistance through direct or indirect contact, consumed food/feed and through the environment is empirical in reduction of failures while treating bacterial infections.

Keywords: antibacterial resistance, *Escherichia coli*, *tetA* gene, tetracycline.

Introduction

The presence of antibiotic-resistant bacteria has reached epidemic proportions in recent years. The spread of antibiotic-resistant bacteria in the environment is dependent on the presence and transfer of resistance genes among microorganisms, and selection pressure to keep these genes in a population. One of the most controversial applications of antibiotics, however, is for growth promotion in livestock, and this application has raised concerns about its contribution to the presence of resistant bacteria in humans [1]. Tetracycline is a broad-spectrum antibiotic that inhibits bacterial protein synthesis by preventing aminoacyl-tRNA from binding to the bacterial ribosome [2]. Tetracycline is the drug commonly used along with anti-mycoplasmal drugs in commercial chickens to suppress the problems associated with *Mycoplasma* and other bacterial infections. Resistance to the antibiotic is conferred by one or more of the 36 currently described *tet* genes, which encode one of three mechanisms of resistance: An efflux pump,

a method of ribosomal protection, or direct enzymatic inactivation of the drug. Efflux mechanisms appear to be more abundant among Gram-negative microorganisms while ribosomal protection mechanisms are more common among Gram-positive organisms [3].

Gram-negative *Escherichia coli* is one of the resident microbiota (commensal) of the gut in animals. Several studies have examined tetracycline resistance among bacteria; most have employed clinically isolated bacteria [4] or populations specifically isolated for their ability to grow in the presence of tetracycline [5]. These studies do not give appraisal of the presence and types of *tet* genes that are present in commensal bacteria in the environment. Recent studies have shown that antimicrobial-resistant bacteria are present in different wild animals that have not been exposed to antibiotics [6,7]. Moreover, the resistance genes of bacteria from wild birds may result in increased numbers of resistant bacteria in livestock and humans [8].

Hence, the present study was planned to detect *tetA* gene and resistance against various antibiotics among *E. coli* obtained from healthy non-clinical layer chickens reared in five different locations in around Namakkal, South India.

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Materials and Methods

Sample collection

Twenty-four cloacal samples (n = 24) were collected from healthy non-clinical layers of different age group in five different commercial layer farms located in and around Namakkal, South India during September and November 2013. The detection of *E. coli* was performed by placing the swabs in buffered peptone water at 37°C for 24 h, followed by aerobic culture on MacConkey agar and eosin-methylene blue agar at 37°C for 24 h. The isolates were identified as per the method previously described [9].

Antimicrobial susceptibility testing

Resistance to tetracycline was tested by the disc diffusion by aerobic culture in Mueller Hinton agar using antibiotic discs (M/s Hi-Media, Mumbai, India). The discs employed were gentamicin, amikacin, ciprofloxacin, enrofloxacin, pefloxacin, norfloxacin, cephalexin, ampicillin, amoxicillin, co-trimoxazole and tetracycline. The susceptibility/resistant patterns of *E. coli* isolates to antibiotics were determined based on the size of the zones of inhibition and categorised as susceptible or resistant [10].

Detection of tetracycline resistance gene *tetA*

In *E. coli* isolates found to be resistant to tetracycline, polymerase chain reaction (PCR) was used to detect *tetA* gene encoding this resistance. Bacterial DNA was isolated from a 24-h culture on blood agar. Isolation of DNA was performed by lysis of bacterial cell suspension at 95.5°C for 10 min, followed by centrifugation. The supernatant was used as template DNA. The *tetA* gene was amplified by PCR with forward primer *tetAC F* - 5'CGCYTATAT YGCCGAYATCAC-3' and reverse primer *tetAC R* - 5'CCRAAWKCGGCWAGCGA-3' which were designed based on regions showing high homology in *E. coli* genome with respect to relevant region [11] portion of the master mix (M/s Bio-Basic Inc., Canada) contained 1.5 U of *Taq* polymerase, 20 nmol of each deoxynucleoside triphosphate, 0.1 µL of the template DNA, 100 pmol of each primer, and 10 µL of the reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 15 mM MgCl₂). PCR amplification included 30 thermal cycles of 60 s at 94°C, 45 s at 55°C, and 90 s at 72°C with an additional extension of 300 s at 72°C.

Results

E. coli was recovered from all the cloacal swabs collected from healthy non-clinical layer chickens. All the isolates produced characteristic reaction in biochemical tests (Table-1). Antibacterial resistant pattern against various antibiotics are summarized in Table-2. In antibacterial susceptibility testing, 21 isolates (88%) were resistant to tetracycline antibiotic in disc diffusion method. Out of 24 isolates, 88% were sensitive to cephalexin, 79% to chloramphenicol, 58%

to ciprofloxacin, 58% to ampicillin, 54% to amoxicillin, 54% to enrofloxacin, 54% to gentamicin, 43% norfloxacin, 29% to amikacin, 16% to co-trimoxazole, 12% to pefloxacin and 12% to oxytetracycline.

All the tetracycline resistant *E. coli* isolates (n = 21) were subjected for PCR amplifying the efflux gene *tetA* (417 bp). Only seven (29%) isolates showed the presence of *tetA* gene (Figure-1).

Discussion

The prevalence of *E. coli* in 88% of fecal samples of layer chickens in the present study was higher than the previous records [12,13]. We investigated the resistance pattern exhibited *in vitro* to various antibiotics by *E. coli* isolates recovered from healthy non-clinical layer chickens. Almost the vast majority *E. coli* isolated from chicken were resistant to tetracycline and sulphamethoxazole+trimethoprim. This might be explained by the fact that both are heavily used in the poultry industry in India and could be the result of inadvertent use [14]. This suggests that the extent of resistance to an antibiotic is associated with the extent of its use. The high antibiotic resistance rate of organisms isolated from animals is not a phenomenon unique to Indian poultry industry. The report found that 67% avian *E. coli* strains were resistant to sulphamethoxazole+trimethoprim that is 16% less than the finding in the present study and that resistance to the new fluoroquinolones was also increasing in

Table-1: Results of biochemical tests used for identification of *Escherichia coli*.

Gram-negative, small rod
Pink colonies on MacConkey agar
Colonies with green metallic sheen on eosin-methylene blue agar
Negative in citrate test
Negative in oxidase test
Positive in indole test
Positive in methyl red test
Negative in Voges-Proskauer test
Positive for catalase production
Positive for lactose fermentation
Positive for urea hydrolysis
Positive for nitrate Reduction
Positive for gelatin hydrolysis
Positive for case in hydrolysis



Figure-1: Agar gel electrophoresis of polymerase chain reaction products with amplified *tetA* gene. Lane 1: 100 bp DNA marker; Lane 5 and 6 showing amplicons of 417 bp and samples in other lanes are negative.

Table-2: Antibiotic resistance pattern of avian *E. coli* isolates.

Antibiotic discs (concentration in mg)	No. of resistant isolates	Percentage
Gentamicin (10)	11	46
Amikacin (30)	17	71
Ciprofloxacin (30)	10	42
Enrofloxacin (30)	11	46
Pefloxacin (30)	21	88
Norfloxacin (10)	14	58
Cephalexin (30)	3	13
Ampicillin (30)	10	42
Amoxicillin (30)	11	46
Co-trimoxazole (25)	20	83
Tetracycline (30)	21	88

E. coli: *Escherichia coli*

Spain [15]. One report revealed multidrug resistance (MDR) in *E. coli* recovered from Irish cattle [16]. Daini and Adesemowo (2008) [17] found the resistance of *E. coli* clinical strains from Nigeria in 54 and 88% strains against gentamicin and tetracycline respectively, which is in agreement with the current finding. The high percentage of resistance to pefloxacin (88%) and amikacin (71%), which are rarely used in the poultry, is raising lot of questions as to why there is a high level of resistance to such antibiotics in natural non-clinical birds and how the bacteria acquired resistance against the antibacterials. If these antibiotics are to be used, it is used only for treating bacterial infections not amenable to other commonly applied antibiotics such as enrofloxacin, ciprofloxacin and gentamicin. MDR had been reported previously [13] where in all *E. coli* isolates exhibited resistance to more than six antibiotics that did not differ from the findings of the present work.

Only seven (29%) *E. coli* isolates showed the presence of *tetA* gene despite percentage of tetracycline-resistant isolates was 88% in the present study. It can be assumed that tetracycline resistance in *tetA* negative isolates might be encoded by other genes such as *tetB*, *tetC* and *tetD* or ribosomal protection encoded by *tetM*, *tetO*, *tetQ* and *tetS* genes than the gene monitored in this study. Not only did those animal hosts that presumably had continuous exposure to tetracycline have a higher percentage of tetracycline-resistant *E. coli* isolates, but also those isolates carried a greater diversity of resistance genes. Moreover, these isolates often had more than one tetracycline resistance determinant. This suggests that the human activity provides suitable environments that select for resistant strains and encourages the transfer of genetic information from unrelated bacterial species [12]. Koo and Woo (2011) [18] have reported that 98.3% of meat-borne *E. coli* containing at least one of the *tetA* to *tetD* genes was able to transfer tetracycline resistance to a tetracycline-susceptible recipient strain of *E. coli*. Interestingly, two isolates carried both *tetA* and *tetB*, but only *tetA* was transferred to the recipient strain. It can be assumed that the *tetA* gene can be spread more

easily in the environment than *tetB*. Antimicrobial resistance can spread to humans and animals via direct or indirect contact, consumed food/feed and through the environment. Therefore, it is important to analyze the epidemiology and mechanisms of emergence and spread of antimicrobial resistance [19].

Conclusion

Based on the findings, it can be stated that the presence of *tetA* gene among tetracycline-resistant bacteria in healthy non-clinical food-producing animals such as layer chickens assumes significance in human medicine as *tetA* gene could easily be spread to other bacteria and similar kind of spread can be extrapolated in transfer of resistance for other antibacterials essential for treating bacterial infections in human. We call for surveillance of more resistant conferring genes in resident microbiota among many food producing animals as it would reveal more than what emerged in the findings of the present study. We suggest prudent use of antibiotics for performance enhancement in food producing animals such as layer chickens and hygienic measures while handling animal food products to avoid crossing over of resistance genes from harmless bacteria to pathogenic bacteria.

Authors' Contributions

AB and KS have designed the plan of work and carried out the laboratory work. ME and PS have collected the relevant samples and have assisted in writing the manuscript. All the authors have read and approved the manuscript. AB revised the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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