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Antimicrobial resistance pattern of *Escherichia coli* strains isolated from poultry farmers and poultry slaughterers in Morocco

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Abstract

Aim: The objective of this study is to characterize the antibiotic resistance pattern of *Escherichia coli* isolates from the fecal samples of poultry workers (farmers and slaughterers), and to study the possible dissemination of resistant *E. coli* from poultry to humans.

Methods: Sixty-four *E. coli* strains isolated from the fecal samples of poultry workers (33 from poultry farmers and 31 from poultry slaughterers) and 35 isolates from a control group workers were tested for antibiotic resistance by agar disk diffusion with 11 antimicrobial agents.

Results: Resistance of *E. coli* isolated from poultry workers to tetracycline, ampicillin and norfloxacin were significantly ($p < 0.05$) higher than those isolated from the control group. All *E. coli* isolates were susceptible to cefotaxime, and most of them are susceptible to gentamicin, amikacin, cefoxitin and ertapenem. Multidrug resistance is alarmingly high in all groups, but was highest in poultry farmers isolates (84%) and poultry slaughterers isolates (80%). Approximately 25% of the *E. coli* isolates from poultry workers showed resistance to four or more antibiotics.

Conclusion: This study suggests that occupational exposure to antimicrobial-resistant *E. coli* through animal contact in the broiler chicken industry may be an important route of entry antimicrobial-resistant *E. coli* into the community.

Keywords

Escherichia Coli; Poultry Workers; Antibiotic Resistance; Multidrug Resistance; Morocco

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Introduction

Antibiotic resistance has been emerged as one of the world's most important public health problems [1, 2].Use of antibiotics in human and animal medicine, especially their misuse, has been associated with the selection and spread of antibiotic resistant strains in human beings and animals [3, 4].This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individuals or populations [5]. Antibiotics are used in animals as well as in humans for both prevention and treatment of infections. In animal husbandry they are also used as growth-promoting agents mixed with feed. These agents are widely used in the poultry industry of Morocco. A recent survey conducted by El-Youbi *et al.* [6] reported that all private veterinarians in Eastern Morocco have found abusive practices of using avian antibiotics.

Fecal flora of poultry contains a relatively high proportion of resistant bacteria; this is due to the high antibiotic selection pressure on bacterial flora of these birds [7]. During the slaughtering process of poultry birds, there can be occurring of fecal contamination through the guts of these birds with multiresistant bacteria especially *E. coli* [8, 9]. The human intestinal tract is the ideal place where antibiotic resistant bacteria can transfer their resistance genes to the endogenous human flora [10].

Many studies have shown that intestinal tract of human volunteers were colonized with resistant *E. coli* originated from poultry [11, 12]. Other investigations have revealed that farm animals are a potential reservoir of antibiotic resistant bacteria [13, 14]. In contrast, some authors [15, 16, 17] have reported that the antibiotic resistance transfer between animals and humans was limited because animal strains colonizing the alimentary tract less readily than human ones. However, the actual impact of antibiotic resistance bacteria isolated from workers who are in frequent contact with animals is not well known.

This study aimed to investigate and compare the antibiotic resistance profiles of *E. coli* isolates from poultry workers of both farmers and slaughterers and a control group of healthy individuals living in the same region.

Materials and Methods

Collection of fecal samples

Our study was carried out from January through April 2016, in Ouarzazate -Morocco. We collected fecal samples from healthy persons. A total of 33 samples from poultry farmers and 31 from poultry slaughterers. For the comparison, 35 fecal samples were collected from the same area from healthy persons who are practicing other professions except healthcare workers. To avoid cross-risk factors, we have excluded people who had been hospitalized within the last six months and people who received antibiotic treatment a month prior to be included in this study.

Antibiotic susceptibility testing

The isolation and purification of *E. coli* strains was made on MacConkey agar and the identification was performed using API20E system (Biomerieux, Marcy l'Etoile, France). The isolates were screened for their antibiotic resistance to the following antibiotics (Marnes-La-Coquette, France): Amoxicillin-clavulanic acid (AMC) 20+10 µg; cefoxitin FOX 30 µg; cefotaxime CTX 30 µg; tetracycline TE 30 UI; gentamicin (CN) 15 µg; amikacin AK 30 µg; ertapenem (ETP) 10 µg; cephalothin (KF) 30 µg; ampicillin (AM) 10 µg; trimethoprim/sulphamethoxazole (SXT) 1.25/23.75 µg and norfloxacin (NOR) 5 µg. The antibiogram was performed by the agar diffusion method following the guidelines of the Antibiogram Committee of the French Microbiology Society (CA/SFM, 2014) [18]. Quality control was carried out using *E. coli* strain ATCC 25922. For each individual samples, one *E. coli* isolate was

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selected randomly for the purpose of testing its antibiotic susceptibility.

Statistical analysis

The antimicrobial susceptibility data are expressed as percentages or frequency of the human isolates. The χ^2 (khi-deux) test was used to estimate overall difference between the percentages of resistance between *E. coli* isolates from different groups. In all cases, $p < 0.05$ was regarded as statistically significant.

Results

A total of 99 fecal samples were examined. Response rates to participate in the study was in poultry farmers and poultry slaughterers were 73.3% and 68.8%, respectively. Antibiotic resistance rates among *E. coli* isolates found in each group were shown in **Table 1**. Of the three examined population groups, the highest rate of resistance to almost all tested drugs was detected infecal samples of poultry farmers, followed by those from poultry slaughterers and much lower in the control group, respectively. However, this high rate of resistance was only significant for tetracycline, ampicillin and norfloxacin ($p < 0.05$), and most of the tested isolates showed high antimicrobial resistant to tetracycline .

All *E. coli* isolates were susceptible to CTX, and most of them are susceptible to CN, AK, FOX and ETP. However, one isolate (3%) from poultry workers showed resistance to cefoxitin and ertapenem, and one isolate (3%) from poultry farmers were additionally resistant to gentamicin and amikacin. The percentage of multiple drug resistance patterns in *E. coli* isolates of each group is shown in **Figure 1**.

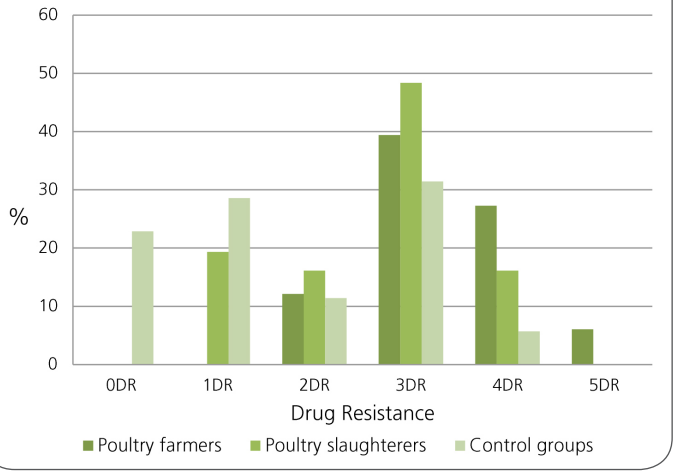
Multidrug resistance (MDR) was defined in this study as resistance to at least one agent out three or more antimicrobial classes [19]. The highest rate of multidrug resistance (72.7%) was found in poul-

Table 1. Comparison of antibiotic resistance rates of E. coli isolates from poultry farmers, poultry slaughterers and control group.

Antimicrobial agents	Antibiotic resistance of isolates		
	%		
	Poultry farmers	Poultry slaughterers	Control groups
	n = 33	n = 31	n = 35
Amoxicillin-clavulanic acid (AMC 20+10 µg)	42.42[a]*	38.7[a]	31.42[a]
Cefoxitin (FOX 30 µg)	3.03[a]	3.22[a]	0[a]
Cefotaxime (CTX 30 µg)	0[a]	0[a]	0[a]
Tetracycline (TE 30 UI)	93.93a	93.54[a]	71.42
Gentamicin (CN 15 µg)	3.03[a]	0[a]	0[a]
Amikacin (AK 30 µg)	3.03[a]	0[a]	0[a]
Ertapenem (ETP 10 µg)	3.03[a]	3.22[a]	0[a]
Cephalothin (KF 30 µg)	36.36[a]	25.80[a]	20[a]
Ampicillin (AM 10 µg)	45.45[a]	38[a]	14.28
Trimethoprim/ sulphamethoxazole (SXT)	39.39[a]	32.25[a]	25.71[a]
Norfloxacin (NOR 5 µg)	27.27[a]	25.80[a]	5.71

*: Rates are not significant (P > 0.05).

Figure 1: Antibiotic multiresistance patterns of *E. coli* strain in poultry farmers, poultry slaughterers and control group.



try farmers isolates, followed by those from poultry slaughterers (64.5%) and significantly lower in the control group (37.1%). Approximately 25% of the isolates of poultry workers harboured *E. coli* strains resistant to four or more antibiotics.

The antibiotic resistance pattern most frequently observed was resistance to TE/AM/SXT (12.1%) in the poultry farmers and (9.7%) in the poultry slaughterers. The highest number of resistance antibiotic markers was five (TE/AM/AK/CN/NOR) and (TE/AM/SXT/AMC/NOR), in two *E. coli* isolates from poultry farmers, respectively.

Discussion

To the best of our knowledge, this is the first based study in Morocco investigated the prevalence of antimicrobial resistant *E. coli* from poultry workers. Our data showed that resistance rates remained significantly higher in *E. coli* isolated from poultry workers than those from control groups. The results presented here confirm similar studies from other countries showing that poultry farmers and poultry slaughterers workers were at higher risk for colonization with antimicrobial-resistant *E. coli* [10].

The presence and frequency of tetracycline resistant in *E. coli* in this study agree with findings of other studies on antibiotic resistance in *E. coli* [14, 15]. Tetracycline is a commonly used as first line antibiotic for many domestic animals and as a growth promoter or as an infection control agent, and is often used before the antibiotic resistance profile of a pathogen has been determined [20, 21].

In Morocco, the tetracycline is one of the most prescribed antibiotic in avian medicine [6]. In addition, other studies by us [22] reported that resistance in *E. coli* strains of poultry meat is higher to tetracycline (80%) than to trimethoprim/sulphamethoxazole (33.3%), nalidixic acid (26.6%) and amoxicillin (20%). Lower resistance rates were found by EL Allaoui *et al.* (5 to 12.5%) for ertapenem, aztreonam and gentamicin [23]. These results suggest that the extent of antibiotic resistance is associated with the extensive of its usage. Bacterial resistance to tetracycline is plasmid mediated with a wide variety of genetic determinants[22].

According to Van den Bogaard [24], dissemination of resistant bacteria and/or their resistance plasmids from broilers to poultry workers is the most likely explanation for the high antimicrobial resistance rates observed among fecal *E. coli* of the farmers and slaughterers.

The major factor for selecting antimicrobial resistance in bacteria is the misuse of antibiotic, in addition to crowding and poor sanitation in poultry farmes. These three factors are typically related to intensive poultry farming, and explain the high prevalence of resistance in fecal *E. coli* of poultry workers in this and other studies [1].

In this study, carriage of multidrug-resistant *E. coli* was found significantly more common in poultry workers. Such high rate of multidrug resistance may apparently be occurred due to indiscriminate usage of antimicrobial agents [1]. During slaughtering process multidrug resistant *E. coli* strains can be transferred often from the gut of poultry carcasses and contaminate its meat [8,24].

The findings of our study confirm the effect of exposure to antibiotic resistance bacteria at the work place. This can be also associated with transfer of multiple antimicrobial resistance to commonly used drugs in the poultry farming. Bongers *et al.* [25] noted a significantly higher rates of *E. coli* resistant isolates to oxytetracycline and ampicillin in poultry industry workers than those not working with birds [26].

Conclusion

The data obtained in this study indicate that occupational exposure to antimicrobial-resistant *E. coli* from animal contact in the broiler chicken

industry may be an important route of entry of antimicrobial-resistant *E. coli* into the community. We recommend to establish a strategy which can slow down the spread of antibiotic resistant bacterial strains by applying different preventive measures, such as control of antibiotic use, training and education of workers to practice safety measurements.

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