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Research Article

Antimicrobial Resistance in *Escherichia coli* Isolates from Healthy Poultry, Bovine and Ovine in Tunisia: A Real Animal and Human Health Threat

Abstract

A total of 174 *E. coli* isolates collected from healthy poultry, bovine and ovine recovered between December 2009 and June 2013 in different geographic location in Tunisia, were assessed and examinated for resistance to antimicrobial agents. Avian isolates showed the highest rates of antibiotic resistance: tetracycline (74.7 %), trimethoprim/sulfamethoxazole and amoxicillin with the same rate of resistance (57 %). Prevalences of resistance to the same four antimicrobials in bovine isolates were 33.3 %, 65 %, 30 %, 28.3 %, respectively. However, for ovine isolates, low resistance rates were observed, except for tetracycline (40 %) and amoxicillin (22.85 %). Only one ESBL-producing isolate from chicken was detected. In addition, seventy-seven (44.2 %) isolates were resistant to three or more classes of antibiotics and were considered multidrug resistant (MDR). Interestingly, avian *E. coli* isolates were more resistant than bovine and ovine ones. These results provide novel insights into the epidemiological characteristics of poultry, bovine and ovine *E.coli* isolates in Tunisia, and suggest the need for the prudent use of antimicrobial agents in husbandry and the urgent need to establish a national antibiotic resistance monitoring program.

Introduction

Multi-drug-resistant bacteria have emerged as a major concern for human and veterinary medicine. To fight against this worrisome problem, reliable data on the rates of antimicrobial resistance in bacteria isolated from both human and animal are required. Therefore, it is necessary to develop monitoring programs to survey for the objective the extent of antimicrobial resistance [1,2]. Continued surveillance of the antimicrobial susceptibility profiles of foodborne pathogens, including *Escherichia coli*, has been strongly recommended to identify emerging antimicrobial-resistant phenotypes [3]. *E. coli* is a common component of the gut flora of food animals that can serve as an indicator for the acquisition of resistance to various antibiotics by enteric organisms [4,5]. The use of *E. coli* as the indicator bacteria is also appropriate because changes in the antibiotic resistance of this species may serve as an early warning of the development of resistance by related pathogenic bacteria [5]. Although, antimicrobial therapy is an available tool for treating clinical infections, antibiotic resistance in pathogenic bacteria from human, animal and environmental sources is recognized as a global problem in public health. Especially, the high degree of resistance to common antibiotics, the worldwide emergence of multidrug resistant phenotypes (MDR) and extended spectrum β-lactamases (ESBLs)-producing *E.coli* strains are of increasing concern [6,7]. A number of studies have reported that antimicrobial drugs commonly used for the treatment of human infections are misused in animals either for therapy or prevention, which has significant clinical implications for both human and veterinary medicine [8,9].

Resistant bacteria can compromise public and animal health and lead to severe economic losses in animal production [10,11].

Nowadays, the resistance to antimicrobial agents increased significantly for *E. coli* [12]. Initially, this resistance was described for certain antimicrobial agents such ampicillin, trimethoprime, sulphonamides and tetracycline [13]. However, since many years, large phenotypes of resistance have been observes such as resistance to all beta-lactams, aminosides, and quinolones.

Many studies on antimicrobial resistance in *E. coli* strains isolated from human origins in Tunisia have been published. In addition, same studies have been published, where genetic characterization of ESBL-producing *E. coli* isolates from animal origins and from food products of animal of origin. However,

little is known about the antibiotic resistance in commensal *E. coli* from livestock. Indeed, it is very important to know the actual epidemiology of antibiotic susceptibility of *E. coli* from livestock in order to establish a guide for Tunisian veterinarians for the reasonable use of antibiotic in husbandry. For these reasons, we carried out this study to investigate antimicrobial resistance in *E. coli* isolates from healthy poultry, bovine and ovine during a period of 4 years and 6 months (June 2009 to December 2013).

Materials and Methods

Bacterial isolates and epidemiological background

Two hundred feces samples from various healthy animal species (bovine (n=50), ovine (50) and poultry (150)). Samples were collected from several regions in Tunisia (Siliana, Kef, Sidi Bouzid, Beja, Nabeul, Bousalem, Mateur, and Sousse) between June 2009-December 2013, Five grams of feces were incubated in Brain Heart Infusion (BHI, Becton-Dickinson) for 1 h at 37°C and then subcultured on Mac Conkey Agar (MSA, Becton-Dickinson) for 18-24h [14]. One colony from each positive sample with typical *E. coli* morphology was picked up randomly for further identification. Then, isolates were identified by classical bacteriological tests (Oxydase test, catalase test, Gram staining) and Api20E (Bio-Mérieux, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by the agar disk diffusion method on Mueller-Hinton Agar (Bio-Rad, France) plates according to recommendation of *Clinical and Laboratory Standards Institute* guidelines [15]. The antibiotics tested were the following: amoxicillin (25 µg), amoxicillin/clavulanic acid (20 µg + 10 µg), ticarcillin (75 µg), ticarcillin/clavulanic acid (75 µg + 10 µg), piperacillin (75 µg), cefoxitin (30 µg), ceftazidim (30 µg), cefotaxim (30 µg), imipenem (10 µg), streptomycin (10 UI), gentamicin (10 UI), kanamycin(30 UI), tobramycin (10 µg), tetracycline (30 UI), chloramphenicol (30 µg), colistin (50 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1,25 µg + 23,75 µg), and fosfomycin (50 µg) (Bio-Rad, France). *E. coli* ATCC 25922 was used as a control strain. The double-disc synergy test with cefotaxime or ceftazidime in the proximity to amoxicillin-clavulanic acid was used for the screening of Extended-Spectrum Beta-lactamases (ESBL) [16].

Statistical analysis

Statistical testing was performed using SPSS version 12.0. P < 0.05 was considered statistically significant.

Results

Identification of isolates

Amongst the 200 feces samples, 174 isolates of *E. coli* were collected. They were from: poultry (79), bovine (60) and ovine (35).

Antibiotic resistance rates

Occurrence of antibiotic resistance in the 174 *E. coli* isolates of is presented in table 1. High rates of resistance were observed for tetracycline (53.4 %), streptomycin (51.7 %), amoxicillin (40.2 %), and trimethoprim/sulfametoxazole (39 %). Moderate rates of resistance were observed for nalidixic acid (28.7 %) and ciprofloxacin (18.4 %). However, low frequencies of resistance were observed for amoxicillin-clavulanic acid (9.8 %), gentamicin (6.3 %), cefotaxime (2.3 %) and ceftazidime (1.7 %) (Figure 1). Interestingly, only one ESBL-producing *E. coli* isolate of avian origin was detected.

Analysis of resistance profile of the 174 isolates, showed that 37 isolates (21. 3 %) were susceptible to all antibiotics, 33 (19 %) were resistant to one antibiotic, 27 (15,5 %) were resistant for two antibiotics, and 77 (44,2 %) were multidrug-resistant (resistant to three or more families of antibiotics).

Rates of antibiotics resistance according to origins

For poultry isolates, the highest rates of resistance were observed for tetracycline (74.7 %), followed by streptomycin, trimetoprime/sulfametoxazole, and amoxicillin (each 57 %), nalidixic acid (54.4 %) and ciprofloxacin (34.2 %). Whereas, much lower resistances were observed for cefotaxime, gentamicin (5.1 %), and ceftazidime (3.8 %) (Table 2). For bovine isolates, streptomycin showed an important resistance

Table 1: Frequencies of antibiotics resistance in *E. coli* isolates.

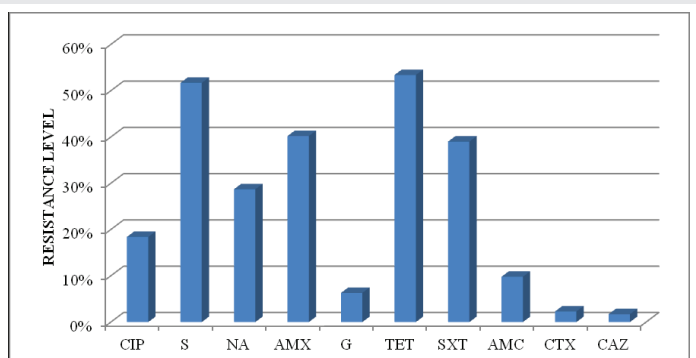| Antibiotics	Resistant isolates, n (%)
Acide nalidixic (NA)	50 (28.5)
Ciprofloxacin (CIP)	32 (18.4)
Streptomycin (S)	90 (51.7)
Gentamicin (G)	11 (6.3)
Tetracycline (TET)	93 (53.4)
Trimethoprim/sulfamethoxazole (SXT)	68 (39)
Amoxicillin (AMX)	70 (40.2)
Amoxicillin/clavulanic acid (AMC)	17 (9.8)
Cefotaxime (CTX)	4 (2.3)
Ceftazidime (CAZ)	3 (1.7)

Figure 1: Frequencies of antibiotics resistance in 174 E. coli isolates. Ciprofloxacin (CIP); Streptomycin (S); Nalidixic acid (NA); Amoxicillin (AMX); Gentamicin (G); Tetracycline (TET); Trimethoprime/sulfamethoxazole (SXT); Amoxicillin-clavulanic acid (AMC); Cefotaxime (CTX); Ceftazidime (CAZ).

(65 %) followed by tetracycline (33.3 %), trimetoprim-sulfametoxazole (30 %), and amoxicillin (28.3 %). However, for ovine isolates, we observed low resistance rates, being, limited to tetracycline (40 %) and amoxicillin (22.85 %) (Figure 2). Taken together, we observed that avian *E. coli* isolates were more resistant than bovine and ovine ones, especially for tetracycline, trimethoprim/sulfametoxazole (p=10^{-5}), amoxicillin (p=10^{-4}), nalidixic acid (p=10^{-8}) and ciprofloxacin (p=510^{-5}).

Discussion

The aim of our study was to identify the profiles of antibiotics resistance for a collection of *E. coli* isolates (174) recovered from apparently healthy poultry, bovine and ovine. Our work showed high rates of resistance to tetracycline and streptomycin. Average rates of resistance were noted for amoxicillin, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacin. Low frequencies were observed for the resistance to amoxicilline/clavulanic-acid, gentamicin, cefotaxime and ceftazidime. A single ESBL producing strain was detected from chicken. High rates of resistance to tetracycline and streptomycin have been reported previously [17]. These antibiotics were the oldest molecules very widely used in veterinary medicine [18]. Indeed, tetracycline was usually

used in the treatment of omphalites, respiratory infections, whit lows and interdigites dermatitis in ruminants as well as respiratory diseases and enteritis in poultry; it was also used as a growth promoter. Streptomycin is used for the treatment of diarrhea and streptococcal infections. These two molecules (streptomycin and tetracycline) are used very frequently and for a long time in the poultry sector. Studies made by other authors [14,19] were in agreement with our findings. Indeed, they showed a high rates of resistance to tetracycline (90.8-95.2 %), and streptomycin (46 %). High rates of antibiotic resistance in *E. coli* towards tetracycline were reported at most species breeding [8,20-22]. Although frequencies of streptomycin resistance vary according to countries and conditions of breeding, the reported rates were generally very high whatever the type of animal species [8,20-22].

A moderate rate of resistance was noted for trimethoprim-sulfamethoxazole (39 %), similarly to other studies from many countries (66.1-88.6 %) [23-26]. However, a very high frequency of resistance to trimethoprim -sulfamethoxazole (80 %) was reported in our previous work [27]. Trimethoprim is used in medicine against a wide specter of bacteria including *E. coli* and other enterobacteria. Furthermore, the combination with sulfonamides increases its efficiency. However, the use of this antimicrobial in the animal feed, as well as its use in uncontrolled way in human medicine, during a prolonged time can entail the development and the transmission of genes encoding this resistance marker [27].

The mechanisms of resistance to beta-lactams in *E. coli* from animal origins is mainly encoded by extended spectrum beta-lactamases (ESBL) [28-33]. In this study, a single isolate producing ESBL from poultry origin, was identified. In Tunisia, the presence of isolates producing ESBL was reported for the first time by [34], from poultry meat, by using a selective protocol for the isolation of these isolates. However [35], did not identify ESBL-producing isolates from 164 isolates by using a non-selective protocol. [36], were the first researchers in Tunisia to report ESBL-producing *E. coli* from avian feces using a selective protocol. Moderate rates of resistance were observed for quinolones, nalidixic acid (28.7 %) and ciprofloxacin (18.4 %). Actually, worldwide, high rates of quinolones-resistance are increasingly reported in *E. coli* from animal origins. Indeed, high rates of quinolones-resistance (18.2 % - 92.5 %) were observed worldwide [14,19,37]. For ovine isolates, the frequencies of resistance were low and almost all isolates were susceptible to the tested antibiotics, except for tetracycline (40 %) and to amoxicillin (22,85 %).

Our study showed that antibiotics resistance rates in *E. coli* varied according to the animal species. Indeed, and for all antibiotics, except for streptomycin in bovine isolates, avian isolates were the most resistant ones, especially for tetracycline, streptomycin, trimethoprim/sulfamethoxazole, and amoxicillin. On the other hand, in cattle, the resistance rates were lower and concern only streptomycin, tetracycline and trimethoprim/sulfamethoxazole.

The results concerning the frequencies of antibiotic resistance in poultry are in agreement with other studies

Table 2: Frequencies of antimicrobial resistance in *E. coli* isolates according to origins.

| Antibiotics	Poultry (N=79)		Bovine (N=60)		Ovin (N=35)		Total n (%)	Test χ²
	n	%	n	%	n	%		
Ciprofloxacin	27	34.2	2	3.3	3	8.6	32 (18.4)	S
Nalidixic acid	43	54.4	4	6.7	3	8.6	50 (28.7)	S
Streptomycin	45	57	39	65	6	17.1	90 (51.7)	S
Gentamicin	4	5.1	5	8.3	2	5.7	11 (6.3)	NS
Trimethoprim/ sulfameth	45	57	18	30	5	14.3	68 (39)	S
Tetracycline	59	74.7	20	33.3	14	40	93 (53.4)	S
Amoxicillin	45	57	17	28.3	8	22.8	70 (40.2)	S
Amoxicillin/clav acid	12	15.2	2	3.3	3	8.6	17 (9.8)	NS
Cefotaxime	4	5.1	0	0	0	0	4 (2.3)	NS
Ceftazidime	3	3.8	0	0	0	0	3 (1.7)	NS
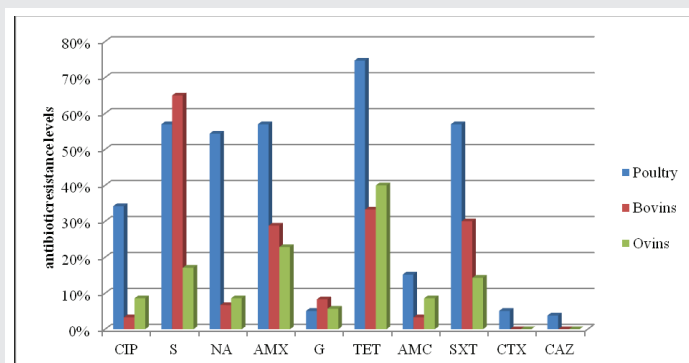
Figure 2: Distribution of antibiotic resistance in E. coli isolates according to their origins. Ciprofloxacin (CIP); Streptomycin (S); Nalidixic acid (NA); Amoxicillin (AMX); Gentamicin (G); Tetracycline (TET); Amoxicillin-clavulanic acid (AMC); Trimethoprim/sulfamethoxazole (SXT); Cefotaxime (CTX); Ceftazidime (CAZ).

[14,19,38,39]. Those studies reported high rates of resistance to amoxicillin / ampicillin (25.8 %–50.4 %), tetracycline (90.8 %–95.2 %), trimethoprim–sulfamethoxazole (66.1 %–88.6 %), and streptomycin (46 %). Also, it seems that *E. coli* isolates from bovine and ovine origins are mainly more susceptible to antibiotics than avian isolates [40]. This might be explained to the regular use of antibiotics, in avian industries, for therapeutic or preventive purposes. This common practice mainly leads to the selection of resistant clones and, afterward, for intra– and inter–spread of these clones by fecal contamination.

The multiresistant bacteria, which accumulate resistance to several antibiotics of different families, can leads to therapeutic impasses. A simultaneous resistance to several families of antibiotics is considered as a major problem in animal and human health. Indeed, amongst our isolates, 77 (44.2 %) isolates were resistant to 3 families of antibiotics or more.

In conclusion, antibiotic susceptibility of 174 *E. coli* isolates collected from healthy poultry, bovine and ovine showed high frequencies of resistance to tetracycline, streptomycin, amoxicillin, and to trimethoprim–sulfamethoxazole with the presence of a single isolate producing ESBL. The highest rates of resistance were observed in poultry isolates. These results can be explained by the excessive usage of antibiotics in the industrialized poultry sector and to the practices of avian extensive breeding. However, the low rates of antibiotic resistance observed in *E. coli* isolates from bovine and ovine is probably linked to the scarce use of antibiotics in the Tunisian's bovine and ovine husbandries. Taken together, antibiotic resistance in avian *E. coli* isolates is alarming and possible dissemination of such isolates to human and environment is worrisome.

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