Current Epidemiology of Non-β-Lactam Antibiotics-resistance in *Escherichia coli* from Animal Origins in Tunisia: A Paradigm of Multidrug Resistance

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Abstract

Worldwide, resistance to antibiotics is a major health problem. Nowadays, multiples lines of evidence confirmed that food, in particular poultry products, may acts as reservoir of antibiotic-resistant bacteria or genetic elements encoding antibiotic resistance. Commensal *Escherichia coli* colonizing animals designated to human consummation (poultry, bovine, pork) often harbors mobile genetic elements that can be transferred in vivo to infectious pathogens. In addition, antibiotic resistant *E. coli* can be easily transferred to human via food chain. The objective of this work was to review the published data on the prevalence of antibiotic resistance and their genetic supports in *E. coli* of animal origins in Tunisia.

Keywords: Antibiotic-resistance, *Escherichia coli*, animal, Tunisia

Introduction

Antimicrobial resistance (AMR) is one of the most serious public health concerns of the twenty-first century. It is of particular concern the phenomenon of resistance carried out by some Gram- positive and negative bacteria such as methicillin-resistant Staphylococcus aureus, glycopeptides-resistant *Enterococcus faecium*/*Enterococcus faecalis* and beta-lactam-resistant Enterobacteriaceae [1]. AMR has been largely limited to bacteria from human clinical setting; however, antibiotic-resistant bacteria of animal origin have been increasingly reported worldwide [2]. Consequently, foods of animal origin are considered as reservoirs of antibiotic-resistant zoonotic bacteria. Nowadays, most experts agree that the contribution from food animals is a concern and many evidences seem to indicate that the importance of the food-animal reservoir is larger than we estimated a decade ago. Worldwide, beta-lactam-resistant *Escherichia coli* producing Extended Spectrum Beta-Lactamases (ESBLs), cephalosporinases or carbapenemases have been increasingly reported from food animals as well as from many wildlife species [3,4]. In Tunisia, high frequencies of antibiotic resistant *E. coli* from human as well as from animal or foods of animal origin have been increasingly reported during the last decade. Recently we have published a review reporting the occurrence of ESBL-producing *E. coli* from animal and foods of animal origin [5] highlighting the importance of this worrisome phenomenon in Tunisia. However, studies on resistance to other critically important antimicrobial families are also of interest to improve our knowledge about *E. coli* of animal origins. This might be useful not only for Tunisian health authorities and politician deciders but also for neighbour countries especially European countries that we share with them large economic exchanges including food products. Therefore, in this work we aimed to review the published data on the prevalence of AMR (other than beta-lactams resistance) and their genetic supports in *E. coli* of animal origins in Tunisia.

The level of the AMR problem in Tunisia

Aminoglycosides, tetracyclines, quinolones, trimethoprim, and sulfonamides are mainly used in the treatment of *E. coli* infections in humans and animals [6]. Therefore, resistance to these antibiotics is of great concern. Nowadays, increasing rates of resistance are reported worldwide in Enterobacteriaceae especially in *E. coli* regardless of their animal or human origin [7,8]. In this context, all reported results about antibiotic susceptibility of *E. coli* isolates from animal sources in Tunisia showed high rates of resistance to the above-mentioned antibiotics [9,10]. Similarly to the global trend, particular high rates of resistance were observed against tetracycline, streptomycin, trimethoprim/sulfonamides, sulfonamides, and nalidixic acid. In unpublished survey in our Institute of Veterinary Research of Tunisia, we found that the level of resistance to different antimicrobials significantly varied according to the source of the isolates. *E. coli* isolates from poultry meats and faeces have the highest resistance rates than those from bovine and sheep. This finding might be a consequence of the selective pressures imposed by antimicrobial use in different food animal production and

processing environments. In addition, the high levels of resistance in poultry may be partly due to the specific practices in avian industry, in which antimicrobials for disease control and prevention are overused [11,12].

Tetracycline resistance

Tetracycline resistance has been observed in 43% [13], 89% [9], and 95.2% [10] of *E. coli* isolates from food samples of animal origin (poultry, sheep, beef) (**Table 1**). This finding is in agreement to other studies reporting high rates of tetracycline-resistant *E. coli* from animal origins, especially in pig and chicken, ranging from 17.1 % (cattle) to 70.6 % (pigs) in different European countries (France, Italy, UK, Germany, Ireland, Greece) [8,14] and reaching 89.63 % (poultry) in China [15]. This finding might be explained by the fact that tetracyclines, including chlortetracycline and minocycline, have been amongst the oldest antibiotics and have been used for growth promotion for poultry [16]. In addition, its efficacy, low cost, and the lack of side effects make it the most popularly used antibiotic in livestock farming. Its widespread and imprudent use caused a high prevalence of tetracycline-resistant bacteria nowadays. Resistance to tetracycline can occur by various mechanisms including active efflux of tetracycline, the production of ribosomal protection proteins, decreased drug permeability, target mutation, and enzymatic degradation of the antibiotics [17]. However, active efflux of tetracycline predominates in Enterobacteriaceae, and at least 26 tet-type genes have been reported to encode efflux-pumps [17]. Amongst those determinants, tetA and tetB alleles have been mainly reported to predominate in E. coli regardless their origin, followed by tetC gene [15,18,19]. It can be assumed that the tetA gene can be spread more easily in the environment than other tetB and tetC-genes. Similarly, in the Tunisian studies, tetracycline-resistant E. coli mainly harboured tetA and tetB genes, being found in ranges of 37.9 % to 77.5% and 16.4% to 32%, respectively [9,10,13].

Table 1 Frequencies and genetic supports of antimicrobial resistance of *E. coli* of animal origins in Tunisia

Antibiotic Agent	Rates of Antibiotic Resistance (%)		Detected Genes
	Tunisia	Worldwide	
Tetracycline	43-95.2	17.1-89.63	tetA, tetB, tetC
Trim/sulf	29-80	9.0-70.6	sul1, sul2, sul3, dfrA1, dfrA17, dfrA5, dfrA12
Streptomycin	29-78	17.1-70.1	aadA1, aadA2, aadA5
Nalidixic acid	33 - 72.3	22-41	*gyrA mutations:Ser83Leu, Asp87Gly, Asp87Asn Asp87Tyr *parC mutations: Ser80Ile, Glu84Gly, Ser80Arg
Ciprofloxacin	7.0-19.9	10.9-62.5	qnrS1, qnrB5, qnrB19
Gentamicin	0-03	0-10	aac(3)-II
Kanamycin	40	-	aph(3')-Ia ; aac(6')-Ib-cr

Resistance to aminosides and trimethoprim/sulphonamides

Resistance to streptomycin, the combination trimethoprim/sulfamethoxazole, and to sulfonamides are also high in *E. coli* of animal origins in our country. Indeed, resistance frequencies of 29% to 78%, 29% to 80%, and 39% to 87% have been reported for streptomycin, trimethorprim/sulfamethoxazole (SXT), and sulfonamide, respectively [9,10,13]. These high frequencies are very similar to reported results worldwide (SXT: 9.0% - 70.6%; Streptomycin: 17.1% - 70.1%) [8,14,15]. Moreover, worldwide, in each studied *E. coli* collection, frequencies of resistance to these three antibiotics are mainly very close, indicating that *E. coli* isolates are concurrently resistant against these antibiotics. According to genetic point of view, these results are expected since genes encoding streptomycin, trimethoprim, and sulfonamides-resistance are usually co-located on the same genetic vehicle such as integrons, plasmids and transposons [20]. Sulfonamide resistance in Gram-negative bacilli generally rises from the presence of the sul1, sul2, and or sul3 genes [21,22]. Historically, sul1 and sul2 genes were the most prevalent ones in *E. coli* from animal and human origin, and sul3 gene scarcely detected. However, recently in Tunisia and in other part of the world, the sul3 gene has been emerged as an important determinant encoding sulfonamide resistance. This finding was attributed to another genetic phenomenon linked to a specific genetic rearrangement in the structure of the typical class 1 integron. Indeed, recently, many studies highlighted the increasing reports of lack of the sul1 gene from the 3'-CS (qacΔE-sul1) of class 1 integron and its replacement by sul3 gene. More importantly, the sul3 gene was found in a transposase-like sequence, qacH-IS440-sul3-orf1-IS26 [23,24] that might have contributed to the maintenance and further spread of sul3 in *E. coli* isolates.

Resistance to streptomycin was mainly encoded by aadA type gene variants (aadA1, aadA2, aadA5) rarely by aadB alleles as reported in Tunisia and in others countries [9,10,13,25-27]. Interestingly, the different aadA alleles have been showed genetically linked to various dfrA alleles (dfrA1, dfrA17, dfrA5, dfrA12) on class 1 integron. This finding explains in part the close rates of resistance against trimethorprim and streptomycin in *E. coli* isolates reported by many studies [8-10,14,15]. Moreover, and considering the occurrence of both these genes (dfrA+aadA) on class 1 integron with 3'-CS containing sul1 or

sul3 genes; such strains are therefore also resistant to sulfonamide [28]. Kanamycin-resistance has been also reported, with moderate level (0-40%) and encoded by aph(3')-Ia ; aac(6')-Ib-cr genes. In contrast to aforementioned rates of antimicrobial resistance, rate of gentamicin resistance was very low, being less than 2 % and mainly encoded by the aac(3)-II gene [9,10,13,25,26,29].

Resistance to fluoroquinolones

In Tunisia, non cephalosporins-resistant isolates showed resistance to nalidixic acid, which varied from 33% to 72.3%, and ciprofloxacin-resistance ranging from 7% to 19.9% [9,10,13]. In the world, the prevalence of fluoro-quinolones resistance in *E. coli* from poultry has been increasingly reported. The mechanisms attributed to quinolones resistance are principally related to mutations in the quinolone resistance determinants regions (QRDR) of gyrA and parC genes [30]. Ser83Leu, Asp87Gly, Asp87Asn and Asp87Tyr substitutions in GyrA protein have been reported, and Ser80Ile, Glu84Gly and Ser80Arg in ParC protein [26,31]. Plasmid-mediated-quinolone resistance (PMQR) was also found to contribute to the increase of quinolone resistance among *E. coli* isolates [30]. qnrS1, qnrB5 and qnrB19 genes have been reported respectively in chicken samples and faecal samples from healthy dog in Tunisia [29,31]. The aac(6')-Ib-cr gene, which encodes a variant of the widespread aminoglycoside acetyltransferase ACC(6')-Ib was also detected in chicken and poultry meat samples [10,29].

Conclusions

Worldwide, a global analysis showed high resistance rates against tetracycline, trimethorprim/sulfamethoxazole, streptomycin and quinolones antibiotics. *E. coli* from pig and poultry were the most resistant ones. This finding is linked to the specific production systems of pig and poultry, where thousands of organisms are assembled in crowded limited space. In such industrialized environments, animals are stressed and highly susceptible to bacterial and viral infections. Consequently, and to overcome economic losses, application of antimicrobial substances is common in pig and poultry farms. Therefore, these antibiotic-rich environments become ideal settings of cross-transmission of resistant *E. coli* isolates and genetic elements encoding antibiotic resistance (integrons, plasmids). *E. coli* of animal and food-producing animals in Tunisia seems to be in the global way of acquiring multi-drug-resistance. The high rates of resistance to tetracycline, streptomycin, quinolones/fluoroquinolones and trimethoprim/sulfamethoxazole are compared to the worldwide situation.

Conflict of Interest

All authors have contributed equally to the realization of this work. None of the contributing authors has any conflict of interests relevant to the subject matter or materials discussed in the manuscript. No funding or other financial support was received.

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