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Impact of Antimicrobials use in chickens on emergence of drug-resistant Campylobacter organisms in humans

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Abstract

The objective of this review is to present the impact of uncontrolled use of antimicrobial agents in chicken husbandry on emergence of drug-resistant *Campylobacter* in humans. The absence of an Antimicrobial Resistance Monitoring System (ARMS) in most developing countries of the world, amidst trade's globalization of animals and animal products, helped in the recent decade in spreading of drug-resistant organisms across the world's food chains.

The emergence of drug resistance in *Campylobacter* organisms was associated with their transmission from animals and their products to humans. This transmission of drug-resistant Campylobacter resulted in serious failure of treatment regimens prescribed to infected humans.

Keywords: Antimicrobials; chicken; drug-resistance; *Campylobacter*; humans.

Introduction

Most developing countries around the world lack a system that monitors the drug-resistance pattern in bacteria that cause serious zoonoses around the globe [1]. The Antimicrobial Resistance Monitoring System (ARMS) should be established in each country, including in its structure selected personnel from The Ministry of Health, Veterinary Medicine Syndicate, Department of Agriculture, and representatives from the WHO (World Health Organization), FAO (Food and Agriculture Organization), and OIE (Office International des Epizooties).

The ARMS main role is to receive zoonoses-causing bacteria isolates from specialized centers in the developing countries for speciation and/or serotyping, and for determining their antimicrobial susceptibility to a wide spectrum of drugs available in the world's market.

An annual report should be prepared by each country, presenting the frequency of resistance to different drugs by the tested zoonotic bacteria, such as *Campylobacter* organisms, with the inclusion of information related to the isolate source, and the region. Such annual report should be

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available through publications that are distributed to libraries around the world, and preferably through an established web site net work to cover most developing countries, or at least a network with a regional coverage. The annual reports should be accessible by large communities of the world, including personnel in the fields of human medicine, veterinary medicine, animal production, food industry, consumers, and touristic industries.

Such availability of data will help physicians, veterinarians, managers in animal production and food processing, consumers, and tourists to be aware of the sources of the zoonotic bacteria and the in-vitro efficacy of the tested drugs. In addition, the presentation of such annual reports will build a pressure on animal and food industry to improve their husbandry and hygiene measures, to reach to a safer food that improves the livelihood of the consumers around the globe.

The reader of this review will be exposed to conclude researches from different parts of the world, related to the problem of drug-resistance in *Campylobacter* organisms that originated from poultry. This exposure will raise the awareness to the seriousness of the abuse of drugs in animal production management, affecting the human health by organisms that are resisting the drugs of choice.

Campylobacter infection in humans

Campylobacter organisms are etiologic agents of diarrhea in humans, and are composed of two predominant species namely, *Campylobacter jejuni* and *Campylobacter coli* [10; 11]. *Campylobacter* infection is rated as the most common cause of bacterial gastro-enteritis in the developed countries [12].

The infection seems self-limiting in two thirds of patients, after an incubation period of three days, leading to symptoms of abdominal pain and diarrhea; however, the other one third of cases suffer of fever, general aches, dizziness, and delirium during around 24 hours before the onset of the known gastrointestinal symptoms. The one third portion of patients manifest a severe pathogenesis by the *Campylobacter*, requiring immediate therapeutic intervention, usually by known drugs of choice, namely fluoroquinolone or macrolide antimicrobials [22].

The early clinical trials with fluoroquinolone showed an apparent efficacy against *Campylobacter* infection that led to a recommendation to use this drug as prophylaxis during travels of Europeans to outside their continent [23; 24;.25; 26; 27].

Veterinary abuses of quinolones in poultry

The two predominant species of *Campylobacter* in human infections (*C. jejuni* and *C. coli*), have their main reservoir in poultry [11], allowing their inclusion with other members defined under 'food pathogens'. The Fluroquinolone 'enrofloxacin' was introduced into poultry in the year 1987, just one year before the ciprofloxacin was introduced as a drug for humans [28]. The use of enrofloxacin in poultry spread to almost every country, due to its high efficacy in treatment of the most predominant secondary infections in poultry, caused by *E. coli* [28], especially the colibacillosis that follows a common primary infection by *Mycoplasma gallisepticum* [29]. The use of enrofloxacin in the first five years of its introduction to poultry resulted in 29% of *Campylobacter* isolates building resistance to nalidixic acid, flumequine, enrofloxacin and ciprofloxacin [28]. It is worth noting that in the year 1982 no fluoroquinolone-resistant *Campylobacter* strains were isolated from human cases in Netherland; however, resistance to this drug started to increase drastically in human isolates after 3-4 years of enrofloxacin introduction to poultry [30].

A controlled experiment by Jacobs-Reitsma *et al.* in 1994 showed a direct effect of the application of fluoroquinolone on emergence of resistance in *Campylobacter* recovered of chickens. This led the authors to recommend a reassessment of the use of fluoroquinolones in animal husbandry.

The United Kingdom delayed the introduction of fluoroquinolone in its poultry until 1994, while the use of enrofloxacin in poultry of mainland Europe started earlier in 1987. This helped in comparison of the frequency of *Campylobacter* resistance to enrofloxacin in UK chicken versus chicken of the other mainland European countries. The frequency of the enrofloxacin resistance in *Campylobacter* isolates of the UK-chickens was seven times less than that of the other mainland European-chickens [31], confirming the hypothesis of emergence of drug-resistance in poultry isolates of *Campylobacter* due to introduction of enrofloxacin medication on poultry farms.

It is worth noting that many other studies have attributed the emergence of drug-resistance in *Campylobacter* to the use of antimicrobial agents in poultry production [32]. These alarming documented research led to banning of the use of fluoroquinolone in poultry husbandry, effective the year 2005.

The research documented in Belgium, Austria, Minnesota (USA), Brazil, and Turkey pointed at the emergence of drug-resistance in *Campylobacter* isolates after the introduction of fluoroquinolones as a medication inpoultry. In Belgium, alarming high rates of drug-resistance in *Campylobacter* recovered from chickens (44.2%), suggested that the use offlu-

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oroquinolones in poultry is most likely behind the increasing drug-resistance in human isolates [5]. The rate of isolations of *Campylobacter* organisms from chicken broilers at Styrian slaughter house in Austria was 54%, and the frequency of resistance to ciprofloxacin was 62.2%, suggesting that such a high frequency of drug-resistance was due to the fact that enrofloxacin was the most commonly used drug in broiler production in Austria [6]. In Minnesota, a central north state of the USA, reported a rapid rise in quinolone resistance of human *Campylobacter jejuni* that coincided with the licensing of fluoroquinolones in poultry; moreover, an epidemiology was concluded, based on comparing subtypes of drug-resistant chicken versus human isolates of *C. jejuni*, proving that 'the use of fluoroquinolones in their poultry had a primary role in increasing resistance to quinolones among the tested *C. jejuni* isolates recovered from humans [7].

The conclusion reached in Brazil, following a documentation of high frequency of resistance to fluoroquinolones in *C. jejuni* isolates recovered from children, especially in the absence of previous history of children's treatment with this drug, suggested an animal origin of the drug-resistant *C. jejuni* due to veterinary use of enrofloxacin for treating poultry bacterial diseases [9].

Last but not least, the documented research from Turkey, including data between 1992-2000, proved that the first fluoroquinolone-resistant *Campylobacter* isolate recovered from broiler chickens was in the year 1992, occurring after two years of enrofloxacin inclusion in treatment programs of Turkish poultry farms. A significant rise in resistance of *Campylobacter* occurred in the year 2000, in which 75.5% of the isolates were resistant to enrofloxacin and 73.0% to ciprofloxacin. The authors concluded that 'The uncontrolled use of fluoroquinolones in animals in Turkey is behind this rise in resistance [8].

It is worth noting that the fluoroquinolone member used in farm animals was enrofloxacin (Brand name: Baytril), while that used for humans was ciprofloxacin (Brand name: Cipro). The previous mentioned researches from different countries proved that the use of Baytril, as prophylactic for chickens is implicated in increasing resistance to Cipro. In addition, the bacteria that develop resistance to the Macrolide, used in chicken under the name Tylosin, are often cross-resistant to erythromycin that is used against *Campylobacter* infection in humans, and for treatment of people that are allergic to penicillin.

Unfortunately, the fluoroquinolone is still used on a wide scale in poultry husbandry of many Middle Eastern countries, in the absence of periodic reports to monitor the buildup of drug resistance in the poultry and human isolates of *Campylobacter*.

Mechanism of Quinolone and Macrolide Resistance

The long term use of quinolone and macrolide in poultry husbandry led to mutations in the *Campylobacter* organisms present in aviant intestine, which was transmitted on a wide scale into the poultry industry.

The fluoroquinolones, such as enrofloxacin, target the DNA gyrase enzyme in the bacteria. A mutation in the gyrA-encoding subunit of the DNA gyrase results in one or more amino acid substitutions [35]. These substitutions exist within the DNA-binding domain. A change in the sequence of amino acids in such a domain may lead to a resistance in the *Campylobacter* organism to fluoroquinolones. This important domain is referred to in literature as the QRDR (quinolone-resistance determining region). Some substitutions of amino acids in the QRDR domain have been characterized and correlated to quinolone-resistance; examples of such substitutions are the Thr-86-Ile substitution [34; 35] and the Asn-203-Ser substitution [34].

The mechanism of Macrolide resistance in *Campylobacter* organisms is also studied, resulting in different conclusions. The Erythromycin, a commonly used macrolide in poultry, inhibits the protein synthesis in *Campylobacter* organisms. This inhibition occurs due to the binding of the drug to ribosomes of this bacterium. The binding leads to dissolution of the tRNA-amino acid chain complex, thus preventing the bacterial growth by binary fission. The build-up of resistance in *Campylobacter* organisms treated with macrolides seems to be accomplished by two mechanisms: the first mechanism is by modification of the macrolide binding-target by a genetic mutation, and the second mechanism is by exclusion of the drug from the *Campylobacter* cell by an efflux process [26]. The most common mechanism for emergence of resistance to macrolide is the first, in which point mutations occur at position 2074 and/or 2075 in the 23SrRNA of the 50S-ribosomal subunit or by the mutations in L4 and L22 ribosomal proteins [26; 36].

The second less frequent mechanism for building resistance in *Campylobacter* organisms to a wider range of antimicrobials is by the Cme-ABC efflux pump. This mechanism results in expulsion or exclusion of the Macrolides and other antimicrobials such as quinolones, from the *Campylobacter* cells. This expulsion pump can promote both intrinsic and acquired resistance to a range of antimicrobial agents [37; 38].

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The understanding of the above mechanisms, that result in emergence of resistance in *Campylobacter* organisms to drugs of choice (quinolones and macrolides), incriminates the point mutations as the most common mechanisms foracquiring drug resistance by these organisms.

These point mutations become stable in the *Campylobacter* populations harboring the poultry host, transferring such resistance trait to their daughter cells, regardless of the measure taken by poultry industry to ban the practice of application of quinolones. Actually, the resistance to quniolones and macrolides in *Campylobacter* isolates of poultry persisted until after the year 2005, the year of banning the use of quinolones in poultry husbandry [13; 14; 15].

Drug-resistance post the banning of Quinolone

The drug resistance in *Campylobacter* isolates, post the banning of quinolone application in poultry husbandry in the year 2005, is persisting.

In the year 2006, just one year following the banning of quinolones applications in poultry, sporadic reports documented different levels of quinolone and macrolides resistance in poultry isolates recovered from different parts of the world [16; 17; 18; 19]. Two years following the banning of quinolone use in poultry, reports started to commend the success of this banning approach in reducing the resistance to quinolones in avian isolates [20]; following such a success, more efforts were directed towards the macrolide abuse in poultry, especially the emergence of *Campylobacter* isolates with resistance to erythromycin [21].

The sporadic reports in the four years, between 2008 and 2011, related to frequency of resistant avian *Campylobacter* isolates to quinolones and macrolides included major works by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS, 2011). CIPARS identified greater than 10% emerging prevalence of ciprofloxacin-resistant *Campylobacter* isolates recovered from chicken marketed in British Colombia and Saskatchewan provinces. This prevalence had an increasing trend between 2007 and 2010.

In April of 2011, EFSA and the European Center for Disease Prevention and Control (ECDC) reported latest results of antimicrobial resistance, as submitted by EU countries (ECDC, 2011). Unfortunately, there was a high resistance to ciprofloxacin, ranging from 33% to 78% in chickens, chicken meat, pigs and cattle. EFSA's report indicated that resistance in these zoonotic bacteria persisted after 2005, which was most likely due to continuing practice of using these drugs by the European farmers.

The National Antimicrobial Resistance Monitoring System of the United States Department of Agriculture (NARMS, 2009) reported between 1998-2009 the resistance in chicken *Campylobacter* isolates to 10 antimicrobials that fall under 6 antimicrobial classes namely the Aminoglycosides (Gentamicin), Lincosamides (Clindamycin), Macrolides/Ketolides (Azithromycin, Erythromycin, Telithromycin), Phenicols (Chloramphenicol, Florflenicol), Quinolones (Ciprofloxacin, Nalidixic acid), and Tetracyclines (Tetracycline). The highest consistent resistance in *Campylobacter*, in the last 4 years of the report's data was to tetracycline ranging between 49.6-56.6%, followed by Nalidixic acid (8.8-33.3%), Cipofloxacin (8.8-32.1%), Erythromycin and Gentamicin (0.0-1.3%), and Azithromycin (0-1%). The *Campylobacter* recovered from chickens had no resistance in the last 4 years of the report to clindamicin, telithromycin, and florfenicol.

In conclusion, the abuse of drugs in poultry that results in resistance of human *Campylobacter* organisms to drugs of choice is a neglected issue in many developing countries, resulting in a significant negative impact on the livelihood of their people and on tourists that visit such countries. It is recommended to establish a Regional Antimicrobial Resistance Monitoring System (RARMS) in the Middle East, composed of selected personnel from the Ministries of Health, Veterinary Medicine syndicates, and Departments of Agriculture, and representations from WHO (World Health Organization), FAO (Food and Agriculture Organization) and OIE (Office Internationale des Epizooties). The RARMS main role will be to receive *Campylobacter* isolates from specific stations in all Middle Eastern countries for speciation and/or typing and for determining their susceptibility to a wide spectrum of antimicrobials. An annual report should be published to include the frequency of resistance to different drugs by *Campylobacter* organisms, the isolates source, region, and the country of the Middle East. This report could be downloaded on a special web site of the RARMS, for an easy access by the human medical communities, veterinarians, food industry, local people of the Middle East, and tourists.

The alarming data of this review, related to the emergence of resistance to drugs of choice by poultry isolates of *Campylobacter*, require an immediate decision to establish RARMS for the benefit of human and animal livelihood of the Middle East.

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