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Resistance to tetracycline in *Escherichia coli* isolates from poultry meat: epidemiology, policy and perspective

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Food borne transmission of antibiotic resistant strains of *Escherichia coli* from contaminated food has been recognised as an important hazard for human health in the past few decades and pathogenic strains of *E. coli* have long been considered as serious zoonotic hazards. Poultry meat is attractive for consumers worldwide, so the measures to preserve the safety of poultry meat are important issues. Tetracyclines are one of the most frequently used antibiotics in poultry farming and are still used in human medicine. The widespread use of tetracyclines in poultry farming may have resulted in the development and transmission of resistance strains from poultry to humans via the food chain. The relationship between the use of tetracyclines and the presence of resistant strains of *E. coli* in poultry meat, mainly due to the irregular use of antibiotics, is reviewed in this paper. The need for prudent use of antibiotics, particularly those which are used in human medicine is highlighted. Continuous monitoring and reporting on incidents in the future should improve the current regulations.

Keywords: antibiotic resistance; Escherichia coli; monitoring; poultry meat; recommendations; tetracycline

Introduction

Poultry meat has a high nutritive value which is of a great importance for human nutrition. Puvača *et al.* (2014) stated that the consumption of all meat types is decreasing with the exception of poultry, which has increased by 80% in the last three decades. The relatively low and competitive price of poultry meat, lack of

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religious and cultural barriers and nutritional quality are the main reasons for the fact that poultry meat is very attractive for consumers worldwide (Magdelaine *et al.,* 2008), so the measures to preserve the safety of poultry meat are very important issues.

Tetracyclines have been widely used in poultry production for decades (Wassenaar, 2005). Notwithstanding the fact that they are used therapeutically in order to improve health and welfare of poultry, they have been used for prophylactic purposes and as growth promoters in order to improve growth rate and feed conversion efficiency. Certain antibiotics used in poultry production, including tetracyclines, are often the same, or belong to the same class, as those used in human medicine (Schwarz *et al.*, 2001). The main consequences of such use of antibiotics is the propagation of antibiotic resistance as a result of continuous positive selection of resistant bacterial clones, whether these are pathogens, commensal or environmental bacteria (Wassenaar, 2005). Results obtained by Diarra *et al.* (2007) showed that up to 75% of antibiotics pass through unaltered in faeces, so their regular use in poultry feed increases the risk of resistant pathogens being excreted by poultry. These resistant pathogens then become available in the environment, and can be spread to humans via food or water as well as by environmental contamination from poultry litter and direct contact with animals (Ljubojević *et al.,* 2016a; 2016b).

E. coli is used as an indicator organism to evaluate the hygienic quality of food products and the dissemination of antibiotic resistance (Capita *et al.,* 2002). Specifically, tetracycline-resistant *E. coli* is used due to its high frequency of occurrence (Koo and Woo, 2011).

In the last two decades, the use of prophylactic antibiotics in poultry has changed, as growth promoters have been severely restricted or banned in some countries. Local, national and international management campaigns and policies have encouraged prudent use and limited unnecessary exposure to antibiotics, with the final goal of preserving their effectiveness for serious diseases (Landers *et al.,* 2012). This has led to great differences between countries in regard to prevalence of antibiotic resistance (Van den Bogaard *et al.,* 2001; Adelowo *et al.,* 2009; EFSA, 2016). Furthermore, since practices differ between countries across the world there is an opportunity to determine the broad effects of antibiotic use by comparing the frequency of tetracycline resistance among poultry meat from different parts of the world.

Several reviews of antibiotic resistance of *E. coli* have recently been published (Schroeder *et al.,* 2004; Todorović *et al.,* 2015; Ljubojević *et al.,* 2016a; 2016b) but the problems with tetracycline resistance in *E. coli* in poultry meat have not been specifically addressed.

The aims of the present review are to compare the results from available literature regarding the prevalence of tetracycline resistance in *E. coli* strains isolated from poultry meat in different countries. Moreover, the most important international policies on use of antibiotics in poultry have been reviewed. Recommendations for preventive measures and monitoring of the use of antibiotics is proposed.

The use of tetracyclines in human clinical practice and poultry husbandry

Tetracyclines represent a family of broad-spectrum antibiotics and tetracycline, chlortetracycline, and oxytetracycline are used in the treatment of bacterial, chlamydial, rickettsial and protozoal infections (Roberts, 2003). They are among the most popular and the most frequently used antibiotics in poultry farming but these compounds are still used in human medicine due to their efficacy, relatively low cost and lack of side effects. Tetracycline is a frequently used, first line antibiotic in poultry and is often used before any resistance profile of the pathogen has been determined (Miles *et al.,* 2006). Thus, such extensive use has led to natural selection for resistant bacteria and could result in horizontal transfer of resistance determinants from poultry to humans as well as to the environment.

Worryingly, tetracycline resistant isolates of *E. coli* from poultry are more likely to become resistant to additional antibiotics (Van den Bogaard *et al.,* 2001). Furthermore, resistance may be conserved in bacterial populations over time, regardless of selection pressure, which might result in an overall increase over time. Resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants which makes it easier for a susceptible bacterium to acquire resistance factors by conjugation (Sunde and Nordstrom, 2006; Koo and Woo, 2011) or transformation (Miles *et al.,* 2006). The rapid dissemination of tetracycline resistant determinants within a bacterial population is due to the location of tetracycline genes on mobile elements (Roberts, 2003).

The role of *E. coli* in dissemination of tetracycline resistance

E. coli are the most common commensal bacteria present in human and poultry digestive systems and one of the most important reservoirs of antibiotic resistance (Ljubojević *et al.,* 2016a; 2016b). Besides this, *E. coli* are important pathogens and can cause cellulitis, septicaemia and colibacillosis in poultry and being linked to extra-intestinal pathogenic *E. coli* strains in humans, such as urinary tract infections (Manges, 2016). The majority of infections caused by *E. coli* in humans are not harmful, but some infections may be lethal. Furthermore, it could be a source of contamination of poultry meat during evisceration after slaughter and processing, during contact with contaminated water or food handling (Schroeder *et al.,* 2004).

Resistance in *E. coli* from poultry has been reported worldwide and generally is associated with the use of antibiotics (Mayrhofer *et al.,* 2004; Álvarez-Fernández *et al.,* 2013). It is well established that the level of resistance in commensal *E. coli* can be used as an indicator of the selection pressures due to the use of antibiotics as well as the resistance patterns and problems that can be expected in pathogenic bacteria. Monitoring resistance among commensal *E. coli* is of great importance, not only in humans but also in poultry and meat thereof, in order to detect the possible route of transfer of resistant bacteria or resistant determinants from poultry to humans.

Links between the use of tetracycline and bacterial resistance and the role of poultry meat in the spread of resistance to humans

Positive correlations between the use of antibiotics in veterinary medicine and the appearance of antibiotic resistance in bacteria isolated from humans have been reported (Van den Bogaard *et al.,* 2001). Schroeder *et al.* (2004) stated that any use of antibiotics could lead to selective pressure favouring emergence of resistant bacteria. However, it should be emphasised that sub-therapeutic use can be an additional factor contributing to the emergence of antibiotic resistant bacteria (Schwarz *et al.,* 2001). A direct threat to public health may arise from the possibility for transfer of antibiotic resistance genes among humans, animals and the environment (Ljubojević *et al.,* 2016a; 2016b).

Cooke *et al.* (1970) provided evidence that food producing animals, including poultry, were a reservoir for *E. coli* nearly fifty years ago. Levy *et al.* (1976a) proved that applying oxytetracycline-supplemented feed to chickens facilitated the emergence of tetracycline-resistant *E. coli* both in chickens and farm workers and, in further work, Levy *et al.* (1976b) described the spread of an antibiotic resistance plasmid, pSL222-6, in *E. coli* from chickens to human handlers. Moreover, poultry meat may be contaminated with human-borne *E. coli* via the hands of workers during slaughter, processing, at retail and during food preparation. This make it likely that a prevalence of antibiotic-resistant *E. coli* found in meat and meat products has arisen from clinical antibiotic use in humans and, conversely, poultry meat may transfer resistant strains of *E. coli* to human volunteers, as demonstrated by Linton *et al.* (1977). They showed that antibiotic-resistant strains of *E. coli* from chickens colonised and survived the human alimentary tract via food consumption. Furthermore, Linton (1986) traced antibiotic-resistant *E. coli* from the gut contents of chickens to carcasses, and showed that it colonises the gut of human volunteers handling and eating meat. Van den Bogaard *et al.* (2001) reported the spread of antibiotic resistance plasmids in *E. coli* from chickens to human handlers. Cogan *et al.* (1999) reported that bacteria from food can contaminate kitchens during meal preparation, and cooks can acquire resistant *E. coli* from poultry carcasses without consuming the food. Huang *et al.* (2001) suggested that students from USA who travelled to Guadalajara, Mexico became colonised with antibiotic-resistant *E. coli* probably due to poor sanitary conditions and the recurrent and heavy exposure to antibiotic-resistant indigenous flora as a result of contaminated food and drink.

On the other hand, Smith (1969) concluded that human and poultry isolates belong to two distinct pools of resistant *E. coli* and that the antibiotic resistance transfer between animals and humans was limited, and that animal strains colonised the alimentary tract less readily than human ones, concluding that animals are not an important source of resistant *E. coli* in man.

Schroeder *et al.* (2004) stated that there is no documented case of antibiotic treatment failure of infections shown to be directly related to ingestion food borne *E. coli* and that, without a definitive link between antibiotic resistant *E. coli* in food and clinical treatment failure, the extent to which antibiotic-resistant *E. coli* in poultry meat represent a threat to human health remains speculative and controversial.

The prevalence of tetracycline resistant *E. coli* in poultry meat

Due to the emergence of resistant bacteria, the European Commission banned the use of antibiotics as growth promoters in animal feed. Moreover, consumer pressure has forced the poultry industry to produce animals without using antibiotics as growth promoters in many countries (Puvača *et al.,* 2015a). However, Dibner and Richards (2005) noted that removal of antibiotics as growth promoters has led to reduced growth performance and increased incidence of poultry diseases. Since 2014, the mandatory monitoring of antibiotic resistance in commensal *E. coli* from food producing animals has been laid down in the legislation (EFSA, 2016). Sáenz *et al.* (2001) examined food products of animal origin obtained from 14 local supermarkets and poultry shops in La Rioja (Spain) and found a high frequency (53%) of tetracycline resistance in samples which included chicken hamburgers, sausages, mince, skin, caecum, breast meat, pre-cooked chicken foods and turkey products.

The results of a study conducted by Van den Bogaard *et al.* (2001) strongly indicated that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans commonly occurs. They concluded that the major factor selecting for resistance in bacteria is antibiotic use, as well as crowding and poor sanitation.

Mayrhofer *et al.* (2004) analysed 288 samples of poultry meat and 266 samples of turkey meat and found that, next to quinolone, resistance to tetracycline was seen most often in pathogenic *E. coli* isolated from poultry meat from supermarkets, butchers, street markets and slaughterhouses throughout Austria. They conclude that the antibiotic resistance profile of pathogenic food isolates reflected animal treatment with antimicrobial substances, as tetracycline is the drug used most often in animal husbandry.

Miranda *et al.* (2008) examined 30 samples each of organic chicken meat, conventional chicken meat and conventional turkey meat in Spain and observed that isolates from organic chicken meat were less resistant than isolates from conventional chicken meat to doxycycline, and were less resistant than isolates from turkey meat to doxycycline. They noted that levels of resistance obtained for isolates from organic chicken were significantly lower than those obtained from conventional chicken and turkey for the antibiotics more commonly used in poultry production, such as quinolones, tetracyclines and sulphonamides. Álvarez-Fernández *et al.* (2013) obtained 120 poultry carcasses, 30 samples each of organic chicken, conventional chicken, conventional turkey and conventional quail from eight retail outlets in the Province of León in North-Western Spain and found that resistance to tetracycline was 40.0% in conventional chickens, 46.7% in organic chicken, 66.7% in conventional turkey, 93.9% in conventional quail 93.3% with an average of 61.7%. They found that the relative high resistance to tetracycline in *E. coli* isolated from poultry meat from organic system of rearing was unexpected and surprising and explained that by the fact that tetracyclines have been used on poultry farms in Spain for treatment of infectious diseases for a long time. This may have led to the possibility that *E. coli* evolved to become resistant to tetracyclines, which contributed to widespread distribution in animals as a reservoir, regardless the type of production and the usage of antibiotics.

The occurrence of resistance to tetracycline in indicator *E. coli* from poultry meat in countries reporting MIC data in 2013 was 11.2% in Denmark, 42% in Germany, 58.7% in Hungary and 35.2% in Slovenia (EFSA, 2015). The level of resistance to tetracycline in indicator *E. coli* from turkey meat was reported in Norway and was found to be 17.5% (EFSA, 2015). Others have reported the occurrence of tetracycline resistance *E. coli* from poultry meat in countries from MIC data in 2014 was 42.7% in Switzerland, 11.9% in Denmark and 36.3% in Germany (EFSA, 2016), and other reports from Germany showed 61.6% from turkey meat (EFSA, 2016).

In many countries, the majority of antibiotics used are administered to livestock, and in the United States as well as in many other countries it is for prophylactic purposes or to stimulate growth promotion (Mathew *et al.,* 2007). Schroeder *et al.* (2003) isolated *E. coli* from raw meat from retail supermarkets in Greater Washington, DC, USA including 51 chicken and 50 turkey samples and 212 whole chickens and 194 turkey breasts and noted that 59% of the isolates displayed resistance to tetracycline.

In the majority of African countries, the use of antibiotics in poultry production is unregulated and prescription is not required even for human use. Olonitola *et al.* (2015) showed there is a shortage of data related on the emergence of antibiotic resistant pathogens among African countries, but undoubtedly people in Africa suffer from high rates of antibiotic resistant infections. For example, antibiotics is commonly used as additive in poultry feed in Nigeria (Adelowo *et al.,* 2009) for prophylaxis, therapy and diseases control. Ayeni *et al.* (2016) stated that tetracycline is one of the most frequently used antibiotics in Nigeria both in human and veterinary medicine because of its relatively low price and availability. Other authors from Nigeria (Kabir *et al.,* 2004; Adelowo *et al.*, 2009; Olonitola *et al.,* 2015; Ayeni *et al.,* 2016) provided evidence that tetracycline resistance is common on poultry farms and highlighted the need for strict regulation on tetracycline usage. Adelaide *et al.* (2008) examined multi-resistance and presence of virulence related genes in *E. coli* isolates from healthy broilers at slaughter time at the Tigoni processing plant in Limuru, Kenya. They reported that tetracycline resistance ranged from 44.4% to 96.4% on all the farms and with 75.9% at the slaughterhouse. Soufi *et al.* (2009) examined 55 *E. coli* isolates acquired from chicken and turkey meat obtained from two slaughterhouses in Tunis and found that 89% showed resistance to tetracycline. They noted that tetracycline resistance was predominately mediated by the tetA gene and that 60% of isolates harboured integrons.

Van *et al.* (2008) examined the current profile of antibiotic resistance and virulence of *E. coli* isolated from foods commonly sold in the market place in Vietnam. They found that 84.2% were resistant and found tetA in 71.43% of isolates, tetB in 28.57% and tetC in 14.29%. Lei *et al.* (2010) collected samples from fresh or chilled chickens at 12 open markets and supermarkets in six cities in Southern China and recovered 29 *E. coli* isolates from chicken. They noted that isolates from animal food products showed moderate, low or very low rates of resistance to most of the tested antimicrobials, and only showed high resistance to tetracycline (86.5%). In addition, they found high levels of tetracycline resistance in food producing poultry with 95.2% in chickens and 96.6% in ducks, geese and pigeons.

Koo and Woo (2011) noted that tetracyclines were the most common used (43-51%) antibiotic for livestock farming in Korea between 2002 and 2006, and most were used as growth promoters or prophylactics. Such practises in Korea were partially limited in 2005, and antibiotics such as oxytetracycline and chlortetracycline were entirely banned for non-human uses as of January 2009 in Korea. Koo and Woo (2011) isolated *E. coli* strains from meat products which were collected through the National Antimicrobial Resistance Management Program in Korea. They collected 55 tetracycline-resistant *E. coli* originating from poultry. They found tetA in 26.4% of isolates, tetB in 17.4%, tetC in 0.8%, tetD in 0.8% and tetA and tetB concurrently in 0.8% of isolates.

Obeng *et al.* (2012) reported that only chlortetracycline is registered for egg-producing birds and a wider range including amoxicillin, neomycin, lincomycin, spectinomycin, and oxytetracycline is registered for use in broilers in Australia. Caudry and Stanisich (1979) isolated *E. coli* from the fluid obtained after thawing each of five frozen chicken carcasses from retail stores in Melbourne. They found that multiple resistance was more common than single resistance, and that resistance to tetracycline was the most frequently encountered and occurred in range from 19.5 to 40% of the *E. coli* tested from each sample. Bensink and Botham (1983) investigated antibiotic resistance in coliforms isolated from freshly slaughtered and chilled poultry at retail outlets in Brisbane, Australia. They found that approximately 85% isolates examined were resistant to at least one antibiotic, and that 60% of isolates were resistant to tetracycline.

Conclusions

Antibiotic resistance undoubtedly represents a serious public health threat. Taking the available published results together, it appears that poultry meat is an important risk for exposure to antibiotic resistance and virulence potential. The use of antibiotics in poultry may be the reason for high rates of resistance, and overcrowding and poor sanitation may contribute to the spread of antibiotic and virulence genes between populations. Urinary tract infections in humans may be associated with poultry consumption (Manges, 2016), which endorses the need for more rigorous surveillance and improved farming practices that can reduce the carriage of resistance genes and minimise the likelihood of horizontal gene transfer of resistance genes to other bacteria in the food chain.

Because poultry meat can be an important reservoir of tetracycline-resistant *E. coli*, the need for consumer education on good hygiene practices should be emphasised.

Preventive measures at home include carefully cooking of poultry meat, prevention of cross-contamination from meat products by washing hands and kitchen surfaces during meal preparation and hand washing after contact with animals, their faeces or environments. Training on safe food handling and proper cooking are important to reduce or eliminate the risk from antibiotic resistant and pathogenic bacteria originating from raw poultry. The education of farmers, veterinarians, public, and health practitioners is important in order to ensure the proper use of tetracycline and to ensure that antibiotics are not be used without veterinary approved need for specific diseases.

To reduce resistance levels in pathogenic bacteria, surveillance, monitoring of resistance, prudent use, education, research and the use of alternatives such as specialist feed ingredients and vaccination are recommended by the WHO (Mayrhofer *et al.,* 2004). A number of studies have highlighted the potential use of various phytogenic additives, herbs, species and their essential oils into poultry feed as alternatives to antibiotics (Puvača *et al.,* 2015b; 2015c).

Undoubtedly, tetracycline use in poultry production cannot be stopped, as it represents a valuable tool for reducing poultry diseases as well as suffering. Having that in mind, decisions about what antibiotics to use and how to use them in every occasion must be made with consideration of their potential impact on human health. The intensive poultry production industry needs to optimise poultry production performance and minimise economic loses, as well as ensuring the safety of poultry meat via the control and elimination of food borne hazards. Phytogenic additives, herbs, pre- and probiotics and essential oils present promising alternatives to antibiotic growth promoters in poultry industry. Preserving the effectiveness of antibiotics is vital to protecting human as well as animal health. Continuous monitoring and reporting on incidences in the future should improve the current regulations.

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