Antibiotic Resistance of Enterococci, Coagulase Negative Staphylococci and *Staphylococcus aureus* Isolated from Chicken Meat

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Abstract

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We determined the antibiotic resistance of enterococci, coagulase negative staphylococci, and *Staphylococcus aureus* isolated from chicken meat samples. The antibiotic resistance of the isolated strains was estimated by the Kirby-Bauer disk diffusion method (according to the NCCLS document M2-A9 suggestions). It was found that all strains of *Enterococcus* spp. were resistant to tetracycline, 75% of them were resistant to ciprofloxacin, and 50% of them were resistant to erythromycin, vancomycin, and chloramphenicol. Also all strains of *S. aureus* were resistant to tetracycline and 25% of *S. aureus* strains were resistant to erythromycin and chloramphenicol, whereas all strains of *S. aureus* were sensitive to teicoplanin and 25% of them were sensitive to vancomycin and ciprofloxacin. As for the isolate of coagulase negative staphylococci (CNS), 68.1% of them were resistant to erythromycin, 77.2% of them were resistant to tetracycline, 59% of them were resistant to vancomycin, 9% of them were resistant to teicoplanin, and 27.2% of them were resistant to both chloramphenicol and ciprofloxacin. As a result, it was found that most of the strains (all of *S. aureus* and *Enterococcus* spp., also 77.2% CNS) were resistant to tetracycline.

Keywords: *Staphylococcus* spp.; *Enterococcus* spp.; susceptibility test; identification; antibiotic

Antimicrobial resistance is an important public health concern worldwide. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production (Normanno *et al*. 2007). Antibiotics have been extremely useful for the treatment of infectious diseases in the last 50 years and have become indispensable for the treatment of many diseases formerly known as lethal (Pesavento *et al*. 2007). The use of antibiotics and other antimicrobial agents throughout the food chain contributes to the emergence of resistant bacteria that can be passed directly to humans after ingestion (Demirtürk & Demirdal 2004; Ruzauskas *et al*. 2010). Antibiotics have been used in animal feed to prevent diseases and improve performance for more than fifty years. Continuous use of antibiotics in animal feed led to the development of antibiotic resistance in pathogenic bacteria (Robredo *et al*. 2000; Lange & Brokking 2005).

There are thousands of species colonising animals, but only recently were antibiotic resistant strains isolated from several food production animals, including pigs, cattle, chicken, and other animals. Raw chicken meat is often consumed in many countries including Turkey, so antibiotic resistant strains in chicken meat are a risk factor in the food chain. Sufficient and valid data are an indispensable component in the assessment of a possible health risk related to antibiotic resistant bacteria-contaminated chicken meat (Duman 2007; Irlinger 2008). Turkey, however, lacks

information on antimicrobial resistance profiles, resistance gene distribution, and incidence of some bacteria such as enterococci, coagulase negative staphylococci, and *Staphylococcus aureus* from poultry. As a result, antibiotics are used unconsciously to protect chickens against infections and it is observed that the level of antibiotic resistance has been rising more and more (de Boer *et al*. 2009; Kasimoglu-Dogru *et al*. 2010).

The aim of this study was to investigate the antibiotic resistance of enterococci, coagulase negative staphylococci (CNS), and *Staphylococcus aureus* isolated from chicken meat samples collected from various supermarkets in Adana region of Turkey.

MATERIAL AND METHODS

Samples. Fifty samples of raw chicken meat were collected from various supermarkets in Adana. In all isolations, 25 g portions of the sample were homogenised with 225 ml 0.1% (wt/v) peptone water (Bacteriological peptone; Oxoid, Basingstoke, UK) in a blender (Waring Commercial Blender, Orlando, USA). The homogenates were then submitted to serial 10-fold dilutions in 0.1% (wt/v) peptone water and 0.1 ml of each dilution was plated on the surface of plates.

Isolation and identification of enterococci, coagulase negative staphylococci, and S. aureus. Enterococci were isolated on Kanamycin Aesculin Azide (KAA) agar and Bile Aesculin (BA) agar, both from Oxoid (Basingstoke, UK). The plates were incubated at 37°C for 24 hours. Following the incubation, up to five typical colonies (red, maroon, or pink) were transferred to tryptone soya agar (Oxoid, CM0131) supplemented with 0.6% yeast extract powder (Oxoid, L0021) and submitted to Gram staining and tested for catalase production. Gram-positive and catalase negative cocci were tested for the growth in De Mann-Rogosa-Sharpe (MRS) broth (Oxoid, CM0359) at 10 and 45°C, growth in MRS broth supplemented with 6.5% NaCl, and growth in MRS broth of pH 9.6. Also, gas production from glucose was tested (Gomes *et al.* 2008; Kasimoglu-Dogru *et al.* 2010).

Coagulase negative staphylococci were isolated on Mannitol Salt Agar (Merck, Darmstadt, Germany) at 30°C, from yellow or red colonies (typical staphylococci colonies). Preliminary characterisation included Gram staining and tests for cytochrome c oxidase, catalase, and coagulase (Faria *et al.* 2009).

S. aureus was isolated on Baird-Parker agar (Oxoid CM 275) supplemented with egg yolk-tellurite emulsion (Oxoid SR 54), and incubated at 37°C for 24 hours. From each plate, typical colonies of *S. aureus* with similar morphology were isolated and cultured separately on slants of Brain-Heart Infusion (BHI, Oxoid CM 225). The identification was carried out using the following tests: gram staining, production of coagulase, catalase, DNAse, and oxidation and fermentation of mannitol (Food and Drug Administration 1992).

Antibiotic susceptibility test. Antibiotic resistance of the isolated strains was determined by the Kirby-Bauer disk diffusion method (according to the NCCLS document M2-A9 suggestions). For the antibiotic susceptibility test, we used antimicrobial susceptibility test discs (Oxoid, Basingstoke, UK) such as erythromycin (15 µg) (E 15), tetracycline (30 µg) (TE 30), vancomycin (30 µg) (VA 30), teicoplanin (30 µg) (TEC 30), chloramphenicol (30 µg) (C 30), and ciprofloxacin (5 µg) (CIP 5). The strains were cultivated on Mueller Hinton Agar, and then the antibiotic discs were located by means of a dispenser. After incubation (24 h, 37°C), the bacteria strains were evaluated as resistant, mid-grade sensitive, and sensitive according to the criteria of the NCCLS document M2-A9 by measuring the inhibition zones diameters around the antibiotic discs (Gür 2007).

RESULTS AND DISCUSSION

In this study, 4 enterococci, 22 coagulase negative staphylococci, and 4 *S. aureus* strains were isolated from 50 chicken meat samples. Isolated were mostly coagulase negative staphylococci (73.3%) followed by enterococci (13.3%) and *Staphylococcus aureus* (13.3%).

Enterococci can be used as indicators of fecal contamination. They do not only contaminate raw meats but are also associated with processed and heat-treated food materials (Wilson & McAfee 2002; Busani *et al.* 2004). Enterococci are one of the most spread bacteria in food products as their ubiquitous nature and resistance to adverse environmental conditions account for their ability to colonise different habitats. Enterococci are widely spread in raw poultry products (Ruzauskas *et al.* 2010).

Coagulase-negative staphylococci have their technological value residing in desirable reac-

tions (flavour and aroma formation) during the ripening of fermented foods, especially cheeses and sausages. Under other circumstances, some species of coagulase-negative staphylococci can present a medical risk. Some coagulase-negative staphylococci (CNS) may behave as opportunistic pathogens, often introduced by medical devices or colonising exposed wounds (Veras *et al.* 2008; Faria *et al.* 2009).

Staphylococcus aureus is an important foodborne pathogen. It is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from slight skin infections to more severe diseases such as pneumonia and septicaemia. The presence of *S. aureus* in foods commonly indicates contamination that may be directly introduced into the food by workers who have skin lesions containing *S. aureus*, or by sneesing or coughing. Other contamination sources of *S. aureus* are soil, water, dust, and air (Gundogan *et al.* 2005; Güven *et al.* 2010).

Antibiotic resistance of Enterococci strains

Table 1 shows the antibiotic resistance of enterococci strains isolated from chicken meat samples. In this study, it was determined that *Enterococcus* spp. strains were resistant to erythromycin (50%), tetracycline (100%), vancomycin (50%), chloramphenicol (50%), and ciprofloxacin (75%), but they were sensitive to teicoplanin.

A specific cause for concern and a factor contributing to pathogenesis of enterococci is their resistance to a wide variety of antibiotics which can be acquired or found intrinsically. The role of enterococci in the transfer of antibiotic-resistance traits in foods is still under debate and increasing interest in the epidemiology of these bacteria continues worldwide. Several studies still last (Wilson & McAfee 2002; Busani *et al.* 2004).

The resistance often determined among enterococci from food is that against vancomycin and teicoplanin (Giraffa 2003). Also, Riboldi *et al.* (2009) isolated *Enterococcus* spp. from foods in Southern Brazil and detected only one vancomycin resistant *Enteroccoccus faecalis* isolated from colonial cheese.

In another study, Ruzauskas *et al.* (2010) evaluated the antimicrobial susceptibility of enterococci spread in poultry products. They reported that the most frequent resistance was demonstrated to tetracycline (84.5%), tylosin (64.5%), erythromycin (63.8%), and ciprofloxacin (36.2%).

Antibiotic resistance of coagulase negative staphylococci

The antibiotic resistance of coagulase negative staphylococci isolated from chicken meat samples in given in Table 2. Coagulase negative staphylococci strains were resistant to erythromycin (68.1%), tetracycline (77.2%), vancomycin (59%), teicoplanin (9%), chloramphenicol (27.2%), and ciprofloxacin (27.2%).

The hazards associated with CNS can be worsened by the notorious escalade of antibiotic resistance observed during the last decades in these bacteria. Even though CNS opportunistic infections are most of the times regarded as being of environmental origin, the distribution and antibiotic resistance patterns of these organisms in the environment are poorly characterised (Veras *et al.* 2008; Faria *et al.* 2009).

It was found that the results about the antibiotic resistance of CNS isolated from chicken in the study of Duman (2007) are different from those obtained in our study, especially important rising was observed in vancomycin resistance.

Table 1. Antibiotic resistance of enterococci strains isolated from chicken meat samples

No of strains	E 15	TE 30	VA 30	TEC 30	C 30	CIP 5
10	R	R	I	I	S	I
11	I	R	R	S	R	R
12	R	R	S	S	I	R
20	I	R	R	S	R	R

R – resistant; I – intermediate; S – sensitive; E 15 – erythromycin (15 μg); TE 30 – tetracycline (30 μg); VA 30 – vancomycin (30 μg); TEC 30 – teicoplanin (30 μg); C 30 – chloramphenicol (30 μg); CIP 5 – ciprofloxacin (5 μg)

Table 2. Antibiotic resistance of coagulase negative staphylococci isolated from chicken meat samples

No of Strains	E 15	TE 30	VA 30	TEC 30	C 30	CIP 5
1	R	R	S	S	S	I
2	R	R	R	I	R	I
4	S	S	S	I	S	S
6	S	S	S	S	S	S
8	I	I	R	R	I	I
9	S	S	S	S	S	S
13	R	R	R	I	R	I
14	R	R	R	I	I	I
15	I	R	R	I	S	S
16	R	R	R	I	I	I
17	I	R	R	I	I	R
18	R	R	R	I	R	R
19	R	R	R	I	R	R
21	R	R	R	I	S	R
22	R	R	R	I	S	I
23	R	R	S	S	S	R
24	R	R	R	I	I	I
25	I	R	S	I	R	I
26	R	R	S	I	R	I
27	R	R	R	R	I	I
28	R	R	S	S	I	I
29	R	S	S	S	S	I

R – resistant; I – intermediate; S – sensitive; E 15 – erythromycin (15 μg); TE 30 – tetracycline (30 μg); VA 30 – vancomycin (30 μg); TEC 30 – teicoplanin (30 μg); C 30 – chloramphenicol (30 μg); CIP 5 – ciprofloxacin (5 μg)

Antibiotic resistance of *Staphylococcus aureus* strains

The antibiotic resistance of *Staphylococcus aureus* strains isolated from chicken meat samples is shown in Table 3. According to our results, it was found that *S. aereus* were resistant to erythromycin (25%), tetracycline (100%), and chloramphenicol (25%), however, they were sensitive to vancomycin, teicoplanin, and ciprofloxacin.

In addition to *S. aureus* species causing food-borne illness, another problem about *S. aureus* is the transmission of antibiotic resistant *S. aureus* by various foods (milk, sheep/goat meat, chicken products) (Gundogan *et al.* 2005).

Aerestrup *et al*. (2000) found that erythromycin resistance of *S. aureus* species from poultry was higher than that of *S. aureus* species from human. Bertollatti *et al*. (2003) found that *S. aureus* isolated from chicken possessed erythromycin

Table 3. Antibiotic resistance of *Staphylococcus aureus* strains isolated from chicken meat samples

No of Strains	E 15	TE 30	VA 30	TEC 30	C 30	CIP 5
3	I	R	S	I	R	S
5	S	R	S	S	I	I
7	R	R	S	S	I	I
30	I	R	S	I	S	S

R – resistant; I – intermediate; S – sensitive; E 15 – erythromycin (15 μg); TE 30 – tetracycline (30 μg); VA 30 – vancomycin (30 μg); TEC 30 – teicoplanin (30 μg); C 30 – chloramphenicol (30 μg); CIP 5 – ciprofloxacin (5 μg)

resistance in 10.5–12.5%. Erythromycin resistance found in our study was higher than that observed by Bertollatti *et al*. (2003). Vancomycin resistance determined in the study by Pesavento *et al*. (2007) was similar to that found in our study whereas important differences occurred in tetracycline resistance. Duman (2007) found that erythromycin, tetracycline, and chloramphenicol resistances were different from ours while vancomycin and ciprofloxacin resistances of *S. aureus* were similar to ours.

CONCLUSION

The antibiotic resistance of enterococci and staphylococci spread in the poultry products intended for human consumption is frequent for antibiotics that are important in human medicine. As a result of the widespread and inappropriate use of antibiotics, antimicrobial resistance emerges more frequently.

The results of our study showed that chicken meat also caused the spread of antibiotic resistant bacteria. Due to the rising chicken meat consumption in our country in recent years, some prevention should be taken to decrease the prevalence of resistance in the isolates from chicken meat. Production plants should be more modern and useful. More restrictive policies on the use of antibiotics in animals should be performed.

In conclusion, this study confirmed the presence of *S. aureus*, *Enterococcus* spp., CNS, especially the antibiotic-resistant strains, in chicken meat examined, indicating poor sanitary conditions during processing which may create a health risk for consumers. Plants that process chicken meat should conform to hygienic rules and should perform regulation of food safety such as the Good Agricultural Practice (ITA), Critical Control Points and Hazard Analysis (HACCP).

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