

ESBL-Producing Bacteria and MRSA Isolated from Poultry and Turkey Products Imported from Italy

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Abstract

BENINATI C., REICH F., MUSCOLINO D., GIARRATANA F., PANEBIANCO A., KLEIN G., ATANASSOVA V. (2015): **ESBL-producing bacteria and MRSA isolated from poultry and turkey products imported from Italy.** Czech J. Food Sci., 33: 97–102.

ESBL and MRSA-producing bacteria in food-producing animals may contribute to increased incidences of infection in humans. This study was carried out on 38 samples obtained from 32 chickens and 6 turkey products purchased at retail outlets of Hannover (Germany) and imported from Italy. The samples included the thigh, breast fillet, gizzard, sausage, liver, heart, and roll, and were processed for the detection of ESBL producing *E. coli* and MRSA producing *Staphylococcus aureus*. Twenty-six chicken products (68.4%) of the total of poultry products analysed proved to be positive for *E. coli* and for phenotypical detection of ESBL. Six turkey products (100%) were positive for ESBL producing *E. coli*. *Staphylococcus aureus* was found in 4 chicken products (10.52%) that resulted positive in the detection of MRSA. *Serratia* spp. were reported in 4 samples that were also positive for ESBL. Thirty-three *E. coli* isolates from the poultry and turkey products were all resistant to at least one or more of the compounds tested. The highest resistance levels were observed, notably, against ampicillin and cefotaxim.

Keywords: ESBL; MRSA; antibiotic resistance; chicken; turkey; retail products

The organisms producing Extended-spectrum β -lactamases (ESBL) and Methicillin-resistant *Staphylococcus aureus* (MRSA) are clinically relevant and have become important players among antimicrobial-resistant organisms. ESBL have directly influenced a global change in the epidemiology of β -lactamases since the early 1990s in human medicine and since 2000 in veterinary medicine (PITOUT *et al.* 2010; SMET *et al.* 2010). The term extended-spectrum indicates the ability of ESBL producers to hydrolyse a broader spectrum of β -lactam antimicrobials. They are usually inhibited by β -lactamase-inhibitors such as clavulanic acid and tazobactam, which makes a difference between ESBL- and Amp-C (amino-penicillin hydrolysing cephalosporinase)- β -lactamases producing bacteria. ESBLs have been widely reported in several Gram-negative bacteria, but

they are usually linked to the family of *Enterobacteriaceae*, including *Klebsiella* spp., *Salmonella enterica*, *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., and *E. coli* (PITOUT *et al.* 2010). The increase of ESBL producing *E. coli* among humans is worrying since their mechanism of resistance is involved in the failure of the pharmacological treatment of diseases. France, Italy, Spain, Belgium, and Poland have shown the increase of ESBL-producing bacteria in humans (COQUE *et al.* 2008). The first case of ESBL was reported in Germany in 1983 in various *Enterobacteriaceae* isolates from patients in intensive care units. These isolates produced abnormal strains which were resistant to cefotaxime and ceftazidime. Afterwards, different groups were classified of ESBLs according to their aminoacid sequences (CANTÒN *et al.* 2008). Nowadays, more than 500 different

β -lactamase enzymes are currently known, showing the same mechanism of resistance, but with different ranges of substrates and different susceptibility to the inhibitory substances (PITOUT *et al.* 2010). The presence of ESBL genes has been clearly reported in food-production animals and the food chain has been described as a possible pathway from animals to humans (LEVERSTEIN VAN HALL *et al.* 2011). ESBL producing bacteria have been recovered from livestock (swine, cattle, poultry, and turkey), from companion animals (cats, dogs, and horses), and from wild animals. The gastro-intestinal tract of animals is a reservoir for bacteria carrying β -lactamases and a potential source of human pathogens. In particular, ESBL has been described in healthy poultry, in faecal samples of broilers and also in broiler chicken caecal samples and turkey flocks (COSTA *et al.* 2009; BORTOLOAIA *et al.* 2011; RANDALL *et al.* 2011). Recently, a high prevalence (100%) was found in faecal samples of broilers in Germany, while no ESBLs producing bacteria were observed in turkey farms (FRIESE *et al.* 2013). ESBL were found in chicken products from retail outlets in Netherlands and in breast fillets (WARREN *et al.* 2008; OVERDEVEST *et al.* 2011). In 2012, some authors compared ESBL contamination of conventional and organic chicken retail meat samples showing a prevalence of 100 and 84%, respectively, of ESBL producing *E. coli* (COHEN STUART *et al.* 2011). Methicillin-resistant *Staphylococcus aureus* is resistant to all β -lactam antibiotics, penicillins, cephalosporins, carbapenems, and their derivatives. MRSA have a cosmopolitan distribution and are commonly reported in hospitals of many countries such as Europe, America, North Africa, and the Middle- and Far-East. The first detection of MRSA was reported in a hospital of the United Kingdom in 1961 (DOYLE *et al.* 2011). The earliest occurrence of MRSA in farm animals was reported by DEVRIESE and HOMMEZ (1975) and notably in bovine mastitis milk coming from Belgium. MRSA infections have been described with companion animals, with food-producing animals such as cattle, sheep, chickens, pork, veal, rabbits, and also in some wild animals (FACCIOLI-MARTINS & DE SOUZA DA CUNHA 2012; PANTOSTI 2012). Recently, in Germany, MRSA prevalence in faecal samples was of 40 and 25%, respectively, in turkey and broiler farms (FRIESE *et al.* 2013). Now the term “livestock-associated” MRSA (LA-MRSA) is often used to describe the isolates from livestock (KÖK *et al.* 2010). The detection of MRSA in food has also been documented in some

countries in different proportions: Netherlands 2.5%, Canada 6.4%, Spain 1.6%, Italy 3.8%, and Korea 2.4% (CRAGO *et al.* 2012). MRSA strains were isolated from the retail products, in chicken samples such as breast and drumsticks, in turkey as drumsticks, ground, and cutlets (HANSON *et al.* 2011; WATERS *et al.* 2011). The aim of this study is to evaluate the phenotypical prevalence of ESBL and MRSA positive bacteria and their distribution in retail chicken and turkey products. The antimicrobial resistance profiles of the isolated strains were also evaluated.

MATERIAL AND METHODS

This study was carried out on 38 samples obtained from 32 chicken (15 thighs, 5 breast fillets, 4 gizzards, 3 sausages, 2 breasts, 2 livers, and 1 heart) and 6 turkey products (2 livers, 1 gizzard, 1 breast, 1 heart, and 1 roll) purchased at retail outlets of Hannover (Germany) and imported from Italy (Table 1). The products were sampled and transported to the research laboratory under refrigerated conditions and immediately analysed. The preparation of the samples for microbiological analysis was made according to UNI EN ISO 6887-2:2004. The samples were processed for detection of *E. coli* and *Staphylococcus aureus*.

***E. coli* and ESBL detection.** *E. coli* were detected onto MacConkey agar with addition of cefotaxime (1 mg/l), MacConkey agar with the addition of ceftazidime (1 mg/l), and Brilliance ESBL agar (Oxoid, Basingstoke, UK), after incubation at 37°C for 24 hours. Some presumptive *E. coli* colonies were sub-cultured onto Muller-Hinton agar supplemented with 5% sheep blood (MHB, Oxoid), after incubation at 37°C for and, after incubation at 37°C for 24 h, were subjected to some bacterial identification methods that included Gram stain reactions, colonial morphology, oxidase test. The identification was confirmed using the API 20E system (Biomerieux, Nutrigen, Germany). Resistance to antibiotics was determined by disk diffusion on Mueller-Hinton agar according to the diffusion test guidelines (Clinical and Laboratory Standards Institute 2009). An isolate of *E. coli* ATCC 25922 was used as positive reference strain in all tests. All *E. coli* strains were screened with 6 different antimicrobial drugs: cefotaxime (30 μ g), ceftazidime (30 μ g), cefpodoxime (10 μ g), aztreonam (30 μ g), ceftriaxone (30 μ g), and cefotixim (30 μ g). Phenotypical detections of ESBL, Amp-C, and Metallo- β -Lactamases (MBL), were carried out

doi: 10.17221/428/2014-CJFS

by the standard broth microdilution method using the commercial Micronaut microtitre plates (Micronaut-s β -Lactamase VI; Merlin Diagnostika, Bornheim, Germany). The interpretative breakpoint was in accordance with the guidelines of European Committee on Antimicrobial Susceptibility Testing (www.eucast.org). Thirty-three different phenotypes were tested with an array of 14 antibiotics: sulfamethoxazole (SMX), gentamycin (GEN), ciprofloxacin (CIP), ampicillin (AMP), cefotaxime (FOT), ceftazidime (TAZ), tetracycline (TET), streptomycin (STR), trimethoprim (TMP), chloramphenicol (CHL), colistin (COL), florfenicol (FFN), kanamycin (KAN), nalidixic acid (NAL) (Sensititre, Plate Format, EUMVS2, TREK Diagnostic Systems). The results were recorded after 24 h of incubation at 35°C.

Staphylococcus aureus and MRSA detection. *Staphylococcus aureus* was detected on Brilliance MRSA Agar (Oxoid, Hampshire, UK) and was incubated at 37°C for 24 hours. Some presumptive *Staphylococcus aureus* colonies were sub-cultured onto MHB agar and then were incubated at 37°C for 24 h and subjected to some bacterial identification methods including Gram strain reactions, colonial morphology, catalase test and coagulase test. The identification method was used to confirm the Gram-positiveness with the APIstaphy system (Biomerieux, Nutrigen, Germany). The detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) was carried out by means of Micronaut-s MRSA/IFSG GP 4 (Merlin Diagnostika, Bornheim, Germany).

Table 1. Results of β -lactamase test

Samples	ESBL	<i>E. coli</i>	<i>Serratia</i>	Amp-C	MRSA
32 chicken total	26 (81.30%)	26 (81.30%)	2 (6.25%)	9 (28.13%)	4 (12.50%)
15 thigh	14 (93.33%)	14 (93.33%)	–	3 (20%)	3 (20%)
5 breast fillet	5 (100%)	5 (100%)	–	1 (20%)	–
4 stomach	3 (75%)	3 (75%)	–	3 (75%)	–
3 sausages	–	–	–	–	–
2 breast	1 (50%)	1 (50%)	–	–	1 (50%)
2 liver	2 (100%)	2 (100%)	2 (100%)	1 (50%)	–
1 heart	1 (100%)	1 (100%)	–	–	–
6 turkey total	6 (100%)	6 (100%)	2 (33.33%)	1 (16.67%)	–
2 liver	2 (100%)	2 (100%)	1 (50%)	–	–
1 stomach	1 (100%)	1 (100%)	–	–	–
1 roll	1 (100%)	1 (100%)	1 (100%)	1 (100%)	–
1 heart	1 (100%)	1 (100%)	–	–	–
1 Breast	1 (100%)	1 (100%)	–	–	–

ESBL – Extended-spectrum β -lactamases; Amp-C – amino-penicillin hydrolysing cephalosporinase; MRSA – Methicillin-resistant *Staphylococcus aureus*

RESULTS

Seventy-five strains of *E. coli*, 4 strains of *Staphylococcus aureus*, and 4 strains of *Serratia* spp. were detected. Twenty-six chicken products (68.4%) of the total poultry products analysed resulted as positive for *E. coli* and for phenotypical detection of ESBL. The results of β -lactamase test showed that 14 thighs (93.33%), 5 breast fillets (100%), 3 gizzards (75%), 2 livers (100%), 1 breast (50%), and 1 heart (100%) were positive. Also 6 turkey products (100%) were positive for ESBL producing *E. coli*. *Staphylococcus aureus* was found in 4 chicken products (10.52%) that resulted as positive in the detection of MRSA. MRSA producing *Staphylococcus aureus* were found only in chicken the products: 3 thighs (20%) and in one breast (50%). *Serratia* spp. was found in 4 samples, 2 chicken livers (100%), 1 turkey roll (100%), and 1 turkey liver (50%), and it was positive in the phenotypical detection of ESBL. No ESBL producing *E. coli* and MRSA producing *Staphylococcus aureus* were observed in poultry sausages. Nine chicken and one turkey products (28.1 and 16.7%, respectively) showed positivity in the phenotypic AMP-C detection (Table 1). Thirty-three *E. coli* isolates from the poultry and turkey products were all resistant to at least one or more of the compounds tested. The highest resistance levels were observed against ampicillin 100% and cefotaxim 94%. In addition to this, most ESBL producers were resistant to non- β -lactams,

such as tetracycline 85%, chloramphenicol 73%, trimethoprim 45%, fluoroquinolones (ciprofloxacin 39%), colistin 15%, aminoglycosides (gentamycin 3%). The resistance rate to ceftazidime was only 30% in ESBL-producing *E. coli*, with an MIC₅₀ and MIC₉₀ of 2 mg/ml and 32 mg/ml, respectively, in

the chicken products, while MIC₅₀ and MIC₉₀ in the turkey products was of 1 mg/ml and 8 mg/ml. No isolate was resistant to a single antibiotic while all isolates showed multidrug resistance with a range from two to six different antimicrobials. Several phenotypes among the 33 antibiotic-resistant *E. coli* isolates are presented in Table 2.

Table 2. Characteristics of ESBL-producing isolates recovered from chicken and poultry products

Isolate	Origin	Antimicrobial associated resistances
<i>E. coli</i>	chicken liver	AMP, TET
<i>Serratia</i>	chicken liver	AMP, COL
<i>E. coli</i>	chicken gizzard	CIP, AMP, CHL
<i>E. coli</i>	chicken liver	AMP, TET, CHL
<i>E. coli</i>	chicken heart	CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	chicken heart	CIP, AMP, TET, TMP, CHL, COL
<i>E. coli</i>	chicken breast	AMP, TET, CHL
<i>E. coli</i>	chicken thigh	CIP, AMP, CHL
<i>E. coli</i>	chicken thigh	AMP, TET, CHL
<i>E. coli</i>	chicken thigh	AMP, TET
<i>E. coli</i>	chicken thigh	CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	chicken thigh	CIP, AMP, TET, TMP
<i>E. coli</i>	chicken breast fillet	CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	chicken breast fillet	CIP, AMP, TET, CHL
<i>E. coli</i>	turkey liver	CIP, AMP, TET
<i>E. coli</i>	turkey gizzard	AMP, TET, TMP, CHL
<i>Serratia</i>	turkey roll	AMP, TET, CHL, COL
<i>E. coli</i>	turkey heart	AMP, TET, COL
<i>E. coli</i>	turkey heart	AMP, TET, CHL
<i>E. coli</i>	chicken thigh	AMP, TET, CHL
<i>E. coli</i>	chicken thigh	AMP, TET, CHL
<i>E. coli</i>	chicken thigh	CIP, AMP, TET, CHL, COL
<i>E. coli</i>	chicken thigh	AMP, TET, TMP, CHL
<i>E. coli</i>	chicken thigh	CIP, AMP, TMP, CHL
<i>E. coli</i>	chicken thigh	CIP, AMP, TET, TMP
<i>E. coli</i>	chicken breast fillet	AMP, TET, TMP, CHL
<i>E. coli</i>	chicken breast fillet	CIP, AMP, TET, CHL
<i>E. coli</i>	chicken breast fillet	CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	turkey liver	CIP, AMP, TMP, CHL
<i>E. coli</i>	chicken stomach	CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	chicken stomach	AMP, TET
<i>E. coli</i>	turkey breast	GEN, CIP, AMP, TET, TMP, CHL
<i>E. coli</i>	turkey breast	AMP, TET

GEN – gentamicin; CIP – ciprofloxacin; TET – tetracycline; CHL – chloramphenicol; AMP – ampicillin; TMP – trimethoprim; COL – colistin

DISCUSSION

The findings obtained in this study show the high prevalence of ESBL-producing *E. coli* (84.2%) in retail poultry products sold in Germany and imported from Italy. The prevalence of ESBL producing bacteria in our study was similar to the rate found recently in Netherlands (100% for the conventional and 84% for organic samples respectively), while a lower prevalence was observed in Germany (43.9%) with retail chicken meat (COHEN STUART *et al.* 2012; KOLA *et al.* 2012). In this regard, ESBL contamination might occur during the rearing process, slaughtering or finally, through the environment (COHEN STUART *et al.* 2012). In fact, GREGOVA (2012) showed that the occurrence of ESBLs in poultry meat could be related to the environmental microbes of the slaughterhouse, to the processes of scalding, defeathering, and evisceration, and to that the bacteria can be transferred from chickens because of the contact through water and in correct cleaning and disinfecting. Furthermore, the meat products are cooked and then the colonisation in humans by antibiotic-resistant *E. coli* takes place in the course of preparing and eating cooked products at home.

In our survey, the proportion of MRSA was 12.5%, while, recently, a higher rate (37.2%) was reported in food products of poultry origin in Germany (FESSLER *et al.* 2011).

However, the presence of MRSA in food does not constitute a risk for humans to become healthy carriers or being infected after eating or handling food as reported by BIOHAZ (www.efsa.europa.eu). They also considered that a great risk factor for humans is the direct contact with live animals; therefore farmers, abattoir workers, veterinarians and their families are the people with a higher risk of infection.

Further studies are necessary to identify the sources of contamination and to clarify their dissemination on the farms and along the food chain.

The low prevalence of *Serratia* spp. (10.52%) in the retail samples is unlikely to be the result of animal

doi: 10.17221/428/2014-CJFS

contamination, coming rather from soil, water, and plants (SCHWAIGER *et al.* 2012).

Penicillins, the first- to fourth-generations cephalosporins and the β -lactamase inhibitors are an important group of antimicrobial agents used for the treatment of disease, in veterinary medicine, and in food animal production. These antibiotics are often used for prophylaxis, and have been used as growth promoters for more years. Food animals are often treated by the addition of drugs to water or feed. Their use in sub-therapeutic dosing for a long period of time can cause the development of antimicrobials resistant strains. The observation that all strains isolated were resistant to at least one or more of the compounds tested is alarming. We found the highest proportions of resistance to ampicillin (100%) followed by cefotaxime (94%), and being not much lower with tetracycline (85%), and chloramphenicol (73%). Notably penicillin and cephalosporin are the drugs of choice in the avian medicine in many European countries. The intensive use of these antimicrobial agents determined frequently the contamination of food animal production with antimicrobial-resistant *E. coli* strains. The use of antibiotic such as aminoglycosides, fluoroquinolones, and third- and fourth-generation cephalosporins may lead to the treatment failure and can have serious consequences for the human patient.

ESBL and MRSA producing microorganisms represent a significant public health problem in several countries. Further research and the implementation of infection control measures in veterinary hospitals and clinics, as well as in farms and parks, are necessary to reduce the spread of these resistant bacteria.

Acknowledgements. We would like to dedicate this paper to the loving memory of Dear Prof. Dr. Atanasova that made it possible to take part in research on antimicrobial resistance, in which we were able to learn laboratory techniques specifics.

We would also like to thank the Director of Institut für Lebensmittelqualität und -sicherheit, Stiftung Tierärztliche of Hannover Prof. Dr. Günter Klein for kindly given the availability for this partnership.

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Received: 2014–07–31

Accepted after corrections: 2014–11–10

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