Antimicrobial Resistance of Shiga Toxin-Producing *Escherichia coli* Causing Human Illness

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Abstract

Escherichia coli is usually a commensal bacterium of humans and animals. Pathogenic variants cause intestinal and extra intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis and septicemia. Nowadays, the worldwide concern on the spread of this bacterium is mainly connected to the resistance phenomenon. This research was conducted in order to reveal antimicrobial resistance patterns in *Escherichia coli*, causing human illness.

A total of 30 *Escherichia coli* isolates were recovered from patients hospitalized with food-borne infections, during June 2013-May 2014. The majority of the patients declared the consumption of undercooked meat products (especially beef), raw milk and soft cheeses. The isolates were tested through polymerase chain reaction (PCR) for identification and confirmation of pathogenicity and further, for antimicrobial susceptibility to 26 antimicrobials, using the classical disk diffusion method.

All isolates were Shiga Toxin-producing *Escherichia coli* (STEC). All isolates proved to be resistant to at least one antimicrobial agent. Resistance was most frequently observed to ampicillin, amoxicillin and clavulanic acid, ceftazidime, cefepime and norfloxacin.

Our findings demonstrate that Shiga Toxin-producing *Escherichia coli* recovered from hospitalized patients and presumably of animal origin is highly resistant to critically important antimicrobials for human medicine. These data support the ongoing concern regarding antimicrobial resistance, antimicrobial use in veterinary and human medicine and nevertheless, the problem of antimicrobial resistance in the food chain.

Keywords: *antimicrobial resistance, Escherichia coli, food safety, human illness;*

INTRODUCTION

Escherichia coli (*E.coli*) are the most prevalent bacteria in animals and humans and are also an important zoonotic agent, which can be implicated in animal and human infectious diseases (Costa et al., 2008). In humans, *E.coli* can cause a variety of intestinal and extra-intestinal infections, such as diarrhea, urinary tract infection, meningitis, peritonitis, septicemia and gram-negative bacterial pneumonia (Hammerum and Heuer, 2013).

Based on virulence properties, enteric *E.coli* are classified into enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC),

verotoxigenic or Shiga toxin-producing *E.coli* (VTEC or STEC), enterohemorrhagic (EHEC) and enteroaggregative (EAEC), all being causative agents of diarrhea.

STEC represents a major cause of gastroenteritis that may be complicated by haemorrhagic colitis, or the hemolytic-uremic syndrome, which is the main cause of acute renal failure in children (Mora et al., 2005).

E.coli 0157:H7, which belongs to STEC group is the most common serotype isolated from individuals with haemorrhagic colitis. Outbreaks of infection with those bacteria have emerged

due to the consumption of contaminated animal-derived food products (Belongia *et al.*, 1991).

Contaminated food, especially undercooked ground beef, unpasteurized (raw) milk, soft cheeses made of raw milk and even raw fruits and vegetables (such as sprouts) are considered important sources of *E.coli* infection.

Meat and meat products can be easily contaminated by *E.coli* during animal evisceration after slaughter, through contact with tainted water or during meat handling (Alvarez-Fernandez *et al.*, 2013).

Antimicrobial agents are widely used not only in human medicine but also in food animal production; therefore, meat is frequently contaminated with antimicrobial resistant bacteria, including *E.coli*.

Humans can be colonized with *E.coli* of animal origins, and because of resistance to commonly used antimicrobial agents, these bacteria may cause infections for which limited therapeutic options are available (Hammerum and Heuer, 2013).

Furthermore, foods contaminated with antibiotic resistant bacteria could be a major threat to public health, as there is the distinct possibility that genes encoding antibiotic resistance determinants that are carried on mobile genetic elements may be transferred to other bacteria of human clinical significance (Van *et al.*, 2008).

In Romania, like in many other developing countries, antimicrobial resistance epidemiology is still in development and little is known about the potential role of contaminated food (especially meat and meat products) in the dissemination of multidrug-resistant *E.coli*, because very limited research works had been carried out in our country concerning these aspects.

This study was conducted in order to reveal antimicrobial resistance patterns of *E.coli* of presumably animal origin, causing human illness.

MATERIALS AND METHODS

A total of 30 *E.coli* isolates recovered from patients hospitalized with food-borne infections at the Hospital of Infectious Diseases from Cluj-Napoca, between June 2013-May 2014 were examined. The strains were isolated mainly from patients with diarrhea. The majority of the patients declared the consumption of undercooked meat

products (especially beef), raw milk and soft cheeses.

1. Detection of Shiga toxin-producing genes

The isolates were tested through polymerase chain reaction (PCR) with primers specific for the genes encoding Stx1 and Stx2 toxins (stx1 and stx2 genes).

1.1 DNA extraction

The DNA extraction protocol followed the steps previously reported by Lapusan (2012). Briefly, 2-3 presumptive *E.coli* colonies were chosen and immersed in 150 µl Chelex solution (Germany). The Eppendorf tubes were afterwards subjected to high-degree temperatures for cell membrane lysis, releasing the DNA, which was quantified on a Nanodrop ND-1000 spectrophotometer analyzer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

1.2 The PCR method

The PCR reaction mix (25 µl) comprised: 1X PCR green Buffer, 2.5 mM MgCl₂, 5 pmol of each primer, dNTPs each at 200 µM, 2.5 U of Taq DNA Polymerase (Promega, Madison, WI, USA) and 100 ng of genomic DNA. PCR was performed under the following conditions: 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 60°C for 30sec, 72°C for 1 min and a final extension step of 72°C for 7 min.

The primers used for the detection of Shiga toxin-producing genes were previously reported by Tahamtan *et al.* (2010).

2. Antibiotic susceptibility tests

A number of 26 antimicrobials, some commonly used in veterinary medicine and some mainly employed in humans, were tested on the *E.coli* isolates, using the classical disk diffusion method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006). The isolates were tested against ampicillin, cefotaxime, ceftazidime, ciprofloxacin, nalidixic acid, tetracycline, trimethoprim/sulfamethoxazole, amoxicillin and clavulanic acid, chloramphenicol, gentamicin, piperacillin, cefazolin, ceftiofur, cefepime, amikacin, norfloxacin, nitrofurantoin, cefuroxime, tobramycin, cefixime, cefoperazone, imipenem, meropenem, entopenem, levofloxacin and fosfomycin. The CLSI breakpoints were applied for the interpretation of the results.

RESULTS AND DISCUSSIONS

A number of 30 *E.coli* isolates recovered from patients hospitalized with food-borne infections at the Hospital of Infectious Diseases from Cluj-Napoca, were tested for Shiga toxin-producing genes through PCR and also for antimicrobial susceptibility to 26 antimicrobial agents, some commonly used in veterinary medicine and some mainly employed in humans, by using the classical disk diffusion method.

All isolates included in this study carried Shiga toxin-producing genes. PCR showed that 8 isolates (26.66%) carried *stx1* genes, 16 (53.33%) carried *stx2* genes, while 6 (20%) isolates possessed both *stx1* and *stx2* genes.

The analyzed *E.coli* isolates proved to be highly resistant to antimicrobials. All isolates showed resistance to more than one antimicrobial. Overall, resistance was frequently observed to ampicillin (60.66%), nalidixic acid (56.66%) and norfloxacin (56.66%). The *E.coli* isolates included in the study also displayed resistance to ciprofloxacin, tetracycline, gentamicin, cefuroxime, cefotaxime, ceftazidime, cefixime, nitrofurantoin, trimethoprim/sulfamethoxazole and piperacillin. A lower resistance rate was observed to cefoxitin, amikacin and chloramphenicol. All isolates proved to be susceptible to imipenem, meropenem and entopenem (Tab. 1).

The emergence and dissemination of antimicrobial resistance among Shiga toxin-producing *E.coli* may have potential negative clinical implications.

The World Health Organization (WHO) published a report regarding the critically important antimicrobials for human medicine. According to the report, third and fourth generation cephalosporins, as well as quinolones and aminoglycosides are considered to be critically important antimicrobials in the treatment of human *E.coli* infections (World Health Organization, 2007). Moreover, in the case of *E.coli*, first and second generation cephalosporins as well as sulfonamides are regarded as highly important antimicrobials for the treatment of *E.coli* infections in humans. According to our study a high percentage of the isolates proved to be highly resistant to third and fourth generation cephalosporins (cefotaxime, ceftazidime, cefepime, cefixime, cefoperazone) and also to quinolo-

Tab. 1. Percentage of *E.coli* isolates resistant to different antimicrobials

Antimicrobials	Percentage of resistance
Ampicillin	60.66
Cefotaxime	33.33
Ceftazidime	33.33
Ciprofloxacin	40
Nalidixic acid	56.66
Tetracycline	40
Trimethoprim/sulfamethoxazole	33.33
Amoxicillin and clavulanic acid	50
Chloramphenicol	6.66
Gentamicin	40
Piperacillin	33.33
Cefazolin	13.33
Cefoxitin	6.66
Cefepime	23.33
Amikacin	6.66
Norfloxacin	56.66
Nitrofurantoin	16.66
Cefuroxime	40
Tobramycin	23.33
Cefixime	33.33
Cefoperazone	23.33
Imipenem	0
Meropenem	0
Entopenem	0
Levofloxacin	23.3
Fosfomycin	23.33

nes (nalidixic acid) and fluoroquinolones (ciprofloxacin, norfloxacin, levofloxacin).

Fluoroquinolones are critically important for treating serious infections in humans (Van et al., 2008). Treatment of serious human enteric infections with an effective fluoroquinolone can reduce the duration of illness, and most likely prevent complications and adverse outcomes including hospitalization (FDA, 2000). Therefore, resistance to fluoroquinolones can be considered an important public health concern.

A high resistance rate was observed to gentamicine, an aminoglycoside which is also

included in the list of critically important antimicrobials for human therapy. In humans, gentamicin is used in association with β -lactams, for the treatment of severe infections like sepsis or endocarditis.

The highest resistance rate was observed to ampicillin, an antibiotic frequently administered in both human and veterinary medicine.

Even if the origin of *E.coli* that caused the infections has not been properly investigated, it was assumed that it must have been an alimentary one, given the fact that the majority of the patients declared the consumption of undercooked meat products (especially beef), raw milk or soft cheeses.

In many cases, the origin of *E.coli* that cause infection in humans remains unknown, and the significance of the animal reservoir of the antimicrobial resistant *E.coli* has not been quantified. However, treatment options in humans are compromised if the causative bacteria are already resistant to commonly used antimicrobial agents (Hammerum and Heuer, 2013).

Moreover, it is a well known fact that foods contaminated with antibiotic resistant bacteria could be a major threat to public health, as there is the distinct possibility that genes encoding antibiotic resistance determinants that are carried on mobile genetic elements may be transferred to other bacteria of human clinical significance (Van et al., 2008). *E.coli* is a candidate vehicle for such transfer because of its diversity and also because it survives as common flora in the gastrointestinal tracts of both humans and animals. They are sensitive to selection pressure exerted by antibiotic usage and carry genetic mobile elements to achieve such transmission (Van den Bogaard et al., 2000).

The presence of pathogenic and non-pathogenic *E.coli* isolated from food and food products and resistant to different antimicrobials has been previously reported by many authors (Van et al., 2008; Saenz et al., 2004; Pavithra et al., 2013; Sala et al., 2012; Karadal et al., 2013; Hosseini et al., 2013; Colobatiu et al., 2014).

In a previous study we've analyzed *E.coli* isolations for antimicrobial resistance genes. The results of the study proved that *E.coli* isolates recovered from raw poultry meat are highly resistant to antimicrobials and carry antimicrobial resistance genes that could be transferred to other microbes in the food chain, even important human pathogens (Colobatiu et al., 2014).

The isolates included in our study proved to be resistant to a large number of antimicrobial agents, some of them being commonly used in the prophylaxis or treatment of infections in human as well as in veterinary medicine. The widespread non-human use of antimicrobial agents, some of them regarded as critically or highly important for human therapy, creates a reservoir of resistant bacteria or resistance genes that may be transferred to humans, adding to the burden of antimicrobial resistance in human medicine (Hammerum and Heuer, 2013).

CONCLUSIONS

This study was conducted in order to reveal antimicrobial resistance patterns of *E.coli* of presumably animal origin, causing human illness.

Our findings demonstrate that Shiga Toxin-producing *E.coli*, presumably of animal origin and recovered from hospitalized patients, is highly resistant to critically important antimicrobials for human medicine. These data support the ongoing concern regarding antimicrobial resistance, antimicrobial use in veterinary and human medicine and nevertheless, the problem of antimicrobial resistance in the food chain.

Our findings provide a baseline data for further epidemiological studies which lack in information regarding our country.

We underline the necessity of the implementation of a national antimicrobial resistance monitoring programme.

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