

Full Length Research Paper

Antimicrobial resistance of *Escherichia coli* O157:H7/NM isolated from feces of ruminant animals in Iran

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Bacterial resistance to antimicrobial agents is a phenomenon problem, and the understanding of resistance acquisition and transmission can contribute to the development of new strategies to the clinical and socio-economic implication of this phenomenon, especially in ruminant animals. A total of 327 fecal samples of ruminant animals were examined for *E. coli* O157:H7/NM and the antibiotic susceptibility testing was determined by disc diffusion according to the Clinical Laboratory Institute. Isolates were tested for susceptibility to antimicrobial drugs by the Kirby-Bauer disc diffusion method using Müellar-Hinton agar according to the Clinical Laboratory Standards Institute. Twenty-five (7.6%) *Escherichia coli* O157:H7/NH were isolated, 24(96%) were resistant to one or more antibiotic agent. Six (24%) *E. coli* were resistant to one antibiotic agent, 11(44%) to two agents and 28% exhibited multi drug resistance. Gentamycin resistance phenotype was the most common (56.0%), followed by ampicillin (48.0%), erythromycin (40.0%), amoxicillin (16.0%), tetracycline (12.0%), chloramphenicol (8.0%) nalidixic acid (8.0%), and streptomycin (4.0%) and all *E. coli* O157 isolates were susceptible to cefuroxime. Considering the clinical implication of *E. coli* in veterinary medicine, surveillance of the bacterial pathogens became imperative for better understanding and infection control approach.

Key words: *Escherichia coli* O 157, ruminant feces, antibiotic resistance, Iran.

INTRODUCTION

Antibiotics have saved millions of human lives, and their use has contributed significantly to improving human and animal health and well-being. Use of antibiotics in food-producing animals has resulted in healthier, more productive animals; lower disease incidence and reduced morbidity and mortality in humans and animals; and production of abundant quantities of nutritious, high-quality, and low-cost food for human consumption. In spite of these benefits, there is considerable concern from public health, food safety, and regulatory perspectives

about the use of antimicrobials in food-producing animals. Over the last two decades, development of antimicrobial resistance resulting from agricultural use of antibiotics that could impact on the treatment of diseases affecting the human population that require antibiotic intervention has become a significant global public health concern (Oliver et al., 2011). *Escherichia coli* O157:H7/NM is one of the commonest bacterial cause of infective gastroenteritis in the developed world, and frequently causes food borne illness (Rey et al., 2006; Ferens and Hovde, 2011). Cattle is known as a natural reservoir of VTEC organisms (Meng and Doyle, 1998; Farah et al., 2007; Oliveira et al., 2008), including gastrointestinal tract of pigs, chickens, sheep, and other

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ruminants (Abong'o and Mombo, 2009; Franco et al., 2009; Goncuoglu et al., 2010).

Previous studies have shown that the gallbladder may be a site acquisition of resistance and a source for fecal shedding of *E. coli* O157:H7 (Stoffregen et al., 2004; Jeong et al., 2007). During the processing of the carcasses, fecal contamination or transfer of bacteria from the animal's hide to the carcass can facilitate transmission of pathogenic *E. coli* to the meat (Elder et al., 2000; Ferens and Hovde, 2011). Several studies have suggested that foods might be a source of human-acquired antimicrobial-resistant *E. coli*. The food supply is an established vehicle for certain other antimicrobial-resistant and/or pathogenic bacteria—notably, *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *E. coli* O157:H7 (Tauxe, 1997; Mohle-Boetani et al., 2001; Mead et al., 1999; Lanz et al., 2003; Oliver et al., 2011). Meat and poultry products at slaughtering operations can be extensively contaminated with *E. coli* of animal origin, including strains that express extraintestinal pathogenic *E. coli*-associated O antigens, *Campylobacter* spp. and/or are antimicrobial resistant (Linton et al., 1977; Ngwai et al., 2010).

Antimicrobial resistance has emerged in the past few years as a major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are aimed mainly at human pathogens, agents of zoonoses, and indicator bacteria of the normal intestinal flora from animals (Lanz et al., 2003). However, little attention has been paid to the resistance in specific animal pathogens (Lanz et al., 2003). Limited studies on the ecology of *E. coli* O157:H7/NM have been reported, particularly from developing countries and to the author knowledge, the occurrence and antimicrobial resistance of *E. coli* O157:H7/NM in animals in Iran has not been reported. Therefore, the present study was conducted to determine the antimicrobial resistance of *E. coli* O157:H7/NM isolated from feces collected from buffalo, camel, cattle, sheep, and goats slaughtered for meat production.

MATERIALS AND METHODS

Source of isolates

From August 2010 to February 2011, overall 327 fecal samples were collected from healthy water buffalo, camel, cattle, sheep, and goats at slaughter houses in Isfahan, Chaharmahal and Bakhtyari, and Khuzestan Provinces, Iran. The animals included in this study were randomly selected. The fecal samples were collected according to the method described previously (Islam et al., 2008). Briefly, immediately after the animals were slaughtered, a piece of large intestine (~5 cm) containing fecal material, 1 to 1.5 cm away from the rectum, was excised aseptically. The samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and immediately transported to the Microbiology

Laboratory in a cooler with ice packs.

Microbiological analyses

Five grams (5 g) of the fecal samples were inoculated into 5 ml of buffered peptone water and the cultures incubated at 37°C for 24 h. Cultures were streaked onto Mac Conkey sorbitol agar and the plates incubated at 37°C for 24 h. All sorbitol negative colonies were confirmed as *E. coli* O157:H7 with PCR assay by using the O-antigen encoding region of O157 gene (Paton and Paton, 1998) and flagellar H7 gene (*fli C*) generic primers as described previously (Gannon et al., 1997). A total of 25 *E. coli* O157:H7/NM colonies were recovered and kept on slant agar at room temperature. Antibiotic susceptibility testing was determined by disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2006).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Müeller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute (CLSI, 2006). The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 µg), cefuroxime (30 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), amoxicillin (30 µg), ampicillin (10 µg), and chloramphenicol (30 µg). After incubation at 37°C for 48 h the susceptibility of the *E. coli* O157 to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006).

RESULTS

Of the 327 fecal samples examined, 25 (7.6%) yielded positive growth for *E. coli* O157:H7/NM. The resistance pattern of *E. coli* O157:H7/NM isolates to 9 antimicrobial agents tested in this study is shown in Table 1. 24 (96%) of *E. coli* O157:H7/NM exhibited to one or more antibiotics tested, 5(24%) to only antibiotic, 11(44%) to 2 antibiotics and 28% showed a multiresistant pattern. Gentamycin resistance phenotype was the most common (56.0%), followed by ampicillin (48.0%) (48.0%), erythromycin (40.0%), amoxicillin (16.0%), tetracycline (12.0%), chloramphenicol (8.0%), nalidixic acid (8.0%), and streptomycin (4.0%) and all the isolates were susceptible to cefuroxime.

DISCUSSION

E. coli O157 is the most common bacterial etiologic agent of diarrhoea in humans. In the present study, the sensitivity of 25 *E. coli* isolates to 9 antimicrobials was tested. Several researchers have assayed for antibiotic resistance in *E. coli* O157 and *E. coli* O157:H7 isolated

Table 1. Antimicrobial resistance profiles of *Escherichia coli* O157:H7/NM.

Antimicrobial agent	<i>E. coli</i> O157:H7/NM (N = 25) (%)	<i>E. coli</i> O157:H7 (N = 7) (%)	<i>E. coli</i> O157: NM (N = 18) (%)
Amoxicillin	4 (16.0)	1 (14.3)	3 (16.7)
Ampicillin	12 (48.0)	3 (42.9)	9 (50.0)
Cefuroxime	0 (0.0)	0 (0.0)	0 (0.0)
Chloramphenicol	2 (8.0)	2 (28.6)	0 (0.0)
Erythromycin	10 (40.0)	2 (28.6)	8 (44.4)
Gentamicin	14 (56.0)	5 (71.4)	9 (50.0)
Nalidixic acid	2 (8.0)	1 (14.3)	1 (5.6)
Streptomycin	1 (4.0)	0 (0.0)	1 (5.6)
Tetracycline	3 (12.0)	1 (14.3)	2 (11.1)
Total			
Resistance to 1 antimicrobial	6 (24.0)	2 (28.6)	4 (22.2)
Resistance to 2 antimicrobials	11 (44.0)	3 (42.9)	9 (50.0)
Resistance to > 2 antimicrobials	7 (28.0)	2 (28.6)	5 (27.8)

from feces, milk, meat, dairy products and meat products and the reported antimicrobial resistance profiles seem to be study-specific without a clear or emerging general trend (Picozzi et al., 2005; Caro et al., 2006; Sawant et al., 2007; Solomakos et al., 2009; Oliver et al., 2011). The *E. coli* isolates exhibited resistance to ampicillin (48%), ceftiofur (11%), chloramphenicol (20%), florfenicol (78%), spectinomycin (18%), and tetracycline (93%). Multiresistance (resistance to 3 to 6 antibiotics) was seen in 40% of isolates. Findings from the study by Sawant et al. (2007) suggest that commensal enteric *E. coli* from healthy lactating cattle could be an important reservoir for tetracycline and perhaps other antimicrobial resistance determinants.

Meng et al. (1998) among 125 isolates from animals, food and humans, found that 24% were resistant to at least one antibiotic and 19% were resistant to three or more antibiotics. Antimicrobial susceptibility of STEC isolated from organic and conventional dairy farms was investigated (Cho et al., 2007). Resistance to at least one antimicrobial agent was observed in 62 and 48% of isolates from conventional and organic farms, respectively. All 23 isolates from organic farms were sensitive to chloramphenicol, gentamicin, and trimethoprim/sulfamethoxazole. A significant difference was observed in the proportion of STEC that were resistant to spectinomycin (72.4% conventional vs. 39.1% organic farm sources; $p < 0.05$). Variation in the prevalence of *E. coli* O157 isolates from fecal samples reported in other studies may be a result of different sampling techniques employed, seasonal effects (Ferens and Hovde, 2011) and/or laboratory methodologies employed in different studies (bacteriological and biochemical testing vs.

polymerase chain reaction assays).

The results of antimicrobial susceptibility testing in the present study indicate that there is a high resistance of *E. coli* O157 to ampicillin, gentamicin, and erythromycin. These results are comparable to those reported by other investigators (Picozzi et al., 2005; Caro et al., 2007; Cizek et al., 2007; Abong'o and Momba, 2009; Ngwai et al., 2010). *E. coli* O157 isolates displaying resistance to multiple antimicrobials have been also previously reported (Giammanco et al., 2002; Duffy et al., 2006; Walsh et al., 2006; Solomakos et al., 2009). The results of antimicrobial resistance found in this study are correlated to antibiotics that are being used to treat infection in food animals in Iran. Also, a high percentage of *E. coli* O157 isolates were found to be resistant to ampicillin, an antibiotic used in human medicine for the treatment of coliform infections. Due to the high number of antimicrobial-resistant isolates, we recommend that *in vitro* antimicrobial susceptibility testing of *E. coli* be performed and appropriate treatment be instituted especially for those cases of food borne *E. coli* with severe or prolonged symptoms or in immunocompromised patients.

Antibiotic treatment of *E. coli* O157:H7/NM-infected patients are considered controversial. Experts have argued against such treatment because the administration of certain antimicrobial agents such as quinolone antibiotics in *E. coli* O157:H7/NM-infected patients have been shown to increase the risk for hemolytic uremic syndrome development (Wong et al., 2000; Serna and Boedeker, 2008). Monitoring of antibiotic resistance in *E. coli* O157:H7/NM isolates, however, is still useful for both epidemiological purposes and for the monitoring of the spread of antibiotic resistance among different microbial

species. Continuous studies for the surveillance of the antibiotic resistance of *E. coli* O157:H7/NM is expected as future research.

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