Journal of Animal and Veterinary Advances 11 (1): 52-55, 2012
ISSN: 1680-5593

Antibacterial Resistance of Commensal *E. coli* and *E. coli* O157:H7 Strains Isolated from Cattle and Calves Faeces Samples

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Abstract: This study investigated the occurrence and antimicrobial resistance profiles of *E. coli* and *E. coli* O157:H7 isolated from cattle and calves faeces samples in Afyonkarahisar. A total of 45 *E. coli* and 14 *E. coli* O157:H7 species was isolated. All the *E. coli* isolates were found to be resistant to the penicillin. However, *E. coli* O157:H7 species were found to be resistant to the penicillin, ertiyromisin and neomycin. Most of *E. coli* isolates were found to be resistant to the eritromisin (97.7%), neomycin (95.6%), tetracyclin (73.3%) and ampicillin (60.0%). The tetracyclin to the considerably resistance were seen in both pathogens although, ampicillin resistance was relatively higher in *E. coli* than in *E. coli* O157:H7 species. This study observed that cephalothin and cefoxitim resistances were found to highly different the *E. coli* and *E. coli* O157:H7 strains. In addition the lowest frequency of resistance was seen against both cefoxitim and ciprofloxacin (7.2%).

Key words: Cattle, faeces, *E. coli*, *E. coli* O157:H7, antimicrobial resistance, Turkey

INTRODUCTION

Escherichia coli is commonly found in human and animal intestinal tracts and as a result of fecal contamination or contamination during food animal slaughter is often found in soil, water and foods (Schroeder *et al*., 2002). Shiga Toxin-producing *E. coli* (STEC) O157 has emerged as a public health threat following its initial identification as a pathogen in a 1982 outbreak of illness associated with the consumption of undercooked ground beef (Riley *et al*., 1983). Particularly, *E. coli* O157:H7 are known as major etiologic agents in Hemorrhagic Colitis (HC) and Hemolytic-Uremic Syndrome (HUS) in humans (Thielman and Guerrant, 1999). Recent reports showed that antimicrobial resistance of *E. coli* O157 is on the increase (Meng *et al*., 1998; Schmidt *et al*., 1998; Aarestrup and Wegener, 1999; Zhao *et al*., 2001; You *et al*., 2006).

Recent studies have also revealed that *E. coli* which forms part of the normal intestinal flora of humans and animals is capable of inhibiting the growth of other toxigenic strains of *E. coli* which is often associated with food-borne diseases in humans (American Society for Microbiology, 2009). In addition some strains of *E. coli* occasionally emerge as pathogens due to presence of certain pathogenic features and virulence genes which are located on mobile genetic elements and this distinguish them from ordinary commensal strains (Ronsengren *et al*., 2009). Resistance to cephalosporins is mostly coded by plasmids which code for enzymes that inactivate cephalosporins. Normally resistance to flouroquinolones is mediated by chromosomes but recent studies have confirmed that such resistance could also be carried by resistance plasmids (Smith *et al*., 2003).

The objectives of this study were to determine of antimicrobial resistance in commensal *E. coli* and *E. coli* O157:H7 isolates from cattle and calves faeces samples.

MATERIALS AND METHODS

Identification of *E. coli*: A total of 78 faeces samples were collected from cattle in Afyonkarahisar, Turkey. The rectal faeces samples were collected from individual cattle and stored in disposable sterile plastic faeces sample container. Separate rectal gloves were used for each animal to avoid cross contamination. Samples were then placed in an ice-pack container and immediately transported to the laboratory.

Streaked for isolation of *E. coli* directly on McConkey Agar (Oxoid Basingstoke, Hampshire, UK) and incubated for 24 h at 37°C. Two lactose-positive colonies of different colony morphology were collected and restreaked onto McConkey Agar and incubated for 24 h at 37°C. Biochemical tests were performed on all

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isolates according to the Holt *et al.* (1994) and also *Escherichia coli* was identified as oxidase negative, indole positive, Simmons citrate negative, urease negative and hydrogen sulfide negative.

***E. coli* O157:H7 strains:** A total of 14 *E. coli* O157:H7 strains were used in this study which were previously isolated from cattle and calves faeces samples (n:457) in Afyonkarahisar (Kuyucuoglu *et al.*, 2001). Previously characterized EHEC O157:H7 strain EDL 933 (Wells *et al.*, 1983) was used as positive cotrol strain.

Antimicrobial susceptibility testing: *In vitro* antimicrobial susceptibility testing was conducted using the disk diffusion method on Mueller Hinton Agar according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

The antimicrobial susceptibility of *E. coli* and *E. coli* O157:H7 species was tested with penicillin, ampicillin, neomycin, chloramphenicol, eritromisin, tetracycline, cephalothin, cefoxitim, ciprofloxacin and trimethoprim-sulfamethoxazole (Oxoid Ltd, Basingstoke, UK).

Statistical analysis: Chi-square (χ^2) tests were used to determine the relationship between the *E. coli* and *E. coli* O157:H7 species isolated and the antibiotic resistance. The significance level was defined at $p<0.05$.

RESULTS AND DISCUSSION

A total of 45 *E. coli* and 14 *E. coli* O157:H7 species was isolated. The frequency of antimicrobial resistance profiles of all strains to the ten antibiotics is shown in Table 1. All the *E. coli* isolates were found to be resistant to the penicillin. However, all of the *E. coli* O157:H7 species were resistant to the penicillin, ertiyromisin and neomycin ($p<0.05$). Most of *E. coli* isolates were shown resistant to the eritromisin (97.7%), neomycin (95.6%), tetracyclin (73.3%) and ampicillin (60.0%). In this study revealed that *E. coli* and *E. coli* O157:H7 were considerably resistant to tetracyclin. In addition, ampicillin resistance was relatively higher in *E. coli* than in *E. coli* O157:H7 species.

Intensive antimicrobial use during animal production results in the emergence of antimicrobial-resistant bacteria (Witte, 1998; Sunde *et al.*, 1998). Kozak *et al.* (2009) reported that antibiotic resistance among bacteria especially *E. coli* isolated from cattle and other animals is increasing at an alarming rate. Ajayi *et al.* (2011) also showed that *E. coli* recovered from cattle show high prevalence of antibiotic resistance. This study has supported that *E. coli* and *E. coli* O157:H7 obtained from cattle show high prevalence of antibiotic resistance.

Sharma *et al.* (2008) reported that some commensal strains of *E. coli* may be intrinsically resistant to selected antibiotics, the exposure of *E. coli* to various combinations of antibiotics further increased the prevalence of resistant bacteria in cattle Parveen *et al.* (2005) observed that *E. coli* isolated from the manure of cattle had high levels of resistance to commonly used antibiotics such as ampicillin, erythromycin and tetracycline.

In this study *E. coli* isolated from apparently healthy cattle and subjected to antibiotic susceptibility tests revealed that the isolates were more frequently resistant to penicillin, eritromisin, neomycin, ampicillin, tetracycline and ampicillin. Susceptibility and resistance to *E. coli* O157:H7 strains with oxytetracycline have not been reported, a high prevalence of resistance to tetracycline has been reported in isolates recovered from cattle (Zhao *et al.*, 1998; Galland *et al.*, 2001; Schroeder *et al.*, 2002). Ajayi *et al.* (2011) reported that the number of isolates that showed resistance to tetracycline in their study was also high. Tetracycline and its derivatives is a drug of choice for use as feed supplement and in some cases into milk that is used as replacement diet for young

Table 1: Antimicrobial resistance profiles of the *E. coli* and *E. coli* O157:H7 species isolated from bovine and calves faeces samples

	No. of resistance and sensitive strains (%)					
	E. coli (n = 45)			*E. coli* O157:H7 (n = 14)		
Antimicrobials	R	I	S	R	I	S
Penicillin	45 (100.0)	0.0	0.0	14 (100.0)	0.0	0.0
Ampicillin	27 (60.0)	17 (37.7)	1 (2.3)	5 (35.7)	7 (50.0)	2 (14.2)
Chloramphenicol	9 (20.0)	1 (2.3)	35 (77.7)	3 (21.4)	1 (7.2)	10 (71.4)
Eritromisin	44 (97.7)	1 (2.3)	0.0	14 (100.0)	0.0	0.0
Tetracycline	33 (73.3)	3 (6.6)	9 (20.0)	11 (78.5)	0.0	3 (21.4)
Cephalothin	24 (53.3)	19 (42.2)	2 (4.4)	2 (14.2)	7 (50.0)	5 (35.7)
Cefoxitim	2 (4.4)	1 (2.3)	42 (93.3)	1 (7.2)	0.0	13 (92.8)
Neomycin	43 (95.6)	2 (4.4)	0.0	14 (100.0)	0.0	0.0
Trimethoprim-sulfamethoxazole	15 (33.3)	0.0	30 (66.7)	4 (28.5)	0.0	10 (71.5)
Ciprofloxacin	4 (8.8)	1 (2.3)	40 (88.8)	1 (7.2)	0.0	13 (92.8)

R: Resistance; I: Intermediate; S: Sensitive

animals. This study showed that the resistance to tetracycline was the highest for *E. coli* (73.3%) and *E. coli* O157:H7 (78.5%). This findings conform by Ajayi *et al.* (2011) and Schroeder *et al.* (2002).

This study observed that cephalothin and cefoxitim resistances were found to highly different the *E. coli* and *E. coli* O157:H7 strains. In addition the lowest frequency of resistance was seen against both cefoxitim and ciprofloxacin (7.2%). Goncuoglu *et al.* (2010) showed that four (36.36%) of the 11 cattle *E. coli* O157:H7 isolates were resistant to cephalothin.

Hovewer, this study showed a low prevalance of resistance to cephalothin (14.2%) for *E. coli* O157:H7 species isolated from apparently healthy cattle. Also cephalothin resistance rate was similar to Vali *et al.* (2004) findings. In this study, *E. coli* and *E. coli* O157:H7 strains were susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole. This findings were similar to Goncuoglu *et al.* (2010).

CONCLUSION

The present study evaluated the antimicrobial resistances of *E. coli* and *E. coli* O157:H7 species from bovine faeces samples from Turkey. In this study, the researchers observed that resistance to antibiotics particularly penicillin, eritromisin. neomycin tetracycline and ampicillin were very high both *E. coli* and *E. coli* O157:H7 species. Furthermore, cephalothin and cefoxitim resistances were found to highly different the *E. coli* and *E. coli* O157:H7 strains. In addition the lowest frequency of resistance was seen against both cefoxitim and ciprofloxacin (7.2%).

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