

Occurrence and Antimicrobial Resistance of *Salmonella* spp. and *Escherichia coli* Isolates in Apparently Healthy Slaughtered Cattle, Sheep and Goats in East Azarbaijan Province

Payman Zare¹; Hassan Ghorbani-Choboghlo^{1*}; Samin Jaber¹; Saied Razzaghi¹; Maryam Mirzae²; Kazem Mafuni¹

¹Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, I.R. Iran

²Department of Food Hygiene, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, I.R. Iran

*Corresponding author: Hassan Ghorbani-Choboghlo, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, I.R. Iran. Tel: +98-9141780449, Fax: +98-2166933222, E-mail: hghorbani67@yahoo.com

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Background: The increasing prevalence of antimicrobial resistance bacteria in meat-producing animals, especially ruminants, represents a major problem for human and veterinary medicine and also could increase the patients' morbidity and mortality.

Objectives: The current study aimed to identify the occurrence and antimicrobial susceptibility pattern of *Salmonella* spp. and *Escherichia coli* isolated from slaughtered ruminants in East-Azarbaijan province.

Materials and Methods: In this study 160 samples (40 sheep, 40 goats and 80 cattle) were examined to isolate the enteric pathogens. The antibiotic resistance was determined by Kirby-Bauer disc diffusion method using 12 antibiotics.

Results: A total of one hundred and twenty bacteria were obtained and most of these isolates belonged to these following genera: *Escherichia coli* (25%), *Proteus* (18.8%), *Salmonella* spp. (8.8%), *Pseudomonas* spp. (7.5%) and *Yersinia* spp. (6.3%). Eight (57.1%) of 14 *Salmonella* spp. isolates and 26 (65%) of 40 *E. coli* isolates showed resistance to more than four antibiotics, called multiple antibiotic resistance (MAR).

Conclusions: Overall, the obtained results emphasize the need for a surveillance and monitoring system to emerge drug resistance in all pathogenic microorganisms in ruminant and other animals.

Keywords: Ruminants; *Salmonella* spp.; *Escherichia coli*; Drug Resistance, Microbial

1. Background

Both *Salmonella* and *E. coli* are the most important food-borne pathogens that cause substantial, medical, and economical burdens worldwide (1). Numerous studies have reported the direct transfer of antibiotic-resistant bacteria from animals to humans. Many of the antibiotics used for food-producing animals are the same or belong to the same classes used in human medicine. Resistance to one antibiotic in a class often results in resistance to all drugs in that class and increases the problem in future. The identification of the pathogenic bacteria especially enteric bacteria in food stuff is necessary to control this health threat (2).

Antibiotics are used in food-producing animals to treat or prevent diseases or promote the animals' growth; also animal could be a source of food-borne resistant bacteria. Stressful transporting conditions to abattoir may lead to the increase of shedding rate in foodborne bacteria, therefore when the animals arrive at the slaughterhouse the pathogen could be an important component in the

feces of these animals. During the evisceration process, fecal bacteria may accidentally contaminate the meat and meat products (3, 4). It is clear that the gallbladder is an example of adaptation by the microorganism that *Salmonella* spp. are highly resistant to bile, which could be shedding slather process and contaminate the food product (5, 6). Because of the aforementioned reports, a number of actions have been taken to reduce the prevalence of *Salmonella* spp. and other enteric pathogens with public health significance in food-producing animals but microbiological risk assessments are still hampered due to the lack of data (7, 8).

2. Objectives

The primary aim of the current study was to determine the prevalence and antimicrobial susceptibility profile of *Salmonella* spp. and *E. coli* as important food-borne pathogens isolated from apparently healthy slaughtered ruminants in the East-Azarbaijan, North West of Iran.

Implication for health policy/practice/research/medical education:

The first critical component of comprehensive farms, to reduce the burden of food-borne diseases, is to identify the pathogenic bacteria especially enteric pathogen contamination in food products. To the authors best knowledge these data have novelty in Iran and east Azerbaijan province.

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3. Materials and Methods

3.1. Sample Collection

A cross-sectional study on enteric pathogens was carried out from February 2009 to September 2011 in Tabriz and Bonab cities slaughterhouses, North-West of Iran.

In the current study, 160 fecal and gallbladder specimens from 40 sheep, 40 goats and 80 cattle, were separately collected in sterile bags and then were immediately transported to the microbiology laboratory, using an insulated ice bag.

3.2. Microbial Analysis and Phenotypic Identification

One mL of each sample was pipetted and spread with 9 mL sterile double strength PBS onto Nutrient, Rappaport-Vassiliadis, Mac Conkey broth and peptone water (BPW) in a ratio of 1:10(w / v) (Merck Co., Darmstadt, Germany). Briefly, isolation of bacteria was carried out using XLD Medium, SS Agar, EMB Agar, Brilliant Green Agar (Merck Co., Darmstadt, Germany). All the plates were incubated at 37°C for 24 – 48 hours and the number of grown colonies was determined. Then suspected colonies were sub cultured and further identified by biochemical tests. To identify the colonies, different tests such as gram stain, motility, oxidase activity, catalase activity, oxidation / fermentation, glucose acid, glucose gas, pigment production and citrate utilization were applied. At the end, two isolated colonies (*Salmonella* spp. or *Escherichia coli*) were frozen at 70°C in BHI broth containing 20% glycerol for later susceptibility test.

3.3. Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion technique was used to determine the resistant isolates. After overnight incubation at 37°C, the inhibition zone was measured and categorized as resistant according to the CLSI criteria (9). *Salmonella* spp. isolates were tested against (12 of routine and practical antibiotics) ampicillin (10 µg/disc), amoxiclav (30 µg/disc), cefixime (5 µg/disc), colistin (10 µg/disc), ceftriaxone (30 µg/disc), ciprofloxacin (5 µg/disc), chloramphenicol (30 µg/disc), gentamicin (10 µg/disc), kanamycin (30 µg/disc), and tetracycline (30 µg/disc). The disks were purchased from an international company (Merck Co., Darmstadt, Germany). Isolates, which were resistant to four or more antibiotics, were determined as multi antibiotic resistant (MAR).

4. Results

In the current study, the mean of bacterial species were summarized in Table 1. One hundred and twenty bacteria were collected which belonged to these genera: *Escherichia coli* (25%), *Proteus* spp. (18.8%), *Salmonella* spp. (8.8%), *Pseudomonas* spp. (7.5%), *Yersinia* spp. (6.3%), *Shigella* spp. (5%) and *Klebsiella* spp. (3.8%). The total obtained bacteria in the feces samples were higher than those of gallbladder samples. Among the 54 *Salmonella* spp. and *E. coli* isolates were tested against 12 different antibiotics, 8 (57.1%) and 26 (65%) isolates were multi antibiotic resistant, respectively. The most commonly encountered resistant panel was TET-STR-AMP-COL (Table 2).

Table 1. Frequency of Bacterial Species Isolated From Apparently Healthy Slaughtered Cattle, Sheep and Goats

Animals	Cattle		Sheep		Goat		Total		
Samples type bacteria	Gall bladder (n=30)	Feces (n=50)	Gall blad-der (n=15)	Feces (n=25)	Gall blad-der (n=15)	Feces (n=25)	Gall blad-der (n=60)	Feces (n=100)	Totally (n=160)
<i>Escherichia coli</i> , No. (%)	5 (16.6)	14 (28)	3 (20)	10 (40)	2 (13.3)	6 (24)	10 (16.6)	30 (30)	40 (25)
<i>Proteus spp.</i> No. (%)	3 (10)	10 (20)	2 (13.3)	8 (32)	3 (20)	4 (16)	8 (13.3)	22 (22)	30 (18.8)
<i>Pseudomonas spp.</i> No. (%)	0	10 (20)	0	0	0	2 (8)	0	12 (12)	12 (7.5)
<i>Shigellas pp.</i> No. (%)	1 (3.3)	4 (8)	0	0	0	3 (12)	1 (1.6)	7 (7)	8 (5)
<i>Yersinia spp.</i> No. (%)	0	5 (10)	0	4 (16)	0	1 (4)	0	10 (10)	10 (6.3)
<i>Salmonella spp.</i> No. (%)	2 (6.6)	5 (10)	1 (6.6)	2 (8)	0	4 (16)	3 (5)	11 (11)	14 (8.8)
<i>Klebsiella spp.</i> No. (%)	0	3 (6)	0	1 (4)	0	2 (8)	0	6 (6)	6 (3.8)
Total, No. (%)	11 (36.7)	37 (74)	3 (20)	15 (60)	3 (20)	16 (64)	22 (36.7)	98 (98)	120 (75)

Table 2. Distribution of Antimicrobial Resistance of 54 *Salmonella* spp. and *E. coli* Isolates ^a

Bacteria	AMP ^b	AMO ^b	CEF ^b	COL ^b	CEFTRO ^b	CIP ^b	CHL ^b	GEN ^b	KAN ^b	STR ^b	ENR ^b	TET ^b
<i>E. coli</i> (n=40)	21 (52.5)	18 (45)	8 (20)	20 (50)	9 (22.5)	11 (27.5)	14 (35)	17 (42.5)	18 (45)	22 (55)	15 (37.5)	24 (60)
<i>Salmonella</i> spp. (n=140)	8 (57.1)	7 (50)	4 (28.6)	8 (57%)	5 (35.7)	4 (28.6)	5 (35.7)	7 (50)	3 (21.5)	8 (57.1)	6 (43)	9 (64.3)
Total (n=54)	29 (53.7)	25 (46.3)	12 (22.2)	28 (52)	14 (30)	15 (27.8)	19 (35.2)	24 (44.5)	21 (38.9)	30 (55.5)	21 (38.9)	33 (61.1)

^a Abbreviations: AMP, ampicillin; AMO, amoxiclav; CEF, Cefixime; COL, colistin; CEFTRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; ENR, enrofloxacin; TET, Tetracycline.

^b Data presented as No. (%)

5. Discussion

The relative and absolute abundance of livestock considerably differs by country and geographic area. Today, livestock plays a major role in agriculture, economy and meat production system in Iran. The distribution of *Salmonella* serotypes and other enteric pathogens among ruminants varies greatly over time, and differs among geographic regions, age groups, and production systems. Initially sensitive foodborne pathogens have become resistant to the clinically important antibacterial drugs, reported by a variety of molecular mechanisms. These resistant pathogens are mainly transmitted to human through direct contact, and shedding by animals, also the presence of these organisms in meat animals and raw meat products has relevant public health implications (2, 9, 10).

In the current study, a range of bacterial flora were isolated from the (Gastrointestinal tract) GIT samples, indicated the presence of these organisms in the apparently healthy ruminant GIT living in arid regions of Northwest of Iran. A total of 120 bacteria were obtained from the specimens. The isolated bacteria were predominantly *Escherichia coli* (25%), *Proteus* spp. (18.8%), *Salmonella* spp. (8.8%), *Pseudomonas* spp. (7.5%), and *Yersinia* spp. (6.3%). The majority of these isolated bacterial species were ubiquitous and most of the genera matched with those in other animals. This finding shows the magnitude of contamination at food establishments and slaughterhouses which may be due to food associate diseases (5, 11).

Most human salmonellosis cases have a food borne reason, but every year infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm, environments or other public, professional or private settings (12). The prevalence of *Salmonella* spp. was 8.8% in the feces and gallbladder samples collected from two different slaughterhouses.

Clinically, *Salmonella* infection in cattle is typically manifested as watery or bloody diarrhea, and often associated with fever, depression, anorexia, dehydration and endotoxemia. On the other hand *Salmonella* spp. can be localized into the gallbladder of asymptomatic ruminants (13, 14).

According to the results in Table 1, *E. coli* was the most predominant isolate, which is in agreement with the other studies conducted in different regions. The presence of few foodborne bacteria in the livestock may lead to high contamination of foodstuff received by consumer. Although, serotyping of *Escherichia coli* isolates was not applied in this study, isolation of these bacteria should be taken as a considerable threat (15-17).

In the current study *Proteus* spp. isolated from 18.8% of feces and gallbladder samples. Since studies have shown the importance of *Proteus* spp. as an indicator of unhygienic food processing practice (17), the high frequency of *Proteus* species should be considered seriously. *Proteus* spp. was also isolated from food samples and stools of patients with gastroenteritis thus, the role of *Proteus* spp.

as a food pathogen should be investigated (18, 19). Resistance of pathogens to the antibiotics used for the animals or human medicine is of major concern in clinical settings, and will be important in the future (15, 19).

Antimicrobial resistance was found in all types of samples from all species investigated in this study. In antimicrobial susceptibility testing, the highest prevalence of resistance was observed against tetracycline in both *Salmonella* spp. (64.3%) and *E. coli* (60%), also light resistance to cefixime, ciprofloxacin and ceftriaxone were determined. Other researchers reported the antibiotic resistance in enteric pathogens by other animal sources (6, 14, 19). Results of the current study showed that the overall rate of resistant *Salmonella* spp. was higher than those of *E. coli* isolates.

In the current study, a wide range of isolates presented multi antimicrobial resistance. Eight (57.1%) of 14 *Salmonella* isolates, and 26 (65%) of 40 *E. coli* isolates had resistance to more than four antibiotics (MAR). In *Salmonella* spp. and *E. coli*, the most common resistance pattern was the TET-STR-AMP-COL pattern, reflecting the predominant use of these antibiotics in ruminants (Table 2). Given the fact that the presence of pathogens in living ruminants indicates their presence in carcasses, it is desirable that ruminants reaching the slaughterhouse have a low number of orno pathogens (14, 17, 20).

In conclusion, the results showed that the prevalence of *Salmonella* spp. and *E. coli*, as two important enteric pathogens in slaughtered ruminant were 8.8% and 25%, respectively. The obtained data in this study provide helpful insights into the prevalence of food source pathogens and high level of antibiotic resistance in *Salmonella* spp. and *E. coli* that could transmit to humans through meat and meat products. The results also emphasize the need for a surveillance and monitoring system on the incidence and antimicrobial susceptibility of enteric pathogens in ruminants and other meat animals in slaughterhouses.

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Authors' Contribution

All the authors participated in preparing the manuscript, and experiment procedures equally.

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