Occurrence and Antibiotic Resistance of *Salmonella* spp Isolated from Raw Cow’s Milk from Shaharekoud, Iran

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Abstract: The World Health Organization has recently pointed out an alarming increase in the incidence of antibiotic resistant strains of *Salmonella*, which are due to the use of antibiotics in intensive breeding. *Salmonella* is one of the main causes of forborne illness worldwide. Conventional plating methods for the detection of these microorganisms in food are well established. This study was carried out to determine the prevalence and antibiotic resistance of *Salmonella* species from raw cow’s milk samples in Shahrekord city. A total 350 raw cow’s milk samples were collected from commercial dairy herds in Shahrekord city. Using cultural method, 14 of 350 raw cow’s milk samples (4%) were contaminated with *Salmonella*. In the present study *Salmonella* isolates showed resistance to: Nalidixic acid, Cephalothin, Ampicillin, Streptomycin, Neomycin, Chloramphenicol and Tetracycline.

Key words: Antibiotic resistance • Cow’s milk • *Salmonella* • Shahrekord

INTRODUCTION

*Salmonella* are the major pathogenic bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries [1]. Salmonellosis is the most common food borne disease in both developing and developed countries, although incidence rates vary according to the country. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain [2].

The infective dose of *Salmonella* can be as low as 15 to 20 cells, depending upon age and health of host. *Salmonella* causes one of the most common enteric (intestinal) infections in the world wide and is the second most common bacterial foodborne cause after Campylobacter infection. The number of salmonellosis cases has increased significantly throughout the past decade in several countries, *Salmonella enteritidis* has become the most common cause of salmonellosis [3-7].

Infections of humans can be acquired through direct contact with carrier domestic or wild animals or through the consumption of contaminated foods or water (8). Infections with non typhoidal *Salmonella*, also called salmonellosis, are one of the most commonly recorded causes of gastroenteritis in humans. The general symptoms of human salmonellosis are fever, diarrhea, abdominal cramps, nausea, vomiting, chills and prostration. Usually the disease lasts a few days and is self-limited. Occasionally the infection can be more serious, with loss of fluid and electrolytes and can be fatal, especially to the sick, infants and the elderly.
Antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products [8, 9].

Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese and hamburger meat traced to dairy farms. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella* [10]. The ultimate aim of this study was to determine the level of *Salmonella* contamination in raw cow’s milk samples in Shahrekord, Iran.

**MATERIALS AND METHODS**

**Sample Collection:** A total of 350 raw cow’s bulk milk samples (about 500 ml) were collected from commercial dairy herds Shahrekord, (Iran). The samples of milk were transported to the laboratory after being collected in a portable cooler container with ice packs (at 4°C) and microbiological analysis was carried out immediately.

**Isolation and Identification:** Samples were examined for the presence of *Salmonella* by Iranian National Standard method No. 1810 recommended by the Institute of Standards and Industrial Research of Iran (ISIRI) [11]. Twenty-five cc of milk sample was aseptically added to 225 ml of buffered peptone water (Merck) and incubated at 37°C for 24h and constituted the pre-enrichment stage of the isolation. Subsequently, 10 ml of this pre-enrichment culture was added to 100 ml of selenite cystine broth (Merck) as selective enrichment medium and incubated for 24 h at 37°C. After incubation, a loopful of the enriched cultures was streaked onto *Salmonella-Shigella* agar (Merck) and incubated at 37°C for 24-48 h. Non lactose fermenting colonies with or without black centre on *Salmonella-Shigella* agar were the suspected *Salmonella* spp. Such colonies were picked out and sub-cultured for biochemical tests and were identified according to [1], Put another reference.

**Antimicrobial Susceptibility Test:** Susceptibility to antimicrobial agents was tested using the disk diffusion method on Mueller Hinton agar (Merck) plates according to the National Committee for Clinical Laboratory Standards Guidelines [1]. The following 17 different antimicrobial agents were examined against *Salmonella* isolates recovered from raw cow’s milk samples: Trimethoprim (5µg), Furazolidone (10µg), Nalidixic acid (30µg), Ciprofloxacin (5µg), Imipeneme (10µg), Cephalothin (20µg), Cefxime (5µg), Ceftazidim (30µg), Streptomycin (10µg), Ampicillin (10µg), Neomycin (30µg), Tobramycin (20µg), Kanamycin (20µg), Amikacin (30µg), Gentamicin (10µg), Chloramphenicol (30µg) and Tetracycline (30µg). The diameters of zones of inhibition were recorded to the nearest millimeter and classified as susceptible, intermediate and resistant.

**RESULTS**

A total of 350 raw cow’s milk samples were analyzed by conventional culture method for the detection of *Salmonella* spp. Based on this method 14 samples (4%) were found positive with *Salmonella* spp. All strains isolated from raw cow’s milk samples were subjected to antimicrobial resistance testing against 17 different antimicrobial agents. According to the disk diffusion test antimicrobial resistant was showed to: Nalidixic acid, Cephalothin, Ampicillin, Streptomycin, Neomycin, Chloramphenicol and Tetracycline. The results show in Table 1.

A total of 14 *Salmonella* isolates were tested for antibiotic susceptibility; 11 samples (78.57%) resistance to Nalidixic acid (30µg), 3 samples (21.42 %) resistance to Cephalothin (20µg), Neomycin (30µg), Choloramphenicol (30µg) and 6 samples (42.58%) resistance to Ampicillin (10µg) and Tetracycline (30µg).

Of the 14 isolates only 3 isolates resistance to the Nalidixic acid, Cephalotin, Ampicillin, Streptomycin, Neomycin, Chloramphenicol and Tetracycin.

**DISCUSSION**

Salmonellosis is not a reportable disease in Iran; therefore, the actual incidence of this infection in Iran is unknown. Data regarding the prevalence of *Salmonella* must be available for evaluation by the regulatory authorities as a first step to control salmonellosis in humans. Therefore, the ultimate aim of this study was to determine the level of *Salmonella* contamination in raw cow’s milk samples in Shahrekord, Iran.
Table 1: Resistance of *Salmonella* spp isolated from raw cow milk to different antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>%Resistance of isolates from raw cow milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim (5µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Furazolidone (10µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Nalidixic acid (30µg)</td>
<td>78.57%</td>
</tr>
<tr>
<td>Ciprofloxacin (5µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Imipenem (10µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Cephalothin (20µg)</td>
<td>21.42%</td>
</tr>
<tr>
<td>Cefexime (5µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Ceftazidim (30µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Streptomycin (10µg)</td>
<td>21.42%</td>
</tr>
<tr>
<td>Ampicillin (10µg)</td>
<td>42.58%</td>
</tr>
<tr>
<td>Neomycin (30µg)</td>
<td>21.42%</td>
</tr>
<tr>
<td>Tobramycin (20µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Kanamycin (20µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Amikacin (30µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Gentamicin (5µg)</td>
<td>0%</td>
</tr>
<tr>
<td>Chloramphenicol (30µg)</td>
<td>21.42%</td>
</tr>
<tr>
<td>Tetracycline (30µg)</td>
<td>42.58%</td>
</tr>
</tbody>
</table>

In this study the prevalence of *Salmonella* in raw cow’s milk was 4%. Previous studies have shown a wide range of estimates for the prevalence of *Salmonella* in bulk tank milk. Steele *et al.* [12] detected *Salmonella* in only 0.17% of bulk tank samples from Ontario, Canada. Murinda *et al.* [13] found *Salmonella* spp. in 2.24% of milk samples from the bulk tanks of 30 Tennessee farms.

The detection of *Salmonella* in 4% of the samples tested indicates that the degree of prevalence of the pathogen in raw milk in Shahrekord is relatively higher than originally believed. Although contamination of dairy products currently accounts for a small percentage of foodborne illness, it is clear that raw milk consumption and the consumption of products made with raw milk present some risk. Although proper pasteurization minimizes these risks to the public, there is a small but growing group of people that consume unpasteurized milk or milk products, either for practical (e.g., farm families) or cultural (e.g., soft ethnic cheeses) reasons, or because of perceived health benefits [8]. Although the levels of *Salmonella* in the milk samples tested here seemed to be very low and the infectious dose for this organism is low, the potential for this organism to grow in improperly stored raw milk and in products made from raw milk presents a public health risk, particularly to susceptible members of the population. Continuing surveys of milk will help to estimate the true level of risk associated with these practices and may help to identify dairy management practices that minimize the contamination of bulk tank milk with zoonotic foodborne pathogens.

Karns *et al.* [8] reported a prevalence of 11.8% from bulk tank milk by PCR method which is higher than the present report. In the other study by Addis *et al.* [14] from 195 dairy cows tested 28.6% were positive from milk samples. Akoachere *et al.* [15] in Cameroon reported a high prevalence (27%) of *Salmonella* among cattle. This may be due to the difference in the living condition, like housing conditions, feeding habits, types of feed given for the cattle, of the two cattle populations.

Antibiotic resistance in *Salmonella* is an emerging problem during the last decades. The intensive use of antibiotics in both human and veterinary medicine, as well as in agriculture has caused bacteria to developed resistance mechanisms against therapeutic drugs [17].

In this study according to the disk diffusion test antimicrobial resistance was showed to: Nalidixic acid, Cephalothin, Ampicillin, Streptomycin, Neomycin, Chloramphenicol and Tetracycline with percentages 78.57, 21.42, 42.58, 21.42, 21.42, 21.42 and 42.58% respectively.

Results of the current study indicated that ciprofloxacin and Imipenem are good antimicrobials showing 100% activity against *Salmonella* spp isolated from cow. This is also comparable with the result reported by Morshed and Peighambari in Iran [19]. The issues of food safety attract more attention from the government and public worldwide in recent years. The incidence of salmonellosis outbreak cannot be neglected due to the overwhelming effects to human. The knowledge about *Salmonella* and its evolution is important to ensure the safety and quality of food.

**REFERENCES**


