Isolation, Serotyping, and Antibigram of *Salmonella* Isolates from Raw Milk Sold at Retail Vending in Erbil City, Iraq

Dhary Alewy ALMASHHADANY* and Ardalan Abdulhamed OSMAN

Knowledge University Research Center, Erbil, Kurdistan Region, Iraq

*corresponding author: dhary.hammed@Knowledge.edu.krd

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Abstract

Pathogenic strains of *Salmonella* cause gastroenteritis in humans globally. This study aimed to determine the prevalence and antibiotic susceptibility pattern of *Salmonella* in raw milk sold in Erbil city, Kurdistan region, Iraq. A total of 470 samples were collected from retail vendors between January and June 2019. Samples were cultured on selective media followed by serotyping and antibiotic sensitivity testing by disk diffusion assay. The results revealed that the overall prevalence of *Salmonella* was 7.9%. The isolates belonged to nine (9) different serotypes: *S. Typhimurium* 18.9%, *S. Anatum* 10.8%, *S. Muenchen* 13.6%, *S. Enteritidis* 10.8%, *S. Senftenberg* 8.1%, *S. Newport* 10.8%, *S. Arizona* 10.8%, *S. Montevideo* 8.1%, and *S. Dublin* 8.1%. The antibiotic resistance profile revealed that 67.6%, 62.2%, 56.8%, and 56.8% of isolates were resistant to levofloxacin, streptomycin, imipenem, and tetracycline respectively. This resistance among *Salmonella* may pose a public health threat that needs active safety measures and response.

Keywords: *Salmonella*, Serotyping, Raw milk, Antibigram, Erbil, Kurdistan region, Iraq.

Introduction

Salmonella is one of the most frequently isolated foodborne pathogens globally. The pathogenic potential of *Salmonella* was documented in 1885 in pigs by two American pioneer scientists; Daniel E. Salmon and Theobald Smith (Octavia and Lan, 2014; Al-Gamal et al., 2019). *Salmonella* is found in the intestinal tract of animals, birds, and wide range of mammalian hosts including humans. As a result, it is widely distributed in nature and capable of surviving for a number of weeks in dry environments and various months in water. To date, more than 2,600 serotypes of *Salmonella* exist. In humans, the pathogenic strains have the potential to cause life-threatening infections, most of which are food-borne diseases (Maserati et al., 2017; Ammar et al.,

2019). *Salmonella* strains are generally classified into two categories; Typhoidal salmonellae and Non-typhoidal salmonellae. The former group includes strains that may cause typhoid fever or paratyphoid fever such as Typhi, Paratyphi A, Paratyphi B, and Paratyphi C, which is carried only by humans. Non-typhoidal salmonellae group includes all other *Salmonella* strains carried by humans, different types of animals, poultry, wild birds, and flies. Non-typhoidal salmonellae are the most common strains implicated in food poisoning (Ferede, 2014; El-Prince et al., 2019).

According to WHO statistics, about 1.3 billion cases of non-typhoidal salmonellosis occur globally each year. Around 80.3 million cases (85.6%) are food borne. The predominant serotypes, the foods associated with non-typhoidal salmonellosis, and

the trends of non-typhoidal salmonellosis are different in different region of the world. The disease is most common in children and reaches its peak during summer and fall seasons. In the developing countries, salmonellosis contributes to diarrhea morbidity and mortality of childhood, as salmonellae are accountable for about 20% of childhood illness cases (Majowicz *et al.*, 2010; Stephen and Barnett, 2016; Katiyo *et al.*, 2019). Some *Salmonella* serotypes are host-specific, while others have a more generic host range. Serotypes that cause asymptomatic infection in animal can result in human infections and vice versa (USDA, 2013; Balasubramanian *et al.*, 2019). High-risk individuals for salmonellosis are infants, elderly, immunocompromised individuals and those who have gastric hypoacidity (Acheson and Hohmann, 2001; Coburn *et al.*, 2007; Gutema *et al.*, 2019).

The major sources of Salmonellosis in humans are different types of food, particularly ready-to-eat food and fast food including different types of meats and meat products. It is known that *Salmonella* remains viable for long periods of time in frozen foods. Milk-borne salmonellosis is often related to the consumption of raw or inadequately pasteurized milk. However, *Salmonella* serovars may also contaminate dairy products during and after the pasteurization process (Almashhadany, 2008; Shafini *et al.*, 2017; El Bagoury *et al.*, 2019).

In Kurdistan region (Iraq), raw milk and dairy products are common served, with ease access, in food outlets especially in restaurants, retail outlets, street vendors, hotel, school, canteen and even small outlets which involved different styles of preparations. Therefore, this work was conducted in order to determine the prevalence and antibiogram profile of *Salmonella* among milk sold at retail vending in Erbil city.

Materials and Methods

Study design and sampling

A total of 470 raw milk samples (125 cows, 110 buffaloes, 115 sheep, and 120 goats) were collected under sterile and hygienic conditions during January to June 2019 in Erbil city according to previously published method (Al-Mashhadany, 2014).

Isolation and Identification of *Salmonella*

Isolation of *Salmonella* from raw milk was done according to previously published protocol

(WHO, 2010). Gram stain was done for all suspected *Salmonella* colonies (Casey *et al.*, 2004; Cheesbrough, 2006), followed by biochemical identification tests according to standardized procedures (Cheesbrough, 2006; WHO, 2010). The biochemical tests included TSI, Urease, Lysine decarboxylase, citrate utilization test, MR-VP, indole test, mannitol fermentation, and motility.

Serotyping of isolates

All *Salmonella* isolates that showed typical biochemical reactions were subjected to serotyping by slide agglutination using Remel® kit according to manufacturer instructions (Remel Europe Ltd., UK).

Antibiotics susceptibility testing

Disk diffusion assay on Mueller-Hinton agar (Oxoid, UK) was employed to evaluate the susceptibility of *Salmonella* isolates to a panel of twelve commonly used antibiotics (HiMedia, India). The guidelines of CLSI were followed to perform the Modified Kirby-Bauer method and to interpret the inhibition zones diameters around antibiotic disks (CLSI, 2011). The tested antibiotics were; amikacin (AK₁₀), amoxicillin (AMC₂₅), cefadroxil (CFR₃₀), cefotaxime (CTX₃₀), ceftriaxone (CRO₃₀), gentamicin (GM₁₅), imipenem (IMP₁₀), kanamycin (KAN₃₀), levofloxacin (LEV₅), streptomycin (STR₁₀), tetracycline (TEC₃₀), and tobramycin (TM₁₀).

Statistical analysis

All data was analyzed via version 21 of SPSS software package. Confidence intervals were calculated by normal approximation method. Chi square test was employed to test for difference between groups. Significance level was set to 0.05.

Results

Prevalence of *Salmonella* in milk

Out of 470 raw milk samples, 7.9% (37) were positive for the presence of *Salmonella*. Of note, 10.4% (13/125) of samples from cow milk and 5.5% (6/110) of buffaloes milk harbored *Salmonella* (Table 1). Based on statistical inference, it is estimated that 5.46% - 10.34% (95% confidence interval) of raw milk among Erbil retail vending is contaminated by *Salmonella* species (Table 1). There is no significant difference between types of milk in terms of *Salmonella* prevalence ($\chi^2 = 1.586$, $p = 0.363$).

Detected serotypes of *Salmonella*

Regarding serotyping of *Salmonella* isolated from different types of raw milk, nine (9) different

Table 1. Prevalence of *Salmonella* among raw milk samples.

Type of Milk	No. of tested samples	Positive samples n (%)	95% CI
Cow Milk	125	13 (10.4)	5.05 - 15.75
Buffaloes Milk	110	6 (5.5)	1.24 - 9.76
Sheep Milk	115	10 (8.7)	3.55 - 13.85
Goats Milk	120	8 (6.7)	2.23 - 11.17
Total	470	37 (7.9)	5.46 - 10.34

Figure 1. *Salmonella* serotypes isolated from raw milk**Table 2.** Temporal distribution of *Salmonella* in raw milk samples

Month	No. of examined milk (No. of positive)				Total examined	No. of positive (%)
	Cow	Buffaloes	Sheep	Goats		
January	21 (1)	17(0)	19(0)	19(1)	76	2 (2.6)
February	20 (0)	19(0)	20(1)	21(0)	80	1 (1.3)
March	22 (2)	18(1)	20(3)	21(1)	81	7 (8.6)
April	21 (2)	20(2)	19(1)	19(1)	79	6 (7.6)
May	20 (3)	18(1)	19(2)	20(2)	77	8 (10.4)
June	21 (5)	18(2)	18(3)	20(3)	77	13 (16.9)
Total	125 (13)	110(6)	115(10)	120(8)	470	37 (7.9)

serotypes were detected. The most common serotypes detected were *S. Typhimurium* (18.9%) and *S. Muenchen* (13.6%), while *S. Dublin*, *S. Montevideo*, and *S. Senftenberg* were the least detected. Other serotypes and their percentages are depicted graphically in Figure 1.

Temporal distribution of *Salmonella*

A change in prevalence rate of *Salmonella* was observed through study period. The highest rates of prevalence were detected in June (16.9%) and May (10.4%), while the lowest rate was found in February (1.3%). Table 3 summarizes

the detection rates in different milk samples in a temporal scale. Of note, spring season and early summer were associated with gradual increase in *Salmonella* prevalence ($r^2 = 0.854$) (Figure 2).

Antimicrobial susceptibility of detected *Salmonella*

All *Salmonella* isolates ($n=37$) were tested against twelve commonly used antibiotics. Total sensitivity was found towards amoxicillin and cefadroxil, while high resistance phenotypes to levofloxacin (67.6%) and streptomycin (62.2%) were found. The detailed

Figure 2. Association between months and prevalence of *Salmonella*

Figure 3. Antibiogram of *Salmonella* species to a panel of 12 antibiotics

antibiogram profile is summarized graphically in figure 3.

Discussion

Salmonellosis is a major cause of human bacterial gastroenteritis in both developed and developing countries. It has been reported by the World Health Organization (WHO) and Food Agriculture Organization (FAO) as the most prevalent and considerable zoonosis since 1950. Several studies have revealed the importance of milk and dairy products in transmission of *Salmonella* and development of salmonellosis in humans (Tacket *et al.*, 1985; Mazurek *et al.*, 2004; Dominguez *et al.*, 2009; van Duynhoven *et al.*,

2009; Giacometti *et al.*, 2015; Putturu *et al.*, 2015; Ung *et al.*, 2019).

In the present study, the prevalence of *Salmonella* among raw milk samples from different animals was 7.9% (Table 1). Our results were consistent with Rastegar and associates in Iran, who found the total isolation rate of *Salmonella* among milk samples to be 11% by culture-dependent approach, while higher rate (17%) was found by PCR method (Rastegar *et al.*, 2013). Moreover, the overall prevalence is also in good agreement with the study conducted by Al Mashhadany in Yemen (Al-Mashhadany, 2014), who found the total prevalence in different milk samples was 7.3%. On the contrary, our results are in constant with

studies from Egypt (15%), Bangladesh (25.7%), and Iraq (36%) (Yasmin *et al.*, 2015; El-Baz *et al.*, 2017; Hasan, 2017). These variations are well-known globally and are influenced by many factors including: geographic, temporal, food type contaminated with salmonellae, and detection method (Besser, 2018).

Regarding *Salmonella* serovars isolated in this study (Figure 1), *S. Typhimurium* was the most prevalent (18.9%), which is consistent with earlier reports from Yemen (Al-Mashhadany, 2014), Egypt (El-Baz *et al.*, 2017), and a recent study isolated *Salmonella* from grilled chicken meat in Erbil (Almashhadany, 2019). It is well-known that environmental spreading of *Salmonella* serotypes is influenced by several ecological factors (Andino and Hanning, 2015). In terms of time-based occurrence of *Salmonella*, the highest prevalence was in June (16.9%) and May (10.4%) (Figure 2). Several studies had connected warm period to high occurrence of *Salmonella*. In USA, the overall case counts of salmonellosis reported to peak in summer (38.6%) and were lowest in winter (14.5%). They also found that *Salmonella* serotypes may vary in their normal reservoirs, environmental, seasonal spreading, and their ability to cause human infections (Judd *et al.*, 2019).

Antibiotics resistance in *Salmonella* increases continually. Indeed, monitoring reports showed a two-fold increase in *Salmonella* resistant phenotypes (from 20%-30% to more than 70%) from early 1990s to 2000s (Su *et al.*, 2004). In this study, the sensitivity testing showed that 67.6%, 62.2%, and 56.8% resistance to levofloxacin, streptomycin, imipenem, and tetracycline respectively (Figure 3). The usage of antimicrobials in food producing animals, such as cattle, buffaloes, ewes, and goats, could result in antibiotic resistant strains including *Salmonella* species. The antibiotic resistance of *Salmonella* Typhimurium isolates recovered from the food chain was studied recently (Wang *et al.*, 2019). The most frequently observed antibiotic resistance patterns found in *S. Typhimurium* were tetra-resistant pattern ASSuT (ampicillin, streptomycin, sulfonamides, and tetracycline), and the penta-resistant pattern ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline). However, a total sensitivity to levofloxacin, ceftriaxone, gentamicin was reported

in *Salmonella* recovered from milk and dairy products in Slovakia (Hleba *et al.*, 2011). The emergence of multidrug resistant *Salmonella* is known to complicate the control of food safety and clinical treatment of infections (Eng *et al.*, 2015).

Conclusion

Salmonellosis is a significant foodborne disease that worth an additional research in Erbil city. The prevalence of *Salmonella* in raw milk samples is alarming. Moreover, detected serovars showed different degrees of resistance to important antibiotics used in food industry and clinical medicine. Molecular and epidemiological studies should follow this study to evaluate the hazard for consumers and inspection of antibiotic resistance determinants dissemination. A four-season study is recommended to determine the changing epidemiology of *Salmonella* in milk.

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