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Prevalence and antimicrobial resistance profile of *Salmonella* isolates from dairy products in Addis Ababa, Ethiopia

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A cross sectional study was conducted on dairy items in Addis Ababa from October 2010 to March 2011 to determine prevalence and antimicrobial resistance profile of *Salmonella*. A total of 384 dairy items, 96 of each item (cheese, milk, butter and yogurt) was sampled. The overall prevalence of *Salmonella* was found to be 1.6% (6 of 384). Prevalence of 3.1, 1.04, 2.1, and 0% was observed from cheese, butter, milk and yogurt, respectively. However, there was no statistically significant difference (P > 0.05) in the prevalence of *Salmonella* among the different sample types. Isolates were tested for the effects of eight antimicrobials by disk diffusion technique; all isolates were resistant to one or more of the tested antimicrobials. Of all isolates, 50% were multiple antimicrobial resistant. 83.3, 50, 16.7, and 16.7% of isolates were resistant to tetracycline, ampicillin, amoxicillin, and chloramphenicol, respectively. However, all the isolates were susceptible to gentamycin, ceftriaxone, ciprofloxacin, and sulfamethoxazole. From this pilot study, we concluded that dairy products are a potential source of *Salmonella* infection with antimicrobial resistance. Furthermore, hygienic management of dairy products and prudent use of antimicrobials are also suggested.

Key words: Salmonella, prevalence, dairy products, antimicrobial resistance, Addis Ababa.

INTRODUCTION

Salmonella is a leading cause of food borne illness (WHO, 1988; White et al., 2001). Globally, more than 93 million cases of gastroenteritis are caused by non typhoidal *Salmonella* with 155,000 deaths each year. Of these cases, 80.3 million cases were estimated to be food borne. Salmonellosis, the diseases caused by bacteria of the genus *Salmonella*, is a common intestinal illness caused by numerous *Salmonella* serovars with clinical manifestations that vary from severe enteric fever to mild food poisoning (Jones et al., 2004) both in animals (Radostits et al., 2007) and humans (Hohmann, 2001). Foods of animal origin particularly meat, poultry, egg, milk and milk products are considered to be the primary source of human salmonellosis (Acha and Szyfers, 2001). Most of these food products become contaminated during slaughter, processing in contaminated environment and because of faulty in transport, handling, storage or preparation.

Salmonellosis takes a healthy tool in human life and suffering, particularly among infants and children, the elderly and other susceptible persons particularly in developing countries where most food industries are not

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well aware of food safety issues and knowledge of modern technologies (Van der Venter, 1999). Good Manufacturing Practices, hygiene, Hazard Analysis Critical Control Point systems and quality control are often limited or absent in such countries. Cold storage facilities are inadequate and quality of water used for food processing may not be suitable. The vast numbers of labor that handle food in factories, as well as on farms are illiterate and untrained. In such countries lack of information leads to lack of appreciation of health significance of unsafe food (Van der Venter, 1999).

Salmonellosis has a wide variety of domestic and wild animal hosts (Acha and Szyfers, 2001). Infection in animal is of importance because of the direct economic consequences of salmonellosis attributable to mortality and morbidity. Of even greater importance are the human health consequences of salmonellosis acquired by direct or indirect contact with animals, which constitute a vast reservoir of these organisms (Libby et al., 2004). Several species of *Salmonella* have been documented to colonize udder and shed at level of 2000 organisms per milliliter of milk (Fontaine et al., 1980).

According to Jayaroo and Henning (2001) *Salmonella* was isolated from 6.1% of bulk tank milk sample from dairy herds in South Eastern Dakata and Western Minnesota. Cheeses made from raw milk have been implicated as sources of several outbreaks (D'Aoust, 1994). The worst food poisoning incident due to *Salmonella* occurred in US in 1985 and there was a cause of 16,289 human cases and 7 deaths as the result of recontamination of pasteurized milk with a potent strain of *Salmonella* Typhimurium. In 1994, there was also national outbreak of *Salmonella* Enteritidis affecting 225,000 people who consumed contaminated ice cream products (Doyle and Cliver, 1990). In *Salmonella*, in addition to concern about the presence of it as a potential food borne pathogen, concern has also been raised about the human health impact of presence of antimicrobial resistance transferred among these organisms (Dargatz et al., 2003), which limits therapeutic options for treatment of disease in human and animals. Studies show that antimicrobial resistance *Salmonella* are increasing due to the use of antimicrobial agents in food animals, which are subsequently transmitted to humans usually through the food supply (White et al., 2001).

In Ethiopia, despite attempts to study prevalence of *Salmonella* mainly in poultry and beef, the status in milk and milk products is still unknown. However, studies made elsewhere indicated that milk and milk products are important source of *Salmonella* particularly among those raw consumers (WHO, 1988; Jay, 2000). Ubiquitous nature of *Salmonella,* unhygienic condition prevailing at the farm levels and food handlers, and habit of consuming milk and milk products in raw suggest that milk and milk products can act as source of *Salmonella* organisms in Ethiopia. Considerable proportion of them might have developed resistance to antimicrobials that are commonly used in both the veterinary and public health. Such a problem might be significant in areas like Addis Ababa where consumption of milk and milk products are high, dairy supermarkets are significant, and handling of milk and milk products take several hours until they reach to consumers. Therefore, this study was carried out to estimate the prevalence of non typhoidal *Salmonella* and to determine the antimicrobial resistance profile of *Salmonella* isolates from dairy products in Addis Ababa.

MATERIALS AND METHODS

Study area and sample size

The study was a cross-sectional study conducted in dairy products (butter, cheese, milk and yogurt) which were purchased from different dairy supermarkets of Addis Ababa. The variable of interest considered as an output variable versus risk factors was *Salmonella* status of dairy items. Types and handling of the dairy items were considered as explanatory variables. List of all 81 currently operational supermarkets in Addis Ababa were collected from Addis Ababa municipality. Each of 45 supermarkets was randomly selected using simple random sampling technique and identified for the study. The sample size required for the study was determined based on sample size determination in random sampling for infinite population using expected prevalence of salmonellosis and the desired absolute precision according to Thrusfield (2005). Accordingly, a total of 384 dairy product samples were collected for this study.

Sampling procedure

A total of 384 samples including butter, cheese, milk and yogurt (each n = 96) was randomly purchased from 45 supermarkets. Milk and yogurt samples were collected in separate sterile bottles of 25 ml capacity while cheese and butter samples were collected in sterile plastic bags. Samples were properly labeled by sample type, name of supermarket and date of sample collection and transported in ice box to microbiology laboratory of Aklilu Lemma Institute of Pathobiology for analysis. Upon arrival, the samples were immediately processed or stored over night in a refrigerator at 4ºC until they had been processed in the following day.

Isolation and identification of *Salmonella* organisms

The isolation of *Salmonella* was performed according to the standard operating procedure set by the Global *Salmonella* Surveillance and laboratory support project of the World Health Organization (WHO) and the National Health Services for Wales (NHS), in which both procedures use ISO-6579 (ISO, 2002) Standard for the isolation of *Salmonella*.

Biochemical tests: Pure cultures obtained from nutrient agar were tested biochemically according to ISO 6579 (2002) (ISO, 2002).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the isolates was performed according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999). From each isolate, four to five biochemically confirmed well-isolated colonies grown on nutrient agar were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 35ºC for 4 h

Table 1. Prevalence of *Salmonella* in dairy items in Addis Ababa dairy supermarkets.

Sample type	Number of samples examined	Positive (%)
Cheese	96	3 (3.1)
Yogurt	96	-
Butter	96	1 (1.04)
Milk	96	2 (2.1)
Total	384	6 (1.6)

Fisher's exact = 0.525.

Table 2. Antimicrobial sensitivity test results of *Salmonella* isolates from dairy items of Addis Ababa supermarket.

Type of antimicrobial	Number of isolates			Total
	Resistant (%)	Intermediate (%)	Susceptible (%)	
Gentamycin (10 µg)	-	-	6 (100)	6
Tetracycline (30 µg)	5 (83.3)	1 (16.7)	-	6
Ceftriaxone (30 µg)	-	-	6 (100)	6
Ciprofloxacin (5 µg)	-	-	6 (100)	6
Ampicillin (10 µg)	3 (50)	1 (16.7)	2 (33.3)	6
Amoxicillin (10 µg)	1 (16.7)	1 (16.7)	4 (66.7)	6
Sulfamethoxazole (25 µg)	-	-	6 (100)	6
Chloramphenicol (30 µg)	1 (16.7)	-	5 (83.3)	6
Total	10 (20.8)	3 (6.3)	35 (72.9)	48

All of the total isolates were resistant to one or more of the tested antimicrobials. Of all isolates half (50%) were multiple antimicrobial resistant while the rest half were resistant to single antimicrobial as shown in Table 3. Three types of resistance patterns were observed, resistance to one, two and three antimicrobials as shown in Table 3.

until it achieved the 0.5 McFarland turbidity standard. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod CM 0337 Basingstoke, England). The plates were held at room temperature for 30 min to allow drying. Antibiotic discs with known concentration of antimicrobials were placed and the plates were incubated for 24 h at 37ºC. *Escherichia coli* ATCC 25922 was employed as strain of quality control. Each isolate tested for a series of eight antimicrobials. The diameters of zone of inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible according to published interpretive chart (NCCLS, 1999)

Data management and analysis

The data were entered and managed in MS Excel work sheet. The analysis was conducted using Intercooled Stata 7. The significance differences between the prevalence of *Salmonella* species in various sample types was determined using Fisher's exact test as the numbers within categories were too small for the Chi-square test.

RESULTS

Of the total 384 cheese, butter, yogurt and milk samples (each n=96), 1.6% (6 of 384) were culture positive for *Salmonella* (Table 1). However, there was no significant difference between the prevalence in the different types of products (Fisher's exact = 0.525, P > 0.05).

Antimicrobial resistance

Of all isolates, 50% were multiple antimicrobial resistant and 83.3, 50, 16.7, and 16.7% of isolates were resistant to tetracycline, ampicillin, amoxicillin, and chloramphenicol, respectively. All isolates were susceptible to gentamycin, ceftriaxone, ciprofloxacin and sulfamethoxazole. The highest level of resistance was observed for tetracycline (83.3%) as shown in Table 2.

DISCUSSION

Prevalence of *Salmonella* in dairy items

In this cross-sectional study on prevalence and antimicrobial resistance of *Salmonella* isolates from dairy products in Addis Ababa, overall 1.6% (6 of 384) *Salmonella* prevalence was detected. *Salmonella* was detected from cheese, butter, and milk with prevalence of 3(3.1%), 1(1.04%), and 2 (2.1%), respectively. However, there was no statistical significant difference in prevalence of *Salmonella* among the different dairy samples (Fisher's exact = 0.525, p > 0.05). Prevalence of *Salmonella* detected from cheese in this study was in agreement with the work of Zewdu (2004) who reported *Salmonella* from cheese with prevalence of 2.1% in his previous study of

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates.

Sample type	Isolate	Antimicrobial resistance pattern
Butter	B	Amp, Caf
Cheese	C1	Te, Amp, Amo
	C2	Te
	C3	Te
Milk	M1	Te
	M2	Te, Amp

Note: B is the one isolate from butter; C1, C2 and C3 are isolates from cheese; M1 and M2 are isolates from milk. Amp is ampicilin; Te is tetracycline; Caf is Chloramphenicol; Amo is amoxicillin.

Salmonella organisms in Addis Ababa. *Salmonella* was detected from milk with prevalence of 2.1%. This result is in agreement with the work of Rohrbach et al. (1992) who reported *Salmonella* in milk with prevalence of 2.68% in France. In addition, Jayaroo and Henning (2001) found high prevalence of *Salmonella* 6.1% in bulk tank milk from South Eastern Dakata and Western Minnesota. The difference in prevalence between this study from that of the study in Western Minnesota might be associated difference in hygienic and farm management practices in the different study areas. According to Radostits et al. (1994) epidemiological patterns of *Salmonella* differ greatly between geographical areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies and consumer habits. However, mentioning the difference in method of detection and variation in sample size as a reason for difference in prevalence cannot be overemphasized.

In the present study, *Salmonella* was detected in a relatively quite low prevalence compared to prevalence detected from other food items and dairy products might not pose a significant health risks to humans. According to D'Aoust (1997) even such level of *Salmonella* might be enough to cause salmonellosis in risk group individuals; newborns, infants, the elderly and immune compromised individuals who are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. However, it is generally accepted that the presence of any *Salmonella* isolate in a food should be regarded as a potential hazard to human (Fathi et al., 1994). Therefore, even if the study indicated low prevalence of *Salmonella* in dairy products, it is a potential hazard for *Salmonella* infection through consumption of dairy products; which is especially important in Ethiopia in general and Addis Ababa in particular where dairy products are in most of the time consumed without appropriate cooking practices.

Salmonella was not detected from yogurt samples. Yogurt or ''Ergo'' is a traditional Ethiopian fermented milk produced by spontaneous fermentation using traditional utensil. The low prevalence of *Salmonella* in milk to prepare the yogurt as indicated in this study might contribute for absence of *Salmonella* in yogurt samples. Partly, it might also be associated the relatively hygienic practice exercised during preparation and marketing of yogurt and due to low pH (Makita et al., 2012). *Salmonella* are destroyed or inactivated during the fermentation of high acid products such as yogurt in which pH value is less than 4.55 (Varnman and Evans, 1991). In Ethiopia, there is a habit of smoking of utensils which are used for preparation of yogurt. This was scientifically justified by Mogessie and Fekadu (1993) and Lemma (2004) that smoking reduces the undesirable microbial contamination which enhances the fate of fermentation and passing the smoke flavor to the milk; this might also contribute for absence of detection of *Salmonella* from yogurt samples. However, the prevalence of *Salmonella* in yogurt in this study cannot enable to disregard yogurt as a vehicle for salmonella infection.

Antimicrobial resistance

Antimicrobial resistant *Salmonella* isolates to commonly used antimicrobials were detected; all isolates were resistant at least for one antimicrobial. Of the total tested isolates; 83.3, 50, 16.7, and 16.7% of isolates were resistant to tetracycline, ampicillin, amoxicillin and chloramphenicol, respectively. However, all the isolates were susceptible to gentamycin, ceftriaxone, ciprofloxacin, and sulfamethoxazole. All of the total isolates were resistant to one or more of the tested antimicrobials; 50% were multiple antimicrobial resistant while the rest half were resistant to single antimicrobial. This finding is in contrast to Zewdu (2004) who reported 25% antimicrobial resistant *Salmonella* isolates from cottage cheese. Detection of antimicrobial resistant *Salmonella* might be associated with their frequent usage both in livestock and public health sectors as these antimicrobials are relatively cheaper and commonly available (D'Aoust, 1997). The effectiveness of gentamycin, ceftriaxone, ciprofloxacin, and sulfamethoxazole in this study might be due to the difference in frequency of usage among the available antimicrobials, the nature of drugs, and their interaction with the bacteria. Different individuals reported antimicrobial resistant *Salmonella* isolates in previous studies from Ethiopia (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Molla et al., 1999; Molla et al., 2003) and from other countries (D'Aoust et al., 1992; White et al., 2001).

The findings of 100% antimicrobial resistant *Salmonella* isolates from examined dairy items samples were remarkable. It represents public health hazard due to the fact that food poisoning outbreaks would be difficult to treat and this pool of MDR *Salmonella* in food supply represents a reservoir for the transferable resistant genes (Diaze De Aguayo et al., 1992). The reasons for the recovery of antimicrobial resistance *Salmonella* isolates was most likely due to the indiscriminate use of antimicrobials (WHO, 1988; Guthrie, 1992), self medication due

to easy access to antibiotics without prescription in public health sector, and administration of sub therapeutic dose of antimicrobials to livestock for prophylactic or nutritional purpose (Acha and Szyfers, 2001). This might also be due to the use of antimicrobials for the promotion of growth and prevention of disease in food animals.

The present study indicated importance of dairy products as potential source of *Salmonella* infection. All the isolates were resistance to one or more of the tested antimicrobials. Tetracycline, ampicillin, amoxicillin, and chloramphenicol are less effective drugs against *Salmonella* isolates which limit therapeutic choice both in animal and human health care management. Therefore, food manufacturers should design comprehensive programs as Good Manufacturing Practice (GMP) and implementation of Hazard Analysis and Critical Control Points (HACCP) system to ensure the freedom of dairy products from this pathogen and concerned individuals should create awareness to food handlers and consumers.

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