Escherichia coli, Yersinia, Shigella, and Enterobacter. Salmonella is responsible for various diseases such as Salmonella, Campylobacter, Listeria, pathogenic mushrooms [5,6]. There are different bacteria that cause foodborne infections, caused by a variety of bacteria, viruses, parasites, and fungi. There are more than 250 different food-borne diseases and most of these diseases are caused by a variety of bacteria, viruses, parasites, and fungi.

Introduction

Milk has been described as a nearly perfect food since it contains the vital nutrients essential to the body, but it is also considered as a good medium for many microorganisms [1]. Raw untreated milk is still used by a large number of farm families and workers. In the raw milk value chain, milk producers, vendors and shop outlets can influence the prevalence of harmful pathogens in milk through poor hygienic, sanitary and agricultural practices [3]. Milk contamination by zoonotic pathogens is often natural but can occur through handling milk in unhygienic conditions [1,4].

Food-borne bacterial diseases are a serious challenge to public health in developed and developing countries. There are more than 250 different food-borne diseases and most of these diseases are infections, caused by a variety of bacteria, viruses, parasites, and fungi. Milk and milk products takes place under poor hygiene, sanitary and agricultural practices [3]. Milk contamination by zoonotic pathogens is often natural but can occur through handling milk in unhygienic conditions [1,4].

Salmonella is transmitted to animals and humans through consumption of contaminated food products (milk, eggs, and meats), direct contact with infected animals, through contaminated equipment such as stainless steel, hanging material, bucket, where milk is collected and stored, are a key mechanism for pathogens to contaminate food products [12]. In livestock, clinical signs typically appear 6-24 hr after exposure and include profuse diarrhea, fever, abdominal pain, and sometimes death.

Keywords: Dairy farms; Isolation; Hawassa; Multiple drug; Antimicrobial resistance; Salmonella

Abstract

A cross-sectional study was conducted from November 2017 to May 2018 on selected dairy farms in Hawassa town to isolate and assess the in-vitro antibiotic biogram of Salmonella from lactating dairy cows. A total of 216 samples were collected from selected dairy farms. All samples were processed bacteriologically following standard procedures outlined by ISO 6579: 2002. The overall prevalence of Salmonella was 12.9% (28/216) of the total samples. Out of total, 64.3% (18/28), 10.7% (3/28) and 25% (7/28) were from lactating cows, personnel’s, and equipments, respectively. Based on antimicrobial susceptibility testing, all isolates were resistant at least to one or more antimicrobials tested. According, 96.4% (27/28), 82.1% (23/28) and 75.0% (21/28) isolates showed resistance for oxytetracycline, kanamycin, and nalidixic acid, respectively. Out of all the resistant isolates, 96.4% (27/28) showed multiple antibiotic resistance (resistance to two or more antibiotics) pattern. Multiple antimicrobials resistance was observed in 66.7% (18/27), 74.0% (20/27) and 25.9% (7/27) from lactating cows, personnel’s, and equipments, respectively. Thus, awareness creation to the public regarding the public health importance of multiple drug-resistant Salmonella species and the consumption of unpasteurized milk and milk products is important.

Introduction

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The prevalence of salmonella infection varies across regions, however, the diseases caused by S. enterica serovars are especially prevalent in developing areas, such as Southeast Asia, Africa and South America that leads to an estimated 20 million cases of humans and 200,000 deaths each year. Challenges such as antibiotic-resistant Salmonella strains also pose a significant threat to deliver reliable therapies [16]. In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of Salmonellosis because of the limited scope of studies and the lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of Salmonellosis. Continuous surveillance of the prevalent Salmonella serovars and assessing their antimicrobial resistance pattern is essential to control the spread of the pathogen [17].

Antibiotic resistance in Salmonella is a rising problem over the past decades. Improper use of antibiotics in both human and veterinary medicine has caused bacteria to develop resistance against therapeutic antibiotics [18,19]. Using antimicrobial agents for cattle has been implicated as a source of human infection with Antimicrobial-Resistant (AMR) Salmonella through direct contact with livestock and consumption of raw milk, meat and contaminated material [20]. Antimicrobial-resistant Salmonella are increasing due to the use of antimicrobial agents in food animals at subtherapeutic level or prophylactic doses that may promote growth and markedly increase the human health risks associated with consumption of contaminated milk and meat products through mutation, acquisition of resistance encoding genes and irrational use of antimicrobials in food animals [21-23].

Accordingly, there are limited studies regarding the assessment of the pathogens isolated from apparently healthy animals at farm level, personnel’s and different types of equipment. Thus, the screening of milk and other dairy products against pathogenic organisms will play a vital role in curtailting human infection. Studying the prevalence and antimicrobial resistance of Salmonella from cattle and in contact with humans in dairy farms is the most important to design methods of minimizing the possible transmission of Salmonella between humans and cattle. Moreover, it important in combating the emergence of antibiotic-resistant strains of Salmonella [11]. Hence, this study was conducted to isolate, identify and assess the multiple drug resistance pattern of Salmonella isolates from selected dairy farms in Hawassa town.

Materials and Methods

Study area

The current study was conducted from November 2017 to May 2018 in selected dairy cattle farms of Hawassa towns. It is located 275 km south of Addis Ababa. Hawassa is situated at an altitude of 1750 m above sea level and according to an estimate, it lies between 6 °83’ to 7 °17’ N and 38 °24’ to 38 °72’ E. Hawassa receives an average annual rainfall of 955 mm with mean annual temperature of 20 °C and the city has a total area of about 50 km² divided into eight sub-cities and 32 kebeles (kebeles are the smallest administrative unit below the sub-city/woreda level) [24].

Study population

The study animals were apparently healthy dairy cows that were located in Hawassa town. The study includes dairy cattle kept under different (extensive, intensive and semi-intensive) management systems as well as farm personnel and equipments. There are different types of farms including small, medium and large scale having dairy cattle ranging from five to twenty two. Besides, the farms were selected purposively based on the availability of lactating cows and the willingness of the owners.

Study design and Sampling technique

A cross-sectional study was carried out from November 2017 to May 2018 to isolate, identify and assess the multi-drug resistance pattern of the salmonella isolates from selected dairy farms. The farms are selected purposively based on the availability and accessibility of study animals. Accordingly, a total of 216 samples were collected from selected dairy cattle farms in the study area.

Sample collection, handling and transportation

Samples were collected aseptically from apparently dairy cows (milk and feces), hands of personnel working in the farms and from equipment (container and buckets). Then the all samples were collected after getting proper consent from the personnel and Hawassa university to perform the research activity. Fecal samples were collected directly from the rectum and put into 50 ml containing a universal screwed capped bottle and 10 ml of milk was collected aseptically from all teats in a sterile test tube after aseptically preparing the teats thorough scrubbing with a cotton moistened with 70% denatured alcohol and the first 3-4 streams of milk were discarded. All types of swab samples (milkers’ hand, container and buckets) were collected before the commencement of the milking process using a sterile wooden cotton swab and were put into a sterile test tube containing 10 ml buffered peptone water used as transport media. All sample types were properly labeled with permanent marker. Then, the samples were immediately transported using an icebox to the Microbiology Laboratory of Hawassa University for further bacteriological examination.

Isolation and identification of salmonella

The isolation and identification of Salmonella was performed at the Microbiology laboratory of Hawassa University by using techniques recommended by International Organizations for Standardization (ISO-6579, 2002) [25]. The detection of salmonella was performed based on the following four successive stages: Firstly, All samples were pre-enriched in non-selective liquid media and processed separately. Then, 1 gm of fecal sample and 1 ml of milk was pre-enriched with 9 ml of Buffered Peptone Water (BPW) and incubated for 24 hrs at 37 °C. Secondly, all samples were transferred to selective media such as Tetrathionate Broth and Rappaport Vassiliadis Salmonella Enrichment Broth. A 1 ml of pre-enriched sample was transferred aseptically into a tube containing 10 ml of Tetrathionate Broth and incubated at 37±1 °C for 24±3 hours. Another 0.1 ml of the culture
obtained in pre-enrichment broth was transferred aseptically into a tube containing 10 ml Rappaport Vassiliadis Salmonella Enrichment Broth (Harmonized) and incubated at 41.5±1 ºC for 24±3 hrs.

Thirdly, Plating out and identification of the samples were conducted using Xylose lysine Desoxycholate (XLD) agar and Salmonella-Shigella (SS) agar plates. A loopful of inoculum from cultures of the selective enrichment media were streaked on to XLD and SS agar plates and incubated at 37 ºC for 24 hrs. Then, all colonies that grow on the XLD medium, produces hydrogen sulfide (H₂S) and colorless colonies with black center on SS medium were streaked onto Nutrient Agar and incubated at 37 ºC for 24 hrs for further confirmation through serious of biochemical tests. Finally, All suspected colonies were subjected to a series of different biochemical tests using the procedure of (ISO 6579, 2002; to confirm salmonella [25]. Triple Sugar Iron Agar (TSIA), Urease, Citrate, Indole, Methyl red and Voges Proskouer (VP) tests were performed on all suspected isolates to confirm the salmonella. All presumptive salmonella Isolate were cultured on Nutrient Agar for further antimicrobial susceptibility testing.

Antimicrobial susceptibility test

The antibiotic susceptibility tests of the Salmonella isolates were performed according to the Clinical and Laboratory Standards Institute (CLSI) method using Kirby-Bauer disk diffusion test on Muller-Hinton Agar (HIMEDIA, India) [26]. Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube containing 5 ml of Saline water and emulsified. The broth culture was incubated at 37 ºC for 4 hrs until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller-Hinton agar plate within a sterile safety cabinet. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic discs with a known concentration of antimicrobials were placed and the plates were incubated for 24 hrs at 37 ºC.

Amoxicillin (AML) (25 μg), Cefoxitin (FOX) (30 μg), Chloramphenicol (C) (30 μg), Gentamycin (CN) (10 μg), Streptomycin (S) (10 μg), Kanamycin (K) (30 μg), Nalidixic acid (NA) (30 μg), Ciprofloxacin (CIP) (5 μg), Oxytetracycline (OT) (30 μg) and Trimethoprim-sulphamethoxazole (SXT) (25 μg), were selected based on availability and their current use in human and veterinary medicine. All the antibiotics were from Oxioid Hampshire, England, and the expiry date was properly checked before application. Zone of inhibition of individual antibiotic agent was interpreted in to susceptible, intermediate, and resistance categories by referring recommended clinical and laboratory standards institute [26].

Data analysis

Data collected from field and laboratory investigations were recorded, and coded using Microsoft Excel 2013 program and analyzed using STATA version 13.0. Descriptive statistics were used to figure out the proportions of Salmonella isolate. Moreover, the antibiotic efficacy of each drug was determined by comparing the
In this study, out of 216, the overall prevalence of Salmonella was 12.9% (28/216). From the overall proportion, 64.3% (18/28), 10.7% (3/28) and 25% (7/28) were isolated from the milk and feces of dairy cows, personnel and equipments, respectively. 19% of Salmonella were isolated from plastic container milk. A higher proportion of Salmonella was isolated from milk samples (12.1%) than fecal samples (7.7 %) (Figure 1).

Antimicrobial susceptibility of Salmonella isolates

In the present study, out of 28 isolates, 27 isolates showed multiple drug resistance. Accordingly, all isolates were susceptible to ciprofloxacin, cefoxitin and trimethoprim-sulphamethoxazole with proportion of 96.4%, 89.3%, and 78.6%, respectively. However, all isolates were 96.4%, 82.1% and 75.0% resistant to oxytetracycline, kanamycin and nalidixic acid, respectively. On the other hand, all isolates were 100% sensitive to gentamycin (Table 1).

Multiple drug resistance patterns of Salmonella isolates

Multiple drug resistance (isolates that were resistant for two or more antibiotics) were detected in 96.4% (27/28) of the Salmonella isolates. Out of these, 66.7% (18/28), 7.4% (2/28) and 25.9% (7/28) isolates were from lactating cows, personnels’ and equipments, respectively. The higher multi-drug resistance pattern was observed in K, NA, OT, with the proportion of 25.9% followed by K, NA, OT, with the proportion of 14.8%. The other 19% of the resistant isolates were resistant to six and more antibiotics (Table 2).

Discussion

In this study, out of 216 samples collected from selected dairy farms in Hawassa town, the overall proportion of Salmonella isolated from dairy cows, personnels’ and equipment were 12.9%. This was higher than the reports of where 7.2% were found in slaughtered small ruminants and environment in Modjo export abattoir [27], 7.1% from apparently healthy slaughtered cattle in Debre Zeit and the study on cheese and milk in Debret Zeit (2.1%) as well as dairy product in Addis Ababa (1.6%) [21,28-30]. However, the current finding was comparable with 10.5% from apparently healthy dairy cows in Modjo [31], 10.76% from lactating cows and in contact humans in dairy farms of Addis Ababa and (11.5%) among chicken table eggs at Kombolcha [18], Ethiopia [32]. The present result was lower than the findings of who reported 20% in raw milk from the Korsa district and Ejeta et al., 2004 who reported 14.7% from minced beef, mutton and pork samples among supermarkets in Addis Ababa [33].

In this study, the prevalence of Salmonella from milk and feces of apparently healthy lactating dairy cows was 64.3%. This was higher than who reported 7.1% from apparently healthy slaughtered cattle [28]. This variation could be due to the test procedures and techniques used since pre-enrichment steps using buffered peptone water was employed in this study and source of sample. Similarly, the report of [34]; from England (0.2% and 4%), from Northern Thailand (3%) and from Cameroon (27%) are much lower than the current study [35,36]. The current result was higher than the prevalence recorded in Iran 4% and in USA 7.3% and in Nigeria (15%) and 10.9% reported in Namibia on bovine and ovine bone-and-meat meal and blood meal samples [19,37,38]. This may be attributed to the variation in agroecological location of the cattle, housing conditions, feeding habits, and types of feed provided for the cattle.

According to the current investigation, Salmonella was isolated from the fecal samples of apparently healthy lactating dairy cows with a rate of 7.7%. This finding was higher than the report of from Egypt where prevalence in on fecal shedding of Salmonella among dairy cattle was 1.56 [39]. However, this result was lower than from the United States (9.7%) [40], from central Texas [41], USA where Salmonella shedding rate from fecal samples of dairy calf was 70%. This huge difference might be in the report from Texas, all isolates were one serotype (S. kinshasa) and this serotype might have specific host requirement.

In the present study, Salmonella was isolated from milkers’ hand swab with a rate of 14.3%. This was higher than the report of (8.9%) from small ruminants slaughtered in Modjo export abattoir [27]. However, it was lower than the work of Beyene et al., 2016 (28.6%) from pooled milkers’ hand swab of personnels’ working in Asella Municipal abattoir.

### Table 2: Multiple antibiotic resistance MAR profile of Salmonella isolates.

<table>
<thead>
<tr>
<th>MAR pattern</th>
<th>Number of isolates</th>
<th>Multiple drug resistance patterns</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two</td>
<td>3</td>
<td>OT, NA</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>OT, K</td>
<td></td>
</tr>
<tr>
<td>Three</td>
<td>7</td>
<td>OT, K, NA</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>OT, K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NA, OT, K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>OT, CIP, C</td>
<td></td>
</tr>
<tr>
<td>Four</td>
<td>1</td>
<td>OT, AML, NA</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>NA, OT, SXT, K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>OT, AML, SXT, K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>NA, S, OT, K</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Five</td>
<td>1</td>
<td>NA, C, OT, K</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>OT, AML, K, NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>FOX, OT, K</td>
<td></td>
</tr>
<tr>
<td>Six</td>
<td>1</td>
<td>FOX, S, AML, K, NA</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>OT, AML, SXT, K, NA, C</td>
<td></td>
</tr>
<tr>
<td>Eight</td>
<td>1</td>
<td>S, C, OT, AML, K, NA, FOX</td>
<td>1 (3.7)</td>
</tr>
</tbody>
</table>
The present study revealed that 96.4% of the isolates were resistant for two or more antibiotics which was comparable with the finding of [31]. However, it was higher than the previous studies conducted in Ethiopia and elsewhere in the world [21,28,43-46]. This difference may be due to the increasing rate of inappropriate utilization of antibiotics in the dairy farms which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria [47,48].

The result of the current research indicated Salmonella isolates were resistant to Oxytetracycline, kanamycin, and nalidixic acid with a resistance rate of 96.4%, 82.1%, and 75% respectively. Similarly, reported that the isolates of Salmonella from food items and personnel from Addis Ababa were resistant to the commonly used antibiotics including streptomycin [21], and oxytetracycline. However, resistance rates to oxytetracycline are very high compared to results documented in America reported 95.6% and 87.8% sensitivity levels [37], respectively and Iran reported 42.58% sensitivity for both antibiotics [19]. In this study, 96.4% of the isolates showed resistance to two or more antibiotics which is lower than a report from Addis Ababa, Ethiopia (83%) [18].

According to the study, Salmonella isolates were susceptible to gentamicin and ciprofloxacin with the rate of susceptibility 100% and 96.4% respectively. This was in agreement with the reports of where Salmonella isolated from apparently healthy slaughtered sheep in Turkey showed 100% sensitivity to these antibiotics [36], with and with the report of in Iran where ciprofloxacin was 100% effective [19,30]. However, it was higher than who reported 73.3% and 83.3% [11], who reported 73% and 95% for ciprofloxacin and gentamycin, respectively [33]. This variation might be due to small sample sizes for the data, nature of the drug, presence of different strains of the bacteria, development of resistant gene, their low-frequency usage for prevention and control of disease in food animals in the study area.

The present study revealed that Salmonella isolates were resistant to tetracycline and ampicillin with a rate of 96.4% and 39%, respectively which disagrees with the report of in Egypt reported that each of the ampicillin and tetracycline was 85.7% effective against Salmonella species isolated in dairy cattle [39]. In addition, in the present study trimethoprim–sulphamethoxazole was an effective drug (78.6%) against salmonella isolates that disagrees with the report by who reported 100% resistance to trimethoprim-sulphamethoxazole [39]. A higher activities of gentamycin (100%) observed in the current study disagree with a study in Texas, USA, reported 85% and this difference might be due to availability and oversee of the drug in the farm of the current study [41]. In the current study, ciprofloxacin was 96.4% effective against all isolates which was in line with a report in Sudan where ciprofloxacin was 100% effective to all human and cattle Salmonella isolates [49]. The result for streptomycin resistance in this study (10.7%) was lower than 13.3% and 25%, which was reported by and [18,33], respectively. Amoxicillin resistance in this study (25%) was higher than 16.7% reported by [30]. The resistance of chloramphenicol in this study 17.9% is consistent with 16.7% reported by and [18,30], and lower than 25% reported by [33].

According to the antimicrobial susceptibility testing, all of the isolates showed multiple drug resistance to at least one or more drugs tested were observed which was in line with the report of [30,33,50]. Moreover, 96.4% of the isolates showed multiple drug resistance for two or more types of antimicrobials. This was higher as compared to the report of who reported 70% and 30% [33], who report 83.3% and 16.3% [50], and who reported 50% and 50% for multiple and single antimicrobial resistance, respectively [30].

In general, antimicrobial use is a key driver of resistance development, which is either overuse for minor infectious, misuse due to lack of access to appropriate treatment and underuse due to inadequate dosing, poor adherence or substandard antimicrobial and lack of financial support to complete treatment course. The present study indicated the importance of cattle products (milk), personnel working in the farms and materials/equipment used as a potential source of Salmonella infection.

Conclusion and Recommendations

In the present study, the isolation of 12.9% Salmonella at dairy farms level showed that dairy cattle and their environment are important sources of milk contamination with the organism, and consumption of raw milk and other unpasteurized dairy products can lead to infection with zoonotic Salmonellosis. The presence of a high proportion of multiple antimicrobial-resistant isolates (96.4%) in the dairy farms to antimicrobials that are commonly used in the veterinary and public health set up in this study further signifies the public health importance of Salmonella in addition to treatment failure. In this study, all the isolated Salmonella revealed resistance at least to one of the antibiotics tested. In general, awareness creation to the public about the public health importance of foodborne diseases and the consumption of unpasteurized milk and milk products is important. Gentamycin and Ciprofloxacin should still be used as a choice to treat Salmonellosis. Further, the molecular characterization of the isolates with emphasis on resistant strains is important to identify mechanisms of antibiotic resistance.

References


