Reta et al. International Journal of Food Contamination (2016) 3:4
DOI 10.1186/s40550-016-0027-5

International Journal of Food Contamination

DATA ARTICLE Open Access

Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia

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Abstract

Background: Milk is a compensatory part of daily diet especially for the expectant mothers as well as growing children. It is virtually a sterile fluid when secreted into alveoli of udder. However, beyond this stage of production, microbial contamination might generally occur from different sources.

Methods: A cross-sectional study was carried out from March 2013-January 2014 in Jigjiga city to assess bacterial contamination of raw milk meant for human consumption and to determine antimicrobial susceptibility patterns of the isolates. A total of 120 raw milk samples were aseptically collected from different sampling points that were hypothesized to be a source of potential contaminations. Data were analyzed using SPSS version 17 computer software. *P*-value of <0.05 was taken as statistical significance.

Results: Overall, the organisms identified and their prevalence rates were *Escherichia coli* 70(58 %), *Staphylococcus aureus* 29(24.2 %), *Shigella Sp.* 21 (17.5 %), *Proteus sp.* 9 (7.5 %) and *Salmonella sp.* 4 (3.3 %). The isolation rates of these identified bacteria from each sampling points are statistically significant in *E. coli* and *Proteus sp.* ($P < 0.05$). High antibiotic resistance for *E. coli* isolates were observed to Doxycycline (42.3 %) and Ampicillin (30 %). *Shigella sp.* was resistant to Ampicillin (38.1 %). *Salmonella sp.* isolates were highly resistant to Amoxicillin (50 %). Out of a total of 29 *S.aureus* isolates, high resistance rate was observed to penicillin G 27(93.1 %) followed by tetracycline 20(69 %), and very low level of resistance to vancomycin 2(6.9 %) and rifampicin 1(3.4 %). Multidrug resistance was also observed in 55.2 % of the total isolates.

Conclusions: Considering the high rate of raw milk contamination with the above isolated bacteria, sanitary practice during collecting, transporting and vending is recommended since the consumption of unpasteurized milk may inflict an important public health risk.

Keywords: Bacterial contamination, Critical sampling points, Raw milk, Antibiotic, Jigjiga

Background

Milk is used throughout the world as a human food at least one form or more. It is virtually a sterile fluid when secreted into alveoli of udder. However, beyond this stage of production, microbial contamination might generally occur from different sources (Mennane et al. 2007). Conditions for contamination of raw milk at different critical points are due to less hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipments (Garedew et al. 2012). Milk is largely made up of water, within which a wide range of nutrients including vitamins, proteins, fats and carbohydrates are suspended. These rich nutritional contents, the production and processing procedures in commercial milk production render it susceptible to contamination by a host of pathogenic microbes that could cause diseases in humans. Therefore, milk is known to be an efficient vehicle for transmission of disease causing agents to humans (Garedew et al. 2012). The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and

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manufacturers to produce and market safe milk and milk products (Adesiyun et al. 1995; Mennane et al. 2007). Milk and milk products have important role in feeding the rural and urban population of Ethiopia owing to its high nutritional value. It is produced daily, sold for cash or readily processed. It is a cash crop in the milkshed areas that enables families to buy other foodstuffs and significantly contributing to the household food security (Abebe et al. 2012). Lack of refrigeration facilities at farm and household level in developing countries of tropical regions with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Godefay and Molla 2000). Milk and milk products may carry toxic metabolites of different pathogenic organisms growing in it. Ingestion of such products contaminated with these metabolites cause food poisoning for consumers. On the other hand the ingestion of viable pathogenic bacteria along with the food product leads to food borne infection (Aneja et al. 2002). The disease causing bacteria in the milk are *Salmonella sp. Mycobacterium bovis, Corynebacterium sp., Clostridium perfringens, Yersinia enterocolitica, Coxiella burnetii, Brucella, Staphylococcus sp., Campylobacter jejuni, Mycobacterium avium, Listeria sp., Escherichia coli,* and coliforms (Fadaei 2014; Olatunji et al. 2009). The total coliforms, *E. coli* and other enteric bacteria are reliable indicators of fecal pollution generally in insanitary conditions of water, food, milk and other dairy products. Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic microorganism which could constitute a public health hazard (Soomro et al. 1996). These microorganisms are usually associated with food borne diseases and outbreaks, as recorded by official health organizations (Bouazza et al. 2012). The presence of these pathogenic bacteria in milk appeared as main public health concerns, especially for those people who still drink unpasteurized raw milk (Claeys et al. 2013). Despite this, the aim of this study was to determine the presence of contaminating microorganisms and their antibiotic resistance patterns in the raw milk produced by individual farmers, collectors and milk vendors in Jigjiga city, eastern Ethiopia.

Methods

Study area, design and study period

A cross-sectional study was conducted in Jigjiga city from March 2013-January 2014. Jigjiga is the capital city of Ethiopian Somali Regional State located at 628 km east of Addis Ababa at 9° 20' north latitude and 42° 47' east longitude. The altitude of the district ranges from 900–1600meters above sea level and receives an annual rainfall of 300–500 mm with the mean minimum and maximum annual temperatures of 20°c and 28°c respectively (CSA 2003). The community in this region is pastoral and agro-pastoralist and there is large milk production from cows, camels and goats. The study populations were raw cow's milk from individual farmers' cows, milk collectors, and milk venders in Jigjiga city.

Collection of raw milk samples at critical sampling points and transportation

Milk samples were collected from points considered to be associated with contamination (critical sampling points). The sampling points were the teat during milking, milking buckets at farm level, transport containers, and selling point up on arrival at the market. Overall, 120 raw milk samples were analyzed: of these, 30 raw milk samples were from teat, 30 from milking buckets, 30 from storage containers, and 30 from selling point up on arrival at the markets. During sampling of raw milk directly from teats, the udder and teats were cleaned and dried before sampling; each teat end was scrubbed gently with cotton swabs moistened with 70 % ethyl alcohol. The first 3-4 streams of milk were discarded, and approximately 10 ml of milk was collected into sterile sampling bottles. The other raw milk samples were collected in the morning following standard safety procedures. Prior to sampling from milking buckets and transport containers, the milk was thoroughly mixed by shaking and 25 ml of milk was transferred into a sterile screw capped bottle. Transportation of samples to the Ethiopian Somali Regional Laboratory was immediately conducted for further processing using ice packs following the standard safety procedures (Robinson 2002).

Bacterial identification and isolation from milk samples

Detection of *E.coli:* All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and biochemical examinations. The samples were inoculated on MacConkey Agar (Difco laboratories, USA) and incubated aerobically at 37°c for 24 h. The plates were observed for the growth of *E. coli*. A single, isolated colony was picked and sub-cultured again on MacConkey agar for purification of the isolate. Simultaneously another single colony with similar characters was picked for the preparation of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. The cultural characteristics of the isolates were confirmed by inoculating the pure colonies on Blood Agar (Oxoide, Germany), Nutrient Agar (Oxoid CM0003, Basingstoke, England), Nutrient Broth and Violet Red Bile Agar (Oxoid CM107). Biochemical tests were performed to confirm the *E. coli* using catalase test, Simmon's Citrate Agar, sugar fermentation on Triple Sugar Iron Agar(Oxoid CM0277, Basingstoke, England), Gelatin liquefaction, Indole Production, Nitrate reduction, Urease production, Voges proskaur, Methyl red and Presumptive test. **Detection of *Salmonella Sp:*** The

isolation and identification involves three steps; 1 ml of milk was pre-enriched with 9 ml of buffered peptone water (Oxoid CM509, Basingstoke, England) and incubated for 24 h at 37°c. A portion (0.1 ml) of the pre-enriched cultured was transferred to 10 ml of selenite cysteine broth (Merck) and incubated at 37°c for 24 h respectively. Finally, from the selective enrichment media the sample was inoculated on to xylose lysine deoxycholate (XLD) agar (Oxoid CM0469, Basingstoke, England) and incubated at 37°c for 24 h. Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish color and a black center were sub-cultured on nutrient agar and confirmed biochemically using triple sugar iron agar (TSI)(Oxoid CM0277, Basingstoke, England), Christensen's urea agar (Oxoid CM53, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England), Voges Proskauer (VP), methyl red (MR)(Micromaster Thane, India), and Indole tests (Becton Dickinson, USA) (Hendriksen 2003). **Detection of *S.aureus*:** Gram staining was performed (Cruikshank et al. 1975) and Gram-positive cocci that occurred in clusters under the microscope were subjected to preliminary biochemical tests (the catalase and oxidase tests). The identities of the isolates were confirmed based on positive results for the DNase test, beta haemolytic patterns on blood agar enriched with 5 % (v/v) sheep blood and the coagulase slide test for *S. aureus* using the (PROLD Diagnostics, Canada). The slide agglutination test was performed according to the manufacturer's instructions. Briefly, cells from a pure colony were placed on the clean area of the slide using a sterile toothpick and a drop of the PROLD reagent was added. These were mixed using the toothpick and the isolates were identified based on the formation of agglutination. An isolates that formed agglutination were recorded as *S. aureus* and maintained at 4°c in 30 % glycerol for further characterization by antibiotic susceptibility testing. **Detection of *Shigella Sp:*** Specimens were plated directly on primary media: Salmonella-Shigella agar (Merck) and Selenite F broth (Mast Diagnostics DM 210, Mast Diagnostics, UK). For those negative samples on primary sold media, sub-culturing from enrichment broth to primary media was performed to improve recovery of the isolates. All of the inoculated media were incubated at 37°c for 18-24h. The non-black colonies observed on the center were suspected positive test for *Shigella sp*. and Klingler Iron Agar (KIA) was used for biochemical differentiation of *Shigella* from other coliform bacteria. Colonies of suspected *Shigella* was inoculated on Salmonella-shigella Agar plate (Merck), deoxycholate citrate agar (DCA) (Oxoid CM 35; Oxoid Ltd, UK) and incubated at 37°c for 24 h. Growth of suspected *Shigella sp.* change in color butt of media its color(red) to yellow and red slope remained as it is because *Shigella sp*. is lactose fermenter in anaerobic condition. **Detection of *Proteus Sp*:** One (1 ml) of milk sample was enriched in 10 ml of Buffer peptone water aseptically and incubated at 37°c for 24 h. Inoculum from the enrichment broth was streaked on Hektoen Enteric Agar (HEA) and MacConkey Agar (Difco laboratories, USA) and incubated at 37°c for 24 h. The cultures were identified on the basis of their morphological, and biochemical characteristics.

Antimicrobial susceptibility testing

The antimicrobial susceptibility patterns of the above detected bacteria were carried out following the Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid CM0337 Basingstoke, England) as described by the Clinical and Laboratory Standards Institute (CLSI 2008). The criteria used to select the antimicrobial agents tested were based on the availability and frequency of prescription for the management of bacterial infections in animals as well as for human in Ethiopia and on the basis of their different structures and mechanisms of action. Antimicrobial susceptibility test was performed for all *S. aureus* isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI 2008). For susceptibility test for *S. aureus*, one anti-microbial from each subclass of antimicrobials which were commonly used for treatment of bovine mastitis or considered as important antimicrobial agents for human were selected for antibiogram based on the criteria of Clinical and Laboratory Standards Institute (CLSI 2008). Thus, antimicrobials used for treatment of bovine mastitis included in this study were erythro-mycin (E/15 μg), cephalothin (KF/30 μg), penicillin-G(10unit), sulphoxazole-trimethoprim (SXT/25 μg), amoxicillin-clavulinic acid (AMC/30 μg), chloroamphenicol (C/30 mg), (Oxoid), tetracycline (TE/30 μg) and gentamicin (CN/10 μg) (Biomerioux). Antimicrobials not used for treatment of bovine mastitis but important for human were oxacillin (OX/1 μg), vancomycin (VA/30 μg), clindamycin (DA/10 μg) and rifampicin (RD/5 μg) (Oxoid). Finally, the diameters of the zone of inhibition around the disks were measured to the nearest millimeter using rulers, and the isolates were classified as susceptible, intermediate and resistant (CLSI 2008). *E. coli ATCC 25922* was used as a quality control organism for the antimicrobial susceptibility test (Hendriksen, 2002). Moreover, isolates showing resistance to three or more antimicrobial subclass were considered as multidrug resistant.

Statistical analysis

The collected data for bacterial contamination analysis were entered and analyzed using SPSS version 17 computer software. Accordingly, descriptive statistics such as percentages and frequency distribution was used to describe/present bacterial isolates and antimicrobial susceptibility which was expressed as percent of resistant,

intermediate and susceptible. *P*-value <0.05 was taken as cut-off for statistical significance.

Results

Isolated bacterial species

Overall, five bacterial targets were identified in the milk sampled in the study area. The bacteria so identified and their isolation rate were *E.coli* 70(58 %), *Salmonella sp.* 4(3.3 %), *Shigella sp.* 21(17.5 %), *Staphylococcus aureus* 29 (24.2 %) and *Proteus Sp.* 9(7.5 %). These are indicative of significant contamination of milk and important human pathogens. The most prevalent organism overall was *E. coli*, while the least prevalent was *Salmonella sp*. In this study, the contamination degree of milk by the isolated bacteria is utterly worsened at each critical sampling point. High contamination level was observed at market point sampled milk. The difference in isolation rate across market chain (critical sampling points) is statistically significant in *E. coli.* ($P = 0.00$) and *Proteus sp*. ($P = 0.016$) (Table 1).

Results of the present study revealed that 49 (40.8 %) of milk sampled had at least two different bacterial organisms, 6 (12 %) from the udder, 10(20.4 %) milking bucket, 12 (24.5 %) from storage container and 21 (42.9 %) from the market point (Fig. 1).

Antimicrobial susceptibility of the bacterial isolates

The antimicrobial susceptibility tests of the bacterial isolates were grossly very variable. About 76.1 % *E. coli* isolates were resistant and it had the highest resistance rates to Doxycycline, Ampicillin and Gentamycin (42.3 %, 30 % and 30 %) respectively. A quarter of *E. coli* isolates (25.4 %) were multidrug resistant (≥3drugs). Similarly higher antimicrobial resistance (74.6 %) was recorded against *Salmonella sp.* isolates as well. The highest resistance rate to *Salmonella sp.* was observed in Amoxicillin (50 %). The highest resistance rate to *shigella* was observed in Ampicillin (38.1 %). All s*higella* isolates were highly susceptible to Co-trimoxazole(81 %). About 14 % of *Shigella* isolates were multidrug resistant-fairly better than *E.coli* isolates. All *Proteus sp.* isolates were 66.7 % sensitive to Ciprofloxacin and showed (55.6 %) resistance to Ampicillin (Table 2).

The observations made in the present study clearly proved that *S. aureus* showed resistance to all antimicrobials tested except for Rifampicin and Vancomycin. These indicate that the problem is highly distributed and disseminated. Moreover, the overall resistance of *S. aureus* isolates, to Vancomycin, Rifampicin, Clindamycin and Gentamycin showed less than 25 % of resistance. The highest resistance rate was observed in Penicillin (93.1 %), followed by Tetracycline (69 %). On the other hand, about 55.2 % (16/29) of *S.aureus* isolates were found to be multidrug resistant (Table 3).

MAR phenotypes of *S. aureus*

Multiple antibiotic resistance (MAR) phenotypes were determined for *S. aureus* (Table 4). The predominant MAR phenotypes for *S. aureus* isolated from this study area were PG-TE -Ox and PG-TE-AC-E-SXZ-Ox in 24.1 % and 17.2 % of the isolates, respectively. Furthermore, MAR phenotypes PG-TE- AC-Ox, PG-TE-AC-E-SXZ-Ox-CN, PG-TE-AC-E-SXZ-Ox- CN-CH and PG-TE-AC-E-SXZ-Ox- CN- VA were obtained in 3.4 % of the isolates. Also PG-TE-AC 6.9 %, PG-TE-Ox- KF 13.8 % and PG-TE-AC-E-Ox 6.9 % were the MAR phenotypes for *S. aureus* isolated from this study area (Table 4).

It is thus evident that MAR *S. aureus* was isolated from all critical sampling points. However, among the isolates from this study area 55.2 % of the isolates develop MAR. Among all MAR phenotypes of *S. aureus*, 40.3 % of them were resistance to six different antibiotics and 7.2 % were resistance to seven antibiotics. Fifty four percent (54 %) of them were resistance to 3 or 4 antibiotics.

Discussion

The outcome of our study revealed that 84.1 % of milk samples were contaminated with at least one bacterium that comprised of *E. coli, Salmonella sp., Shigella sp., S. aureus,* and *Proteus Sp.,* with isolation rates of 70(58 %), 4(3.3 %), 21(17.5 %), 29(24.2 %), and 9(7.5 %), respectively.

The levels of contamination with each isolated bacteria were higher across critical sampling points (from teats, Milking bucket, transportation container, and at market points). Similar findings reported by Daka et al.(2012) revealed that the level of contamination with *S.aureus*

Table 1 Occurrence of isolated bacteria across critical sampling points ($n = 120$) of milk collected from Jigjiga city

Bacteria isolated	Isolate No(%)	No. of positive sample (%)				*P*- value
		Udder ($n = 30$)	Collection Bucket($n = 30$)	Storage Material($n = 30$)	Market point ($n = 30$)	
E. coli	70(58 %)	9(30 %)	16(53.3 %)	22(73.3 %)	23(76.7 %)	0.00
Salmonella sp.	4(3.3 %)	1(3.3 %)	0(0.0 %)	1(3.3 %)	2(6.7 %)	0.140
Shigella sp.	21(17.5 %)	4(13 .3 %)	3(10 %)	4 (13.3 %)	10(33.3 %)	0.069
S. aureus.	29(24.2 %)	6(20 %)	7(23.3 %)	6(20 %)	10(33.3 %)	0.727
Proteus sp.	9(7.5 %)	0(0.0 %)	2 (6.7 %)	3(10 %)	4(13.3 %)	0.016

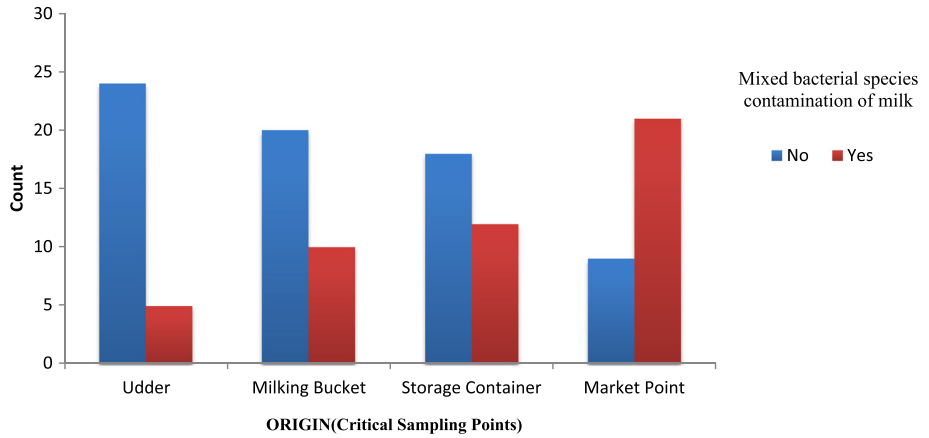

Fig. 1 Isolation of mixed bacteria from milk sampled at different sampling points (n = 120), Jigjiga city

were higher in milk obtained from teat(17.9 %), Milking bucket at farm level(25.7 %), storage containers at milk collection center(26.9 %) and from transportation container(21.8 %). Conditions for contamination of raw milk at different critical points are due to less hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipments, higher environmental contamination during transportation or contamination during waiting along the roadside (Garedew et al. 2012). Based on observations made during the collection of samples, we therefore report that improper hygiene and poor farm management practices contributed to the presence of these isolated bacteria in the milk. In this study area milk was obtained from animals by washing their hands and/or the utensils and containers used. In certain cases, untreated groundwater was used to wash the containers that were used for milking. This may have contributed to the high level of enteric bacteria and *S.aureus* isolated. Improving the hygienic conditions of the milking environment and/or utensils may reduce the prevalence of entropatogenic as well as *S.aureus* in milk and prevent its transmission to humans. Olatunji et al (2009) in Nigeria had reported that higher isolation frequencies of *E. coli* (24.4 %), *S. aureus* (38.2 %), and *Salmonella sp.* (2 %), from apparently normal milk samples from different critical sampling points. It is known that even when drawn under aseptic condition, milk always contains microorganisms which are derived from the milk ducts in the udder, in addition contaminants coming from milking utensils and human handlers (Solomon et al. 2013). Higher isolation frequencies, especially for *E. coli* across market chain was observed in the current study as compared to similar studies performed to assess bacteriological quality of raw milk in Ethiopia (Tassew and Seifu 2011; Tiruneh 1996). This might be due to poor and unhygienic bedding condition in the majority of farms and absence of teat dipping and disinfection practices in the current study. These practices have been known as critical components of mastitis prevention and control program in dairy herds (Galton et al. 1986). Other findings by different researchers confirm that *E. coli* grow well in milk and hence endanger its keeping milk quality (Frazeir and Westhoff 1988). *E. coli* and *coliforms* are often used as indicator microorganisms, and the presence of *E. coli* in milk samples implies a risk that other enteropathogenic bacteria may be present in the sample (Najib 2003; Olatunji et al. 2009; Arafa and Soliman 2013).

Table 2 Antibiotic sensitivity pattern of bacterial isolates in milk samples collected from Jigjiga city

Antimicrobial	*E. coli*(n = 70)			*Salmonella sp.*(n = 4)			*Shigella sp.*(n = 21)			*Proteus sp.*(n = 9)		
	R (%)	I (%)	S (%)	R(%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amoxicillin	15(21.4)	31(44.3)	24(34.3)	2(50)	1((25)	1(25)	2(9.5)	11(52.4)	8(38.1)	1(11.1)	6(66.7)	2(22.2)
Ampicillin	21(30)	28(40)	21(30)	1(25)	1(25)	2(50)	8(38.1)	8(38.1)	5(23.8)	5(55.6)	3(33.3)	1(11.1)
Ciprofloxacin	7(10)	17(24.3)	46(65.7)	1(25)	1(25)	2(50)	2(9.5)	6(28.6)	13(61.9)	0(0.0)	3(33.3)	6(66.7)
Co-trimoxazole	12(17.1)	22(31.4)	36(51.4)	1(25)	2(50)	1(25)	0(0.0)	4(19.0)	17(81)	1(11.1)	3(33.3)	5(55.6)
Chloramphenicol	15(21.4)	26(37.1)	29(41.4)	1(25)	2(50)	1(25)	3(14.3)	7(33.3)	11(52.4)	1(11.1)	5(55.6)	3(33.3)
Gentamycin	21(30)	35(50)	14(20)	1(25)	2(50)	1(25)	4(19.0)	10(47.6)	7(33.3)	2(22.2)	4(44.4)	3(33.3)
Doxycycline	30(42.9)	27(38.6)	13(18.5)	1(25)	2(50)	1(25)	6(28.6)	10(47.6)	5(23.8)	2(22.2)	3(33.3)	4(44.4)

R Resistance, *I* Intermediate, *S* Sensitive, *n* number

Table 3 Antimicrobial susceptibility pattern of *S. aureus* isolates (n = 29) from milk samples collected from Jigjiga city

Antimicrobial	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Pencillin (P)	1(3.4)	1(3.4)	27(93.1)
Chloroamphenicol (CH)	11(37.9)	10(34.5)	8(27.6)
Cephalothin (KF)	15(51.7)	5(17.2)	9(31.0)
Gentamycin (CN)	10(34.5)	12 (41.4)	7(24.1)
Erythromycin (E)	5(17.2)	17(58.6)	7(24.1)
Clindamycin (DA)	9(31.0)	16(55.2)	4(13.8)
Tetracycline (TE)	5(17.2)	4 (13.8)	20(69)
Amoxicillin + clavulanic	19(65.5)	0(0.0)	10(34.5)
Rifampicin (RD)	27(93.1)	1 (3.4)	1(3.4)
Oxacillin (OX)	18(62.1)	2(6.9)	9(31.0)
Vancomycin (VA)	24(82.8)	3(10.3)	2(6.9)
Sulphamethoxazole-timethoprim (SXZ)	19(65.5)	3 (10.3)	7(24.1)

Our results indicated that 24.2 % of the samples were positive for *S.aureus.* This is a favorable finding because, for human health some strains of *S. aureus* are capable of producing heat stable enterotoxins (Asperger 1994). A comparable finding to our result was reported by Abebe et al. (2013) that *S.aureus* prevalence was 15.5 % in raw milk samples. In contrast to this, different literatures revealed a very significant isolation rate of *S. aureus* from raw milk samples (Olatunji *et al*. 2009; Pourhassan and Taravat-Najafabadi 2011; Mohanty *et al*. 2013; Sanaa et al. 2005). Although the prevalence of *S. aureus* has been reported to vary with the size and geographic region of the area sampled, a high proportion of these bacteria in milk relates to poor hygiene practices. Based on observations made during the collection of samples, we therefore report that improper hygiene and poor farm management practices contributed to the presence of *S. aureus* in the milk. In this study low *salmonella sp.* isolation rate with 3.3 % was found. Junaidu et al (2011), Forough et al. (2012) and Sanaa et al.(2005) had reported comparable findings with 2.17 %, 4 % and 1.43 % prevalence respectively. Addis et al. (2011) reported a prevalence of 10.7 % from raw milk which is higher than the present report. In the other study by Addis et al. (2011) from 195 dairy cows tested 28.6 % were positive from milk samples. Akoachere et al.(2009) 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. The detection of *Salmonella* in 3.3 % of the samples tested indicates that the degree of prevalence of the pathogen in raw milk in jigjiga 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 or cultural reasons, or because of perceived health benefits (Karns et al. 2005). 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.

Table 4 The predominant MAR phenotypes for *S. aureus* isolated from milk samples (n = 29) collected from Jigjiga city

MDR patterns	Phenotype	Number observed	Percentage
Three	PG-TE-AC	2	6.9
	PG-TE -Ox	7	24.1
Four	PG-TE-Ox- KF	4	13.8
	PG-TE- AC-Ox	1	3.4
Five	PG-TE-AC-E-Ox	2	6.9
Six	PG-TE-AC-E-SXZ-Ox	5	17.2
Seven	PG-TE-AC-E-SXZ-Ox- CN	1	3.4
Eight	PG-TE-AC-E-SXZ-Ox- CN- CH	1	3.4
	PG-TE-AC-E-SXZ-Ox- CN- VA	1	3.4

The percentage representations of the phenotypes were obtained by dividing the number of a particular phenotype by the total number of multiple antibiotic resistant isolates identified in a given area. *VA* Vancomycin, *PG* Penicillin G, *SXZ* Sulphamethoxazole-timethoprim, *E* Erythromycin, *Ox* Oxacillin, *AC* Amoxicillin-Clavulanic Acid, *TE*Tetracycline, *KF* Cephalothin; *CN* Gentamycin, *CH* Chloroamphenicol

The isolation rates of *proteus Sp.* in this study (7.5 %) is comparable with the report by Junaidu et al (2011) with 8.69 % prevalence. Most of the organisms identified in this study were enteric bacteria indicating probable faecal contamination of the milk as a result of poor

hygiene. The practice of pooling milk from different sources by traders, and the absence of pasteurization generally observed among them could increase the risk posed by such organisms.

In the present study, Doxycycline had the highest resistance rates in *E. coli*. In contrast to this, fairly higher resistant rate was recorded in Ampicillin (100 %) and Amoxicillin (42.11 %) (Thaker et al. 2012). On the other hand the highest resistance rate for *Salmonella sp.* in this report was observed to Amoxicillin (50 %). Different researchers reported antimicrobial resistant *Salmonella* isolates of milk in their previous studies from Ethiopia (Molla et al. 2003; Mekonnen et al. 2005) and from other countries (White et al. 2001). Forough et al. (2012) reported that *salmonella sp.* isolates were resistance to Ampicillin (42.58 %), Tetracycline (42.58 %) and Nalidixic acid (78.57 %). Addis et al. (2011) reported a high resistance rate *salmonella* isolates to ampicillin (100 %). The remarkable degree of resistance to many drugs represents public health hazard due to the fact that food borne 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 were most likely due to the indiscriminate use of antimicrobials (WHO 1988), self-medication and administration of sub therapeutic dose of antimicrobials to livestock for prophylactic purpose (Acha and Szyfers 2001). Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (Forough et al. 2012).

In this study the highest resistance rate for *shigella sp.* was observed to Ampicillin (38.1 %) followed by Doxycycline (28.6 %). In contrast to our finding Sanaa et al. (2005) reported that *shigella* isolated from raw milk were sensitive to Gentamycin (64.3 %) followed by Chloramphenicol (92.1 %), and the highest antimicrobial resistant pattern was observed in Ampicillin and Amoxicillin (92.9 %, 92.9 %) followed by penicillin (42.9 %). In agreement with our result Ayalu et al. (2011) reported that *shigella* isolates were 100 % resistant to Ampicillin and Amoxicillin but sensitive to Chloramphenicol, Gentamicin, and Norfloxacin(41.2 %, 88.2 %, and 94.1 %) respectively. Shiferaw et al. (2012) reported that 74 % *shigella* isolates were resistant to Ampicillin, and 58 % to Streptomycin. On the other hand, All the *Shigella* isolates were resistant to Ampicillin, 94 % to Tetracycline, and 82 % to Ciprofloxacin in a report by Debdutta et al. (2012).

The observations made in the present study clearly proved that *S. aureus* showed resistance to all antimicrobials tested except for rifampicin and Vancomycin (Table 3). These indicate that the problem is highly distributed and disseminated. Moreover, the overall resistance of *S. aureus* isolates to vancomycin, rifampicin, clindamycin and gentamycin showed less than 25 % of resistance and this is similar with the report of Ma et al. (2006) from the dairy farm in Taiwan. The reason why these antimicrobials were less resistant might be they are not frequently used in the study area in veterinary services, and perhaps in human medicine. Similar suggestion was given by Jaims et al.(2002) that the development of antimicrobial resistance is nearly always as a result of repeated therapeutic and/or indiscriminate use of them. However, the present study has demonstrated the existence of alarming level of resistance of *S. aureus* to commonly used antimicrobials (pencillin G and tetracycline) in the study area. This is due to the fact that tetracycline and penicillin are frequently and improperly used antimicrobials in animal and human treatment. The results were in accordance with reports from earlier studies in other countries (Jakee et al. 2008; Edward et al. 2002; Gentilini et al. 2002) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. This is in contrast with the report of Ma et al. (2006) on his report with respect to pencillin and tetracycline in Taiwan. This is not surprising because penicillin G and tetracycline are the most commonly used antimicrobials for the treatment of infection or mastitis in veterinary practice in Ethiopia. Moreover, penicillin resistance is plasmatic and, it spread out very quickly to several other strains. Pereira et al. (2009) showed that 70 to 73 % of *S. aureus* strains isolated from various foods were resistant to β-lactam such as pencillin and ampicillin. Staphylococci are frequently isolated from bovine mastitis which is one of the most common causes for the use of antimicrobial in lactating dairy cows. Similarly, the present investigation indicated that the resistance pattern of penicillin was found to be 93.1 % (Table 3) which is similar to the finding made by Tariku et al.(2011) (87.2 %) in Ethiopia, Landin (2006) (80 %) in Sweden, Gooraninejad et al. (2007) (57 %) in Iran and Myllys et al.(1998) (50 %) in Finland. This is in contrast to findings observed by Adesiyun (1994) who reported 23 % of resistance to pencillin G in West India.

Moreover, the present study showed the resistance of *S. aureus* to tetracycline (69 %), amoxicillin-clavulinic acid (34.5 %), oxacillin (31 %), cephalothin (31 %), chloramphenicol (27.6 %), sulphamethoxazole-trimethoprim (24.1 %), erythromycin (24.1 %), gentamycin (24.1 %), clindamycin (13.8 %) observed in milk samples taken from dairy cows in jigjiga city. This is in accordance with the findings of Tariku et al.(2011) who reported resistance of *S. aureus* to amoxicillin-clavulinic acid (46 %), chloroamphenicol (16 %), vancomycin (3 %), but it disagree with the observation made by Tariku et al. (2011) in the case of tetracycline(0 %), Co-trimoxazole (0 %) and clindamycin (4 %) in dairy farms in Jimma town. The probable explanation could be that *S. aureus* strains

have the capacity to change their resistance behavior to the exposed antimicrobials.

With a particular emphasis to tetracycline, the present observation agrees with preliminary finding conducted by Bayhun (2008) (55.3 %). However, apparent difference was observed in the report of Tariku et al.(2011) (0 %). This is due to the fact that tetracycline is the most commonly used antimicrobial in the treatment of infections in the livestock sector in Ethiopia. Moreover, tetracycline is widely used as growth factors in veterinary medicine for livestock rearing as well in the treatment of bacterial infection occurring in human medicine (Ardic et al. 2005). Furthermore, the resistance profile of *S. aureus* to amoxicillin-clavulinic acid and oxacillin in milk samples was found to be high. This is due to the fact that resistance of *S. aureus* to pencillin G, amoxicillin and oxacillin may be attributed to the production of β-lactamase, an enzyme that inactivates pencillin and closely related antimicrobial. It is believed that about 50 % of mastitis causing *S. aureus* produces β-lactamase (Green and Bradely 2004). Likewise, *S. aureus* showed resistance to vancomycin and clindamycin. This might indicate transfer of resistant strain among environment, livestock and human since this antimicrobials are not used in veterinary practice.

The MAR phenotypes (Table 4) obtained in the study correlated with the percentage of antibiotic resistance. Although the development of resistance to a particular antibiotic depends on the level of exposure to the antimicrobials, (Rychlik et al. 2006) there are many other factors that are involved. We are therefore suggesting that molecular methods be used to characterize these isolates for the presence of antibiotic-resistance determinants, which may provide data to support our conclusions. *S. aureus* is normally resident in humans; therefore, the *S. aureus* present in the cow's milk may have resulted from transmission from humans, which raises questions regarding the hygiene practices followed.

Conclusions

This study revealed that raw cow's milk in the study area could be an important source of infection with a wide range of organisms, particularly enteropathogens. An important source of microbial contamination of the milk is faecal pollution probably from cow dung. There is the need for instituting effective control measures to protect public health. This includes mandatory milk pasteurization by traders and improved hygienic handling of the commodity during milking, ensuring milking is not done on cow dung. The occurrence of multidrug resistance *S. aureus* should be under consideration during selection of antimicrobials for treatment of mastitis especially if the possibility exists in the transfer of resistance in or between microbial species. Moreover, *S. aureus* is a common human commensal, and multidrug resistant *S. aureus* may present without clinical illness. However, when they cause infection they are extremely serious. Furthermore, dairy cows become infected with multidrug resistant *S. aureus*, therefore diagnosis of *S. aureus* does not have implication for treatment only but also it indicates zoonotic transmission since it becomes reservoir for human infection. In practice of indiscriminate use of drugs should be controlled. Further studies that could incorporate isolation of milk contaminating bacteria to the species level should be done to evaluate the imminent danger posed by microbes from milks.

Acknowledgements

Jigjiga University is greatly acknowledged for funding and providing all rounded technical assistance for the smooth accomplishment of this research. The authors are also highly indebted to Ethiopian Somali Regional Health Bureau, Regional Public Health and Research Laboratory for laboratory facilities.

Authors' contributions

MA carried out the conception of the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. TW carried out the laboratory work, sample collection and revision of the manuscript. AN critically revised the proposal, designed the methodology, and reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that there is no financial or non-financial competing interest from anybody or institute. We also want to assure that we did not receive any technical assistant in developing the research concept or preparation of the manuscript.

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Received: 3 February 2016 Accepted: 13 May 2016
Published online: 26 May 2016

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