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Prevalence and Antimicrobial Resistance of Salmonella Isolated from Raw Milk Samples Collected from Kersa District, Jimma Zone, Southwest Ethiopia

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Salmonella sp. is one of the most commonly reported foods borne disease all over the world and developing countries at large. The cattle heath protection is the basic for production of microbiologically safe and sufficient milk and also preferable for consumption by human being. So that, antimicrobial resistant *Salmonella* were the big threat to public health concern. The increasing rate of antimicrobial resistance strains were main reason existing for aggravated bacterial disease. Thus, this study was done to indicate the frequency of antimicrobial resistance *Salmonella* isolates from rawcow's milk in individual farmers and dairy farms of Kersa district that is ready for consumption. A cross sectional study was conducted by collecting rawmilk samples from dairy farms and individual farmers. Isolation and identification was made by serological and different traditional biochemical tests methods. The prevalence of *Salmonella* spp. in raw milk of the study area was 20%. The isolated *Salmonella* spp. were resistant to at least two or more antimicrobials which used in this study. Among tested drugs Nalidixic acid (80%) was most highly resistant; however, most susceptible to Ciprofloxacin (95%).So, the study was aimed to determine prevalence of antimicrobial resistance bacteria and to make the concerned bodies to take corrective measure.

Key words: Antimicrobial resistance, kersa district, raw milk, *Salmonella* sp

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INTRODUCTION

Salmonella species are a major pathogenic bacterium that causes salmonellosis on human being and other organisms in the world (Mrema *et al*., 2006). Sever cases can result in systemic infections and even death. WHO (2007) and CLSI (2005) reports that, *Salmonella* covers 88% the food borne infection. The Garment; fecal wastes from infected animals, storage material and ways of handlings are important sources of *Salmonella* contamination of the raw milk. According to Bauer *et al.* (1996) anti microbial resistance is currently the greatest challenge to the effective treatment of infections globally. For instance, more than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic resistant to at least one type of antimicrobial and more than 50% as resistant to two or more (Dabassa and Bacha, 2012). The use antibiotics during animal production were the main reason for the development of antimicrobial resistant *Salmonella* spp. (IFT, 2003). Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians and misuse by patients (Dabassa and Bacha, 2012). Routine assessment of patterns of emerging antibiotic resistant *Salmonella* strains is of principal importance because such information channeled to physicians and veterinarians help to timely redirect drug use so as to diminish the development and spread of resistance. The study of prevalence and antimicrobial resistance *salmonella* in milk and milk products were at a juvenile stage in Ethiopia. However, studies made elsewhere indicated that milk and milk products are important sources of *Salmonella* particularly among those raw milk consumers (Jay, 2000; Olowe *et al.,* 2007). Different studies indicated that *Salmonella* were highly prevalent in Ethiopia both in veterinary and public setups (Dabassa and Bacha, 2012) even if, reports from apparently raw cow's milk were limited. Thus, this study was aimed to isolate and identify the antimicrobial resistance *Salmonella* from raw cow's milk of Kersa District, Southwest Ethiopia.

MATERIALS AND METHODS

Study area: The study was conducted in oromia regional state Jimma Zone, Kersa Woreda, south western part of Ethiopia from December, 2010 to June, 2011. Based on figures published by the Central Statistical Agency in 2005, the district (woreda) has an estimated total population of 329,629, of whom 162,690 were men and 166,939 women. Agriculture is the major source of economy and it includes mainly the growing of coffee and cattle rearing. The altitude of this woreda ranges from 1740 to 2660 above sea level.

Study population: In Kersa district there are many individual farmers and Dairy farms of milk producers. The four dairy farms from which the study was investigated have 7-25 lactating Holstein-Friesian cross-bred cows. The raw milk produced by individual farmer from local lactating cows is consumed by many farm families in their home whereas the dairy farm owners brought the milk to local consumers , restaurants and cafeterias at Jimma town that is the districts of towns of Jimma.

Sample collection: Preliminary visits were made on the distribution of owners of dairy farms and households vending raw milk in the study area prior to resuming the actual sample collection. Seven area namely, Ankaso, Serbo town, Merewa, Bedabuna, Siphanawi, Seredo and Minko were selected purposively based on their potential for production of milk. The target sampling populations were defined as all households in the study area who owned milk cows. Of the total population, some of the households were selected randomly from a list of farmers registered as milk producers in their respective kebeles.

A total of 100 samples of raw cow milk were separately collected at different occasions using random sampling technique. Individual raw cow milk samples were collected aseptically in sterilized 300 mL screw caped bottles from individual farmers in duplicate and that of dairy farms in triplicate, over a period of 6 months (December to June, 2011). The collected milk samples were transported to Postgraduate and Research Laboratory of Biology Department, College of Natural Sciences, Jimma University, using cold chains. After transportation samples temporarily were kept under refrigerator at 4°C until processed for the detection of *Salmonella* within 3 to 8 h of collection.

Isolation and identification of salmonella: The procedure has been used for detection of *Salmonella* from milk was as per the ISO-6579: 2002 standard. Milk sample was dispersed into suitable non-selective medium (buffered peptone water). One militer of the pre-enrichment culture was transferred into selective enrichment broth (10 mL Rappaport Vassiliadis soy peptone (RVS) and was incubated at 41.5±0.5°C for 18-24 h. Subsequently; the enriched sample was streaked onto each of the Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate agar (XLD) and incubated at 37°C for 24 h. The presumptive *Salmonella* colony on the XLD and BGA was selected and identified by using serologically and a series of biochemical tests including reactions on Lysine Iron Agar (LIA), Triple Sugar Iron agar (TSA), Urea agar, Simmon citrate agar and SIM medium.

Serological tests: The *salmonella* somatic (O) antigens of the isolates were determined by slide agglutination test and flagillar (H) antigens were also identified using a tube agglutination techniques described by (Standard Microbiological Methods of the Member Companies of the Corn Refiners Association (2007) and Ewing (1986).

Antimicrobial susceptibility test of salmonella isolates: The antimicrobial susceptibility testing for *Salmonella* isolates were carried out following the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid CM0337 Basingstoke, England) as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines (NCCLS, 2002). The isolates were tested with their respective concentration (in brackets) for the following antibiotics(all from Oxoid);Chloramphenicol (30 μg), Gentamycin (10 μg), Streptomycin (10 μg), Tetracyline (30 μg), Ciprofloxacillin (30 μg), Kanamycin (30 μg), Nalidixic acid (30 μg) and Amikacin (30 μg) all from Oxoid. A standardized suspension of the bacterial isolates was prepared and adjusted to the 0.5 Mc Farland turbidity standard. Subsequently it was streaked in to the Muller-Hinton Agar; the antibiotic discs were dispensed on the medium and incubated at 35°C for 18 h, followed by measurement of zone of inhibition manually. Finally, the isolates were classified as sensitive and resistant, as described by Vlkova *et al.* (2006).The bacterial characteristics were the main criteria used to select the antimicrobial agents. Moreover, selection was also based on their mechanisms of action. *Salmonella* ATCC 14028 were used as reference strains for quality control of the antibiotics used.

RESULTS

Frequency of isolation of *salmonella*: Twenty samples of the total 100 samples were positive for *Salmonella* isolates. Thus, prevalence of *Salmonella* spp, in raw milk of the study area was 20%. With regards to frequency distribution among selected sites *Salmonella* spp. were not detected in 3 of the dairy farms and 2 kebeles. Comparatively high prevalence of *Salmonella* was encountered in samples collected from Sipanawi and serbo (35.71%, 5/14 each) followed by Ankeso (30%, 6/16). The frequency of isolation of *Salmonella* in dairy farms (8.33%, 1/12) was not comparable to the frequency from individual farms (Table 1).

Polyvalent Flagellar (H) and polyvalent somatic (O) tests were confirmed that 20% of the isolate were *Salmonella* (Table 1) In addition to that, *Salmonella* spp. was also tested for the antibiotic susceptibility. *Salmonella* isolates were showed highly resistance to Nalidixic acid (80%) followed by Tetracyline and Kanamycin (35% each) and Amikacin (30%), Gentamycin, Chloramphenicol and Streptomycin (25% each) and Ciprofloxacin (5%) (Table 2).

Table 1: Frequency of *Salmonella* in raw cows' milk

Positive (%)	Sample source	Frequency of *Salmonella* Sample size (n =100)
Ankeso	16	6 (30)
Bedabuna	14	-
Merewa	16	3 (18.5)
Minko	14	-
Serbo	14	5 (35.71)
Siphanawi	14	5 (35.71)
Dairy farms	12	1 (8.33)
Total	100	20

Table 2: Antibiotic susceptibility patterns of *Salmonella* isolates in raw milk

Antimicrobial -agents	Disk content (μg)	Resistance		Sensitive	
		No.	%	No.	%
Amikacin	30	6	30	14	70
Chloramphenicol	30	5	25	15	75
Ciprofloxacin	5	1	5	19	95
Gentamycin	30	5	25	15	75
Kanamycin	30	7	35	13	65
Nalidixic acid	300	16	80	4	20
Streptomycin	30	5	25	15	75
Tetracyline	30	7	35	13	65

Table 3: Multidrug resistance profiles of *Salmonella* species isolated from raw milk

No. of antimicrobial resistance	Antimicrobial resistance pattern (No. of isolates)	No. of isolates (%)
Two	Nal, Te (1)	4 (20)
	Nal, Chl (2)	
	Nal,Gen (1)	
Three	Nal, Te, Amk (2)	5 (25)
	Chl, Te, Nal (3)	
Four	Tet, Nal, Gen, Amk (1)	5 (25)
	Tet, Str, Nal, Gen (1)	
	Kan, Chl, Nal, Amk (2)	
	Nal, Te, Gen, Str (1)	

Total of 9 Multiple Drug Resistance (MDR) pattern were also observed. The highest MDR noted was Chl/Te/Nal (15%, 3/20). The maximum MDR registered was resistance to four antibiotics with the combination Kan/Chl/Nal/Amk being more frequent (Table 3). In general, MDR to three and four antibiotics dominate the resistance patterns (25%, 5/20 each).

DISCUSSION

Several reports have documented the prevalence and distribution of *Salmonella* in bulk tank (Sandgren *et al.*, 2008). Evidence (Hitoshi, 2006; Mahami *et al.*, 2011; Bauer *et al.*, 1996) indicates that *Salmonella* spp are agents for the cause of mastitis in dairy animals and may have contaminated milk from the udder of infected animals and also reside in the intestinal tract where they cause gastro-enteritis in animals and may have occurred in milk as a result of faecal contamination. In the present study

the prevalence of *Salmonella* spp in raw milk was found 20%.The isolation rate of *Salmonella* in this study was related to reports from Gaborone, Botswana 20% (Aaku *et al*., 2004). However, it was higher than a study conducted by (Dabassa and Bacha, 2012), who reported a prevalence of 7.6 and 13.63%, respectively.

Studies made on *Salmonella* isolation from raw milk and foodborne illness associated with the consumption of *Salmonella* contaminated raw milk had not been clearly documented so far in Ethiopia and Jimma zone in particular. As a consequence of the high antimicrobial use in dairy farms and individual cows, bacterial contaminants carried by milk and milk products often show high levels of antimicrobial resistance (Sandgren *et al*., 2008). *Salmonella* resistant for at least to two or more antimicrobials which were observed in this study (70%) was lower than 83.3% conducted in Ethiopia (Dabassa and Bacha, 2012) and elsewhere in the world (Berge *et al*., 2004) (75%).

These change as results of increasing rate of wrong way-utilization of antibiotics in the dairy farms which maintaining resistance genes in bacteria (Aaku *et al*., 2004). As a result it should be of concern as it raises food safety and ethical issues. In the present study, *Salmonella* isolates were most susceptible to Ciprofloxacin (95%). This result was similar with the result reported by (Hawkey, 2008; Sandgren *et al*., 2008) from Nigeria.

In addition, the data from (Dabassa and Bacha, 2012) has indicated that, the effectiveness of such drugs like ciprofloxacin as the results of the drug were mostly not used for animal treatments. So that, the result of this study indicated that resistance of *Salmonella* isolates to those antibioticslike, Nalidixic acid (80%), Chloramphenicol, Gentamycin and streptomycin (25% each), kanamycin and tetracycline (35% each) and Amikacin (30%). However, *Salmonella* resistance to Tetracycline and Kanamycin (35%each) and Gentamycin (25%) were found higher in the present study as compared to finding of (Dabassa and Bacha, 2012; Mrema *et al*., 2006) who found that 33.3% and 12%, respectively.

In the current study Nalidixic acid showed a least efficacy against *Salmonella* isolates. In addition, the resistance to Nalidixic acid is consistent with the prevalence of 89-92% reported from Kenya (Lakshmi *et al*., 2006). Antimicrobial-resistant *salmonella* in rawmilk may be able to colonize the gut if consumed by humans, thus making infections difficult to treat. Evidence (Mahami *et al*., 2011; Akoachere *et al*., 2009) indicates that the global rise of antimicrobial resistance is mainly due to the exposure of this bacteria in human and veterinary medicine and indiscriminate use of drug for the treatment of both human and animal disease caused by *Salmonella* sp.

CONCLUSION

Examining the prevalence and drug resistance pattern of *Salmonella* from raw milk in dairy farms and individual farmers is the best mechanism to plan methods of reducing the ways of transmission of *Salmonella* between humans and cattle. Likewise, it imperative in fighting the development of drug resistant strains of *Salmonella*. The result obtained in this study (80%) is significantly high to the widely observed food borne salmonellosis in the area. Moreover, medium proportion (35-25%) of *Salmonella* isolates were resistance to two or more of the antimicrobials drugs. This condition creates big problems on human medical treatment. This finding showed that additional exploration is necessary on the prevalence and antimicrobial resistance pattern of *Salmonella*, which is food borne pathogen. In line with the experiential evidences, which indicate the uncontrolled use of antimicrobials for animal and public health treatments were the crucial reason for high rate of antimicrobial resistant *Salmonella*.

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REFERENCES

Aaku, E.N., E.K. Collison, B.A. Gashe and S. Mquchance, 2004. Microbiological quality of milk from two processing plants in Gaborone Botswana. Food Control, 15: 181-186.

Akoachere, J.F., N.F. Tanih, L.M. Ndip and R.N. Ndip, 2009. Phenotypic characterization of salmonella typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. J.Health Popul. Nutr., 27: 612-618.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Berge, A.C., J.M. Adaska and W.M. Sysco, 2004. Use of antibiotic susceptibility patterns and PFGE to compare historic and contemporary isolates of multi-drug resistant *Salmonella* enteric subsp: Enteric server Newport. Applied Environ. Microbiol., 70: 318-323.

CLSI, 2005. Performance Standards for Antimicrobial Susceptibility Testing. 5th Edn., Clinical and Laboratory Standards Institute, Wayne, (PA), Pages: 341.

Corn Refiners Association, 2007. Standard Microbiological Methods of the Member Companies. The Association, Windy, Chicago, IL.

Dabassa, A. and K. Bacha, 2012. The Prevalence and Antibiogram of Salmonella and Shigella Isolated from Abattoir, Jimma Town, South West Ethiopia. Int. J. Pharm. Biol. Res., 3: 143-148.

Ewing, W.H., 1986. Serological Identification of Salmonella. In: Identification of Enterobacteriaceae, Ewing, W.H., (Ed.). 4th Edn. Elsevier Science Publishing Co., New York, USA., pp: 75-96.

Hawkey, P.M., 2008. The growing burden of antimicrobial resistance. J. Antimicrob. Chemother., 62: i1-i9.

Hitoshi, I., 2006. Effect of radiation decontamination on drug resistant bacteria. J. Food Irradiat., 41: 9-13.

IFT, 2003. IFT export report on emerging microbiological food safety issues. Implications for control in the 21st Century. Institute of Food Technologist, S. Lowry /Univ. Ulster/Stone, pp: 14-21.

Jay, J.M., 2000. Food-Borne Gastroenteritis Caused by *Salmonella* and *Shigella*. In: Modern Food Microbiology, James, J.M., (Ed.). 6th Edn. Aspen Publishers, Inc., Maryland, USA., pp: 511-530.

Lakshmi, V., R. Ashok, J. Susmita and V.V. Shailaja, 2006. Changing trends in the antibiograms of Salmonella isolates at a tertiary care hospital in Hyderabad. Indian J. Med. Microbiol., 24: 45-48.

Mahami, T., S. Odonkor, M. Yaro and A. Adu-Gyamfi, 2011. Prevalence of antibiotic resistant bacteria in milk sold Accra. Int. Res. J. Microbiol., 2: 126-132.

Mrema, N., S. Mpuchane and B.A. Gashe, 2006. Prevalence of *Salmonella* in raw meat minced meat, raw fresh sausage and burger patties from retail outlets in Gaborone, Botswana. Food Control, 17: 207-212.

NCCLS, 2002. Approved Standard M7-A4. Method for Dilution Antimicrobial Susceptibility test for Bacteria That Grow Aerobically. 4th Edn., NCCLS, Waynae, Pa.

Olowe, O.A., B.M. Okanlawon, R.A. Olowe, O.T. Adedosu and A.B. Olayemi, 2007. Multiple drug resistant patterns of Salmonella typhimurium infections in Oshogbo, South Western Nigeria. J. Am. Sci., 3: 40-44.

Sandgren, C.H., K.P. Waller and U. Emanuelson, 2008. Therapeutic effects of systemic or intra mammary antimicrobial treatment of bovine subclinical mastitis during lactation. Vet. J., 175: 108-117.

Vlkova, E., V. Rada, P. Popelarova, I. Trojanova and J. Killer, 2006. Antimicrobial susceptibility of bifidobacteria isolated. Livestock Sci., 105: 253-259.

WHO, 2007. Initiative to estimate the global burden of food born diseases. First Formal Meeting of the Food Borne Disease Burden Epidemiology Reference Group (FERG). http://www.who.int/foodsafety/publications/foodborne_disease/FERG_Nov07.pdf