Zoonotic Bacterial Pathogens Isolated from Food of Bovine in Selected Woredas of Tigray, Ethiopia

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Abstract: Zoonotic bacterial foodborne pathogens jeopardize public health, which cause a number of human and animal diseases. The aim of the study is to identify zoonotic bacterial spp. from milk and meat of bovine origin. A cross-sectional study was conducted on 384 samples; milk (n=192) and meat samples (n=192) were collected from different sources in selected Woredas of Tigray, Ethiopia. Then the specimens were cultured and sub-cultured on blood agar. The pure colonies of bacteria were further sub-cultured on BUG media. Finally, the bacteria were identified by BiOLOG Identification system. The results showed that out of the total samples examined, E. coli O157:H7 (10.4%), E. coli, Non O157 STEC (2.6%), enterotoxigenic E. coli (10.6%) S. Typhimurium (10.4%), S. Enteritidis (5.7%), S. Newport (0.26%), S. aureus (9.8%) S. intermedius (6.7%) and S. hycus (4.68%) were isolated as a potential food borne zoonosis. Potential zoonotic bacterial pathogens were identified from bovine food source and this suggests health threat due to consumption of raw animal products. Hence, appropriate control design should be planned to ensure food safety.

Key words: Bacteria, Meat, Milk, Zoonoses

INTRODUCTION

Food safety and in particular safety of products of animal origin, is an increasingly important issue concerning human health. With increase in the consumption of products of animal origin, the risk of food borne diseases of humans also increases. One product that is commonly distributed in raw form is milk. Raw milk is a known vehicle and medium for pathogens such as Escherichia coli, Listeria monocytogenes and species of Campylobacter and Salmonella. Milk can become contaminated in many ways. For example, if the dairy cow has a mammary gland infection (mastitis) or a systemic infection, the pathogen can be passed to the milk. Milk can become contaminated by the faeces of the animals and the hand of the milker usually during hand milking procedure or by equipment used for milk collection and storage [1].

Similarly, Meat and its products are important reservoirs for many of the food-borne pathogens, including Salmonella, Campylobacter, Listeria, E. coli O157:H7 and Staphylococcus aureus.

Food borne diseases remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food [2]. Reliable statistics on food borne diseases are not available due to poor or non-existent reporting systems in most developing countries. Therefore, the study designed to identify major zoonotic bacterial species in raw bovine meat and milk.

MATERIALS AND METHODS

Study Area: The study was conducted in three woredas of Tigray namely, Mekelle, Alamata and Adigrat. These Woredas are selected mainly because of their geographic location difference that may help us to obtain reliable evidence about the magnitude and epidemiology of disease in the region.

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Mekelle town is located 783 km north of Addis Ababa at latitude of 39°38' E and 13°23' N. It has an altitude of 2000-2200 m.a.s.l. The weather condition is hot and dry. The mean annual rainfall of the area is 628.8 mm. The annual minimum and maximum temperature is 11.8°C and 29.94°C respectively [3].

Alamata is located in southern zone of Tigray region and is about 180km south of Mekelle, the capital of the region. It is south most woreda of Tigray region and borders with Amhara region from the South and West. Elevation in the area ranges from 1178 to 3148 m.a.s.l and 75% of the woreda is lowland (between 1500-3445 m.a.s.l). Rainfall is usually intense and short duration and average annual is 831 mm [3].

Adigrat is located on the northern Ethiopia in north eastern part of Tigray. It is about 120 km from the state capital (Mekelle). The area is at an altitude of 2332 m.a.s.l with a temperature range of 10 °C to 26 °C. The total surface area of the town is about 896.95 hectare and the average annual rainfall is 583 mm [3].

**Study Design:** A cross-sectional study was conducted from November 2012 to June 2013 in selected Woredas of Tigray, Ethiopia.

**Sample Size and Sampling Technique:** A total of 384 samples was collected from bovine raw milk and meat in a selected Woredas of Tigray, Ethiopia. The sample size is determined according the formula given by Thrusfield [4] by taking prevalence of 50% so that the maximum sample size should be achieved. Accordingly, the calculated value for sample size is equal to 384. Then equal samples of milk (n1=192) and meat (n2=192) were included purposefully. In sampling of milk and meat samples, simple random probability sampling technique was applied. Consequently, milk and meat were collected until sample size was achieved.

**Sample Collection, Transport and Handling:**

**Milk Samples:** Milk samples was aseptically collected directly from the teats of lactating cows (n=96) and from distribution site (n=96) (immediately before distribution) using sterile sample bottle of 250 mL. The samples were transported using icebox to Microbiology laboratory of College of Veterinary Medicine, Mekelle University, Mekelle. Then milk samples were immediately cultured or stored at 4 °C for a maximum of 24 h until it will be cultured on blood agar media. After culture in college of Veterinary Medicine, the prepared samples were transported with icebox to Microbiology laboratory of Institute Biodiversity Conservation, Addis Ababa for further confirmatory identification.

**Meat Samples:** The raw meat from slaughter house (n=96) during slaughtering and non-pre- packed meat samples from beef will be purchased randomly from selected butcher shops (n=96). Sections of meat (10 cm × 10 cm × 3 cm) from neck of each carcass will be aseptically removed and placed in separate sterile plastic bags to prevent spilling and cross contamination and then it will be immediately transported to Microbiology laboratory of College of Veterinary Medicine, Mekelle University in a cooler with ice packs. After culture in College of Veterinary Medicine, the prepared samples were transported to Microbiology laboratory of Institute Biodiversity.

**Culture and Identification:**

**Milk Sample:** Bacteriological examination was done according to the National Mastitis Control Council [5]. A 0.1 mL of milk was streaked on tryptose blood agar base (Oxoid, UK) enriched with 7% defibrinated sheep blood using the quadrant streaking method for each samples after centrifugation and discarding the supernatant. Blood agar plates were incubated aerobically at 37°C for 24-48 h. The plates were examined for gross colony morphology, pigmentation and hemolytic characteristics after 24-48 h. All suspected cultures of zoonotic food borne pathogen were sub-cultured into blood agar. Then pure colony was be taken and sub-cultured on BUG (BiOLOG Universal Growth Media) at 37 °C for 18-24 h as a primary and secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media was inoculated into 18-20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into of Micro plates with multi-channel pipettes. The Micro Plates was loaded into Omni log tray to be incubated, analyzed and interpreted for 18-24 h as per guidelines of Biolog Universal Guidline [6] and finally identified zoonotic food borne pathogen was printed out.

**Meat Sample:** The microbiological examination should be commenced within 12 h of sample collection. For each meat sample, 25 g was homogenized and 1 g of the homogenate is added to 5 mL of buffered peptone water (BPW- HiMedia Laboratories, Mumbai, India) and incubated. Cultures was streaked on tryptose blood agar base (Oxoid, UK) enriched with 7% defibrinated sheep blood using the quadrant streaking method and the plates was incubated overnight at 37 °C. From each plate
Fig. 1: Percentages of food borne pathogens isolated from bovine milk and meat.

(one plate for each meat sample), 5 to 10 suspected bacterial colonies was selected and sub-cultured onto blood agar. Then pure colony was further sub-cultured on BUG (BiOLOG Universal Growth Media) at 37 °C for 18-24 h as a primary and secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media was inoculated into 18-20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into of Micro plates with multi-channel pipettes. The Micro Plates were loaded into Omni log tray to be incubated, analyzed and interpreted for 18-24 h as per guidelines of Biolog Universal Guidline [6] and finally identified zoonotic food borne pathogen was printed out.

**Quality Control:** Confidence in the reliability of test results is increased by adequate quality assurance procedures and the routine use of control strains. Thus, *S. aureus* ATCC25923 and *E. coli* ATCC-25922 was taken as an important part of quality control for culture and BiOLOG identification for the study.

**Variables:** Independent variables such as types of samples, site of collection, interpreted against dependent variable of zoonotic bacterial isolates.

**Ethical Issues:** Verbal consent was obtained from dairy farms, abattoirs and butcher shop owners/managers.

**Statistical Analysis:** The collected data was entered into EPI data version 3.1 and exported to SPSS version 16 computer software then the data were analyzed. Accordingly, descriptive statistics such as percentages and frequency distribution used to describe/present bacterial

**RESULTS AND DISCUSSION**

Out of 384 of samples examined, 41(10.7%) *E. coli enterotoxigenic* followed by *E. coli* O157:H7 40 (10.4%) and *S. typhimurium* 40 (10.4%) were identified as the predominant species in the area by BiOLOG Identification System (Figure1). The study showed similar types and percentage of isolates in milk and meat samples except *Salmonella. newport* was isolated in milk (Table1).

The result of this study demonstrated that the food samples originated from bovine were contaminated by pathogenic bacteria which if ingested may be deleterious to consumers’ health and may lead to food borne illness or disease. The result of the study similar to that conducted by Jayarao and Henning [7], Murinda et al. [8], Van Kessel et al. [9] and Waak et al. [10] who detected food borne pathogens in bulk tank milk. Results of those studies have shown clearly that prevalence rates of food borne pathogens including *E. coli* and *Salmonella spp.* in milk vary considerably.
Table 1: Prevalence rates of major bacterial zoonoses isolated from bovine milk and meat.

<table>
<thead>
<tr>
<th>Species identified</th>
<th>Milk sample (n=192) no (%)</th>
<th>Meat sample (n=192) no (%)</th>
<th>Overall (N=384) no (%)</th>
<th>OR (%95)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>20(10.4)</td>
<td>20(10.4)</td>
<td>40(10.4)</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>E. coli, Non O157 STEC</td>
<td>3(1.6)</td>
<td>7(3.6)</td>
<td>10(2.6)</td>
<td>0.42</td>
<td>0.3</td>
</tr>
<tr>
<td>E. coli enterotoxigenic</td>
<td>22(11.5)</td>
<td>19(9.8)</td>
<td>41(10.7)</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>21(10.9)</td>
<td>19(9.8)</td>
<td>40(10.4)</td>
<td>0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>14(7.3)</td>
<td>8(4.2)</td>
<td>22(5.7)</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>S. Newport</td>
<td>1(0.5)</td>
<td>0(0)</td>
<td>1(0.26)</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>22(11.5)</td>
<td>16(8.3)</td>
<td>38(9.9)</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>13(6.7)</td>
<td>13(6.7)</td>
<td>26(6.7)</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>10(5.2)</td>
<td>8(4.2)</td>
<td>18(4.6)</td>
<td>1.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

In the present study, the prevalence of different *E. coli* subspecies that is *E. coli* O*:H* O157:H7 (10.4%), *E. coli*, Non O157 STEC (2.6%) and *E. coli* enterotoxigenic (10.7%). These findings are in conformity with reports by other researchers [11-13]. Higher prevalence of *E. coli* was reported by Ali and Abdelgadir [14] and Lingathurai and Vellathurai [15]. In fact, if the methods of production, transportation, handling and sale of milk are entirely unhygienic there is high prevalence of *E. coli* [11].

Salmonellosis is one of the most important zoonotic bacterial pathogen of food-borne infection around the world. The most important serotypes of *Salmonella* are S. Typhimurium (10.4%), S. Enteritidis (5.7%) and S. Newport (0.26%) and they were recovered in the present study. Similar results were reported [16, 17] with 4.6 and 4.2 % prevalence rates of *Salmonella* respectively. Abouzeed *et al.* [16] recorded that the most prevalent serotypes of animal *Salmonellae* is Serovars Typhimurium that similar to the present investigation. This result is significantly high to be a potential source of food borne Salmonellosis.

The present study also indicated the prevalence of coagulase positive *Staphylococcus* species. Accordingly, *S. aureus* (9.9%), *S. intermedius* (6.7%) and *S. hyicus* (4.6%) were identified as potential zoonotic bacteria from milk and meat. Similarly, it was closely comparable with the findings of Bishi [18] and Hussien *et al.* [19] who reported 9 and 10% prevalence of coagulase positive *Staphylococcus* in Addis Ababa, respectively. However, the present findings are lower than that of Workineh *et al.* [20] and Dego and Tareke [21] who reported 39.2 and 40.3% *S. aureus* isolates at Addis Ababa and Southern Ethiopia, respectively.

In general, the present study showed that high frequency distribution of the most pathogenic bacteria from each genus and this suggests a potential health risk due to consumption of raw animal products of bovine origin, it is necessary for public health organizations to be concerned since microorganisms causing food borne hazards and food spoilage can be isolated from raw animal products; thus reduction of contamination is an achievable policy objective.

**ACKNOWLEDGEMENT**

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**REFERENCES**