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Antimicrobial Susceptibility of *Staphylococcus aureus* in Cow Milk, Afar Ethiopia

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Abstract: In most pastoral communities milk is consumed raw without any heat treatment and this can pose a health hazard to consumers. The presence of *Staphylococcus aureus* in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene. *Staphylococcus aureus* is a major problem associated with milk causing number of human and animal diseases. A cross-sectional study was conducted on traditionally managed lactating cows in Afar region to determine the prevalence of *Staphylococcus aureus* in milk and its antimicrobial susceptibility from February to April, 2014. A total of 384 pastoralist lactating cows were tested for mastitis using the California Mastitis Test (CMT). 44 (11.4) were positive for California mastitis test, and on culturing all were showed the presence of Staphylococcus aureus bacteria. The antibiotic sensitivity was done on all isolates against seven commonly used antibiotics in the pastoral community using a standard method. Results showed that S. aureus isolates were very sensitive to Chloramphenicol and Streptomycin, Gentamycin in (100%, 94%, and 90%), respectively. intermediate resistance was recorded for Erythromycin (60%). Isolates also showed high resistant rate to penicillin (100%), Ampicillin (96%), Amoxicillin (92%), and Trimethoprim- sulpha methoxazole (88). On the basis of this result improving the hygienic conditions of milking processes, timely treatment of infected cows and avoiding use of the antibiotics for which the pathogens had shown resistance have been recommended.

Key words: - Pastoral community, California mastitis test, sensitivity, *Staphylococcus aureus*.

1. Introduction

Food-borne diseases are of a major concern, worldwide. The pathogenesis of bacteria causing food-borne poisoning depends on their capacity to produce toxins after or intoxication. Among the bacteria predominantly involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food Loir *et al*., [1]. Its symptoms include sudden onset of nausea, vomiting, abdominal cramps and diarrhea. On heating at normal cooking temperature, the bacteria may be killed but the toxins remains active Thaker *et al*., [2].

Milk and milk products are the prime habitat to complex microbial ecosystems; these are responsible for the broad variations in taste, aroma and texture of milk and milk products Soomro [3].Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment Soomro [3]. Diseases that can be spread through milk include: tuberculosis, typhoid fever, scarlet fever, poliomyelities, undulant fever, septic sore throat, brucellosis and diphtheria Sinell[4]. Pathogenic bacteria can be present in raw milk as a direct consequence of the udder infection. Among the micro-organisms commonly producing mastitis in cows, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli* are pathogenic to man Robinson [5].

Antimicrobial resistance is a major public health problem in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food Erskine *et al*.,[6]. This antimicrobial resistance has been documented by a number of workers in different areas. Several studies suggested that administration of antibiotics to food-producing animals for therapeutic purposes or as growth promoters may be a primary factor in selecting for antimicrobial-resistant bacterial pathogens Barber *et al*.,[7]. Antibacterial therapy of bacterial-induced diseases in cattle has been incriminated as a catalyst for resistance in bacteria isolated from treated animals, other animals within the herd, and food derived from cattle for human consumption Berghash *et al*.,[8]. These days, antibiotic resistant bacteria are emerging as a consequence of drug use in treating the disease mastitis.

Ethiopia has the largest livestock population in Africa which contributes, significantly to the agricultural economy. Livestock contributes to the livelihoods of 60-70% of the Ethiopian population and is responsible for 98% of the milk production Aklilu [9]. Cows represent the largest proportion of cattle in the country according to the Food and Agriculture Organization FAO, [10].

1.1. Objectives

This study was therefore designed to meet the following specific objectives.

- To see the distribution of mastitis in the study area during study period.
- Studying the prevalence of *Staphylococcus aurous* from mastitic cows.
- To test the antibiotic resistance of the bacteria to selected antibiotics.

2. Materials and Methods

2.1 Study Area

The study was conducted in Afar region, Zone one Asayita district. Asayita district is located at North east direction of Addis Ababa with 850 km from capital Addis Ababa. Asayita district was selected because of large cattle population in the region.

2.2 Study design

A cross sectional laboratory-based survey study was employed to determine the prevalence and anti-microbial sensitivity patterns of *Staphylococcus aureus* Isolated from milk samples in Afar region Asayita zone b/n February and April, 2014.

2.3 Sample size

The sample size was determined using an appropriate formula based on the 95% confidence limits and 5% sampling error. n = (Zα/2)2 p (1-p)/d2. In determining the sample size, the p value was taken as 50% (maximum value) because of the absence of recent data on prevalence of *Staphylococcus aureus* in the study area. Thus, the total sample size used in this study, as determined by the above formula, was 384.This formula was deliberately used to get maximum number of samples to examine.

2.4 Milk Collection and handling

Milk samples for bacteriological examination were collected aseptically following the routine procedures as described by Quinn *et al.,* [11]. A total of 384 raw milk samples were collected from 384 lactating cows of pastoralists during the study period. Sampling in pastoral community is too difficult because of the movement of the Pastoralists in search for Pasteur.

2.5 Isolation and Identification

Bacteriological examination was done according to the National Mastitis Council Guideline (1990). The collected samples were transported to the laboratory using ice-box and tested for the presence of mastitis with (CMT) California Mastitis Test. Milk samples from mastitic cows were stored at 4 °C for a maximum of 24 h cultured on blood agar media. All the samples were directly streaked onto 7% sheep blood agar and incubated aerobically at 37 ^{0}C for 24–48 hours. Plates were examined morphologically and for the presence of *Staphylococcus* colonies. Round, smooth and white or yellow colonies and hemolytic pattern were taken and sub-cultured on nutrient agar plates and incubated at 37 ^{0}C for 24-48 hours. Final identification of the organism and species was done based on Gram staining, catalase test, O-F glucose test, oxidase test, sugar fermentation and coagulase test (by using rabbit plasma). The pure isolates in the nutrient slant were preserved and maintained at 4 ^{0}C for further need Quinn *et al.,* [11].

2.6 Antimicrobial susceptibility

The antimicrobial susceptibility test was performed for all (44) isolated *Staphylococcus aureus* isolates identified from milk samples. Standard agar disk diffusion method was employed according to the recommendations of the National Committee for Clinical Laboratory Standards NCCLS [12] using commercial antibiotic disks (Oxoid).

2.7 Data Analysis

The data collected were entered and managed in MS Excel program. SPSS version 16 for windows was used for data analysis. A p-value <0.05 was considered indicative of a statistical significant difference.

3. Results and Discussion

3.1 Prevalence of *S. aureus*

Results showed that 44 (11.4%) were positive for mastitis through CMT test from 384 lactating cows of the pastoral community during this study period. On culturing and laboratory test diagnoses tests all 44 (11.4%) milk samples were positive for the presence of *Staphylococcus aureus* species. This finding of this study was closely comparable with the findings of Bishi [13] and Hussein *et al*, [14], who reported 9% and 10% prevalence in Addis Ababa, respectively.

It is also in contrast with findings of Lakew *et al*, [15], Ndegwa *et al*, [16] and Bedada and Hiko [17] who reported 41.1% and 43.3%, 39.1% in dairy cows, respectively. The possible explanation for this might be that *S. aureus* is a contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures Rowe [18]. Based on observations made throughout the study period in the fields improper practices contributed to the presence of *Staphylococcus S. aureus* incidence is at a considerable higher percentage. Prevalence of mastitis in the study area may be directly related with poor hygienic conditions of the owners during milking processes. Because, it is a potential risk factor that can predispose cows for environmental and contagious mastitis infections.

However, the present findings are much lower than that of Workineh *et al.*, [19], Dego and Tareke [20], who reported 39.2% and 40.3% *S. aureus* isolates at Addis Ababa and Southern Ethiopia, respectively. The finding in this study is still lower than other studies done in Ethiopia, The products specific prevalence of *Staphylococcus* were found to be 47.1%, 58%, 38% 34%, 70.6% and 38% from udders milk, farm tanks milk, farm tanks swab, buckets swab, nasal and hand swab of milkers samples, respectively.

This finding still lesser than other findings in Ethiopia, Mekonnen *et al.* [21] and Alehegn [22], who reported 33% and 29.5% *Staphylococcus* prevalence in tank milk, respectively, in Debrezeit. Factors that could be hypothesized to be causes of contamination of milk in this study include insufficient pre-milking udder preparation and material contamination. The weather condition may contribute for the less prevalence of the bacteria in the study area.

3.2 Antibiotic susceptibility patterns of *Staphylococcus aureus* Isolates

The antibiotic susceptibility test was made to all *S. aureus species* isolated from milk samples. The overall percentage of resistance pattern is shown in (Table.1). Results showed that isolates were highly sensitive to Chloramphenicol, Streptomycin and Gentamycin in (100%, 94%, and 90%), respectively. Intermediate resistance was recorded for Erythromycin (60%). Isolates also showed high resistant rate to penicillin G (100%), Ampicillin (96%), Amoxycillin (92%), and Trimethoprim- sulpha methoxazole (88%). The high frequency of resistance observed with

Penicillin G (90.2%), Erythromycin (70.9%), in similar studies in Ethiopia. This could be due to the free accessibility of pastoralists to certain antibiotics in open market.

	Suscep tibility	AMX No. (%)	AM No.(%)	CH No. (%)	ER No. (%)	GE No.(%)	P No. (%)	ST No. (%)	SXT No.(%)
S. aureus **(n= 44)**	**S**	0	0	44(100)	8(18)	40(94)	0	41(94)	0
	I	3(8)	3(4)	0	26(60)	2(3)	0	3(6)	2(7.8)
	R	41(92)	41(96)	0	10(22)	2(3)	44(100)	0	40(90.2)

Table 1: Antimicrobial drug resistance patterns of *S. aureus* isolates.

AM: Ampicillin, AMX: Amoxycillin, CH; Chloramphenicol, ER ; Erythromycin, GE; Gentamycin, P; penicillin-G, ST; Streptomycine, STX ; Trimethoprim-sulphamethoxazol; S Sensitive; I Intermediate; R Resistant.

4. Recommendations

Plastic containers are widely used in the pastoral community, According to Soomro plastic containers scratch easily and provide hiding places for bacteria during cleaning. Coordinated actions at various stages in the food chain is crucial in preventing such dangerous food borne pathogens. The importance of proper handling and cooking of foods of animal origin are very important in preventing most of food borne diseases. More epidemiological studies are needed in order to determine the distribution of mastitis in pastoral community. Public education is crucial not to drink raw milk or any undercooked animal origin foods. The final recommendation is indiscriminate use of antimicrobial agents and antibiotic sale in the community without the prescription of animal health professional. The use of such under prescribed antimicrobials contribute a lot for the development of drug resistance.

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