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RESEARCH PAPER

Antimicrobial susceptibility of major bacterial pathogens isolated from bovine mastitis in Bench Maji zone, Southwest Ethiopia

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ABSTRACT Bovine mastitis is a potentially fatal mammary gland due to physical trauma or microorganisms infections. It is the most widespread, costly disease in dairy farms and a great concern for many producers in developing countries like Ethiopia. The study was conducted to test the antimicrobial susceptibility of identified pathogens from mastitic cattle. Out of 384 lactating cows examined in the course of the study period, 116 (30.21%) cows had mastitis. The results of bacteriological analysis of cultured milk samples had shown three major strains of pathogenic bacteria namely *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* were isolated from 81 cows, 48 bacterial isolates (59.26 %) were identified as *S. aureus*, 31 bacterial isolates (38.27%) were identified as *Str. agalactiae* and two bacterial isolates (2.47%) were identified *E. coli* (2.47%). The antimicrobial agents used were gentamycin, kanamycin, erythromycin, chloramphenicol, streptomycin amoxicillin, penicillin and ampicillin. The in vitro antimicrobial susceptibility test showed some degree of resistance in varying proportions of isolates. Some of the bacterial isolates exhibited even resistance to two or more antibiotics. S. aureus represented 95.8 % of the total isolates, was found resistant to penicillin while 93.7% of the total isolates were found sensitive to gentamycin. On the other hand, from the total isolates of *Str. agalactiae*, the maximum resistance was recorded in ampicillin (35.5%), and sensitivity was recorded in penicillin (83.9%). All of the *E. coli* isolates were resistant to amoxicillin, ampicillin, and penicillin while all of them were susceptible to gentamycin and streptomycin. The average susceptibility of *S. aureus*, *Str. agalactiae* and *E. coli* strains to all antimicrobials tested in this study were 37.5%, 41.9% and 37.5%, respectively. In comparison, the average resistivity of all antimicrobials tested was 41.4%, 13.3%, and 43.8% of *S. aureus*, *Str. agalactiae* and *E. coli*, respectively. For the bacterial isolates tested for antimicrobial susceptibility, gentamycin, chloramphenicol, and streptomycin were the drugs more active, while the majority of isolates were resistant to amoxicillin, ampicillin, and penicillin. This antibiotic resistance development among these bacteria may pose a problem of concern. Hence, the use of antimicrobials in both the treatment and prevention of intra-mammary infections must be based on in vitro antimicrobial susceptibility test.

KEYWORDS: *antibacterial; bovine; mastitis; predisposition.*

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Introduction

The Ethiopian livestock population is the largest on the African continent, with an estimated 61.51 million cattle, 33.02 million sheep, 38.96 million goats, 1.93 million horses, 9.66 million donkeys, 0.37 million mules, 1.76 million camels, and 59.42 million poultry (CSA 2018/19). This sector has been contributing a considerable portion to the economy of the country and still promising to rally around the economic development of the country. They account for

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12-16 % of total GDP and 30-35 % of agricultural GDP. About 90% of crop production is dependent on animal draft power. Livestock contributes to the livelihoods of 60-70% of Ethiopia's population, most being smallholder farmers (GAIN 2016). Conversely, low annual per capita consumption of milk in Ethiopia (17-19 liter) revealed that current milk production in Ethiopia is insufficient to fulfill the requirements due to many factors (Biffa et al 2005).

Dairy cattle mastitis can reduce milk yield, increase culling rate, incur treatment costs and occasionally result in death from severe infection (Radostitis et al 2007). Seegers et al (2003) indicated that mastitis had been described as the most common and costly disease in dairy production, causing over 38% economic losses due to health problems. Many infectious agents have been identified as the cause of mastitis in dairy cattle. A variety of bacteria can be isolated from bovine mastitis cases *S. aureus and E. coli strains* are the most common causes of contagious and environmental clinical mastitis, respectively. Antimicrobial therapy is commonly implemented for mastitis prevention and control. Unfortunately, despite the best possible antimicrobial treatments, failures of bacteriological cure are common, especially for mastitis and antimicrobial resistance (AMR), which is considered to be one of the reasons for low cure rates (Barkema et al 2006).

Additionally, AMR in bacteria is a public health hazard, and extensive antimicrobial use is considered a potentially important driver of AMR. There was no information on the sensitivity and resistance pattern of major bacterial pathogens of mastitis in the study area. The present study was, therefore, undertaken to determine the sensitivity and resistance pattern of major pathogenic bacteria isolated from bovine mastitis to commonly used antimicrobials in the study area.

Material and Methods

Milk Sample Collection and Laboratory Investigation

According to Quinn et al (2002) procedures of mastitis testing, the lactating cows's milk samples were directly collected using universal sample collection bottles. The first 3-4 streams of milk were discarded. The collecting bottle was held as near horizontal as possible, and by turning the teat to a nearly horizontal position and approximately 10 ml of milk were collected into the container. After collection, samples were labeled and placed in an icebox and transported to the Mizan Regional Veterinary Diagnostic Laboratory, Bench Maji zone (BMZ) of the Southwestern part of Ethiopia. The California Mastitis Test (CMT) was conducted to diagnose the presence of subclinical mastitis (Quinn et al 1999). Milk samples with a test result of CMT 1 to 3 were classified as evidence of subclinical mastitis (Quinn et al 1999; Radostits et al 2007).

Bacterial Isolation and Identification

A bacteriological study was performed on milk samples from the seropositive CMT reactive and mastitis milk for culture. Identification of mastitis pathogens were carried out following microbiological procedures for diagnosis of bovine udder infection described in Quinn et al (1999). One standard loop (0.01ml) of milk was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37°C. The plates were checked for growth after 24, 48, and up to 72 hours to rule out slow-growing bacteria species. A milk sample was considered positive for mastitis pathogens if at least single colonies of a potential pathogen were detected and the positives were identified by biochemical tests. For primary identification, size, shape, color, hemolytic characteristics, Grams reaction and catalase production were used. For confirmation, biochemical tests were used after sub-culturing isolated distinct colonies on selective media. MacConkey agar (Oxoid) and Edward's agar (Oxoid) were used to detect the most aerobic pathogens, enteric bacteria, and *Streptococci,* respectively. Primary identification of *Staphylococci* was based on colony morphology, catalase test, Gram-staining morphology, and differentiated from micrococci based on the oxidative fermentative (OF) test carried out on semi-solid OF medium (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The *Staphylococci* were also tested for production of coagulase enzyme by the tube method as described by Quinn et al (1994). Isolates that produced Gram-positive cocci in clusters and were catalase-positive, glucose-fermentative, resistant to bacitracin, and did not produce coagulase were identified as coagulase-negative staphylococci (CNS). *Staphylococcus aureus* isolates were differentiated from other coagulase-positive staphylococci on the basis of mannitol fermentation on mannitol salt agar (Oxoid). The enteric bacteria were identified using colony morphology, oxidase test, lactose fermentation on

MacConkey agar (Oxoid), **i**ndole production test, citrate utilization Quinn et al (1999). The Interpretation was made according to NMC (1990). The culture was considered negative if no growth occurred after 72 hours of incubation, and plates showing mixed and confluent growths, with no evidence of single discernible colonies, were not investigated further.

Antibiotic Susceptibility and Resistance

Antimicrobial susceptibility testing was carried out with equivalence of 0.5 McFarland turbidity standards by agar disc diffusion method on Mueller-Hinton agar plates following the guidelines of Clinical and Laboratory Standards Institute (CLSI 2008). All the bacteria isolated were tested for their sensitivity to 8 different antibiotics discs, which are commonly used in the study area, gentamycin (G10µg), kanamycin (k30µg), streptomycin (S10µg), erythromycin (E15µg), chloramphenicol (Ch30µg), amoxicillin (A2µg), ampicillin (Am10µg) and penicillin (P10µg). Susceptibility interpretation criteria were based on the CLSI guidelines (CLSI 2015).

Results and discussion

Anti-bacterial Susceptibility of Major Pathogens

Anti-bacterial susceptibility of major pathogen tests was carried out by using the disc diffusion method**.** The mean inhibition zones were recorded for each isolate with all antibiotics used. Table 1 showed that *S. aureus* was found to be highly sensitive to gentamycin (93.7%), streptomycin (91.7%), and chloramphenicol (72.9%), followed by kanamycin (25%), ampicillin (12.5% and amoxicillin (4.1%). However, a few numbers of isolates exhibited high resistance towards penicillin (95.8%), erythromycin and ampicillin (62.5%), amoxicillin (56.3%), and kanamycin (54.2%). The antimicrobial sensitivity of *S. aureus* in this study is comparable with the early findings of Abera et al (2010), who reported high susceptibility to chloramphenicol (100%) followed by gentamycin (91.7%) and streptomycin (86.1%).

In addition, finding a few associated with Begum et al (2007) revealed that *S. aureus* was 82.86% and 37.14% resistant to penicillin and amoxicillin, respectively. Similar types of resistance patterns were also reported by Abera et al (2010) and Birhanu et al (2013) in Adama town and Asella government dairy farm of Oromia Regional state, respectively. However, Mueen et al (2015) reported a higher resistance rate to penicillin (100%), amoxicillin (100%), and erythromycin (75%) in Bangladesh. The resistance of *S. aureus* to penicillin and ampicillin may be attributed to the production of beta-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. It is believed that around 50% of mastitis-causing *S. aureus* strains produce beta-lactamase (Green and Bradely 2004).

The current study also revealed that *Str. agalactiae* was found to be highly sensitive to penicillin (83.9%), chloramphenicol (77.4%), and gentamycin (74.2%), followed by streptomycin (41.9%), kanamycin (32.2%), amoxicillin (16.1%), and finally ampicillin (9.7%). However, these isolates were highly resistant, or a few numbers of isolates exhibited moderate resistance towards ampicillin (35.5%), erythromycin (32.3%), kanamycin, and amoxicillin (19.4%).

As indicated in Table 1, *E. coli* was realized to be highly sensitive to gentamycin (100%) and streptomycin (100%), followed by kanamycin and chloramphenicol (50%). However, these isolates were highly resistant to penicillin, ampicillin, and amoxicillin (100%), followed by erythromycin (50%). The average susceptibility of *S. aureus, Str. agalactiae and E. coli* strains to all antimicrobials tested was 37.5%, 41.9%, and 37.5%, respectively. At the same time, the average resistivity of all antimicrobials tested was 41.4%, 13.3%, and 43.8%of *S. aureus, Str. agalactiae and E. coli,* respectively. This antibiotic resistance development among the bacteria may pose a problem of concern. As a result, the resistance pattern against broad-spectrum antibiotics depicts a worrying situation, which needs special attention.

Table 1 Anti-bacterial susceptibility of the major pathogenic bacteria isolated to different antibiotics.

No	Antimicrobials	*Staphylococcus aureus* Mean (%)		
		Sensitive	**Intermediate**	**Resistant**
1	Gentamycin (G10µg)	45 (93.7%)	3 (6.3%)	0 (0%)
2	Kanamycin (k30µg)	12 (25%)	10 (20.8%)	26 (54.2%)
3	Streptomycin (S10µg)	44 (91.7%)	4 (8.3%)	0 (0.0%)
4	Erythromycin (E15µg)	0 (0.0%)	18 (37.5%)	30 (62.5%)
5	Chloramphenicol (Ch 30µg)	35 (72.9%)	13 (27.1%)	0 (0.0%)
6	Amoxicillin (A2µg)	2 (4.1%)	19 (39.6%)	27 (56.3%)
7	Ampicillin (Am 10µg)	6 (12.5%)	12 (25%)	30 (62.5%)
8	Penicillin (P10µg)	0 (0.0%)	2 (4.2%)	46 (95.8%)
	Mean (%)	18 (37.5%)	10 (21.1%)	20 (41.4%)
No	**Antimicrobials**	*Streptococcus agalactiae* Mean (%)		
		Sensitive	**Intermediate**	**Resistant**
1	Gentamycin (G10µg)	23 (74.2%)	8 (25.8%)	0 (0.0%)
2	Kanamycin (k30µg)	10 (32.2%)	15 (48.4%)	6 (19.4%)
3	Streptomycin (S10µg)	13 (41.9%)	18 (58.1%)	0 (0.0%)
4	Erythromycin (E15µg)	0 (0.0%)	21 (67.7%)	10 (32.3%)
5	Chloramphenicol (Ch 30µg)	24 (77.4%)	7 (22.6%)	0 (0.0%)
6	Amoxicillin (A2µg)	5 (16.1%)	20 (64.5%)	6 (19.4%)
7	Ampicillin (Am 10µg)	3 (9.7%)	17 (54.8%)	11 (35.5%)
8	Penicillin (P10µg)	26 (83.9%)	5 (16.1%)	0 (0.0%)
	Mean (%)	18 (37.5%)	13 (41.9%)	14 (44.8%)
No	**Antimicrobials**	*Escherichia coli* Mean (%)		
		Sensitive	**Intermediate**	**Resistant**
1	Gentamycin (G10µg)	2 (100%)	0 (0.0%)	0 (0.0%)
2	Kanamycin (k30µg)	1 (50%)	1 (50%)	0 (0.0%)
3	Streptomycin (S10µg)	2 (100%)	0 (0.0%)	0 (0.0%)
4	Erythromycin (E15µg)	0 (0.0%)	1 (50%)	1 (50%)
5	Chloramphenicol (Ch 30µg)	1 (50%)	1 (50%)	0 (0.0%)
6	Amoxicillin (A2µg)	0 (0.0%)	0 (0.0%)	2 (100%)
7	Ampicillin (Am 10µg)	0 (0.0%)	0 (0.0%)	2 (100%)
8	Penicillin (P10µg)	0 (0.0%)	0 (0.0%)	2 (100%)
	Mean (%)	18 (37.5%)	1 (37.5%)	0 (18.8%)

Conclusions

The major pathogenic strains isolated were; *S. aureus, Str. agalactiae* and *E. coli*. This indicates that mastitis caused by *S. aureus* (59.26%) is one of the major problems of dairy cows in milk production, followed by *Str. Agalactiae* (38.27%) and *E. coli* (2.47%), respectively. The average susceptibility of *S. aureus, Str. agalactiae and E.coli* strains to all antimicrobials tested in this study were 37.5%, 41.9%, and 37.5%, respectively. In comparison, the average resistivity of all antimicrobials tested was 41.4%, 13.3%, and 43.8%of *S. aureus, Str. agalactiae and E. coli,* respectively. This antibiotic resistance development among the bacteria may pose a problem of concern. For the bacterial isolates tested for antimicrobial susceptibility, *gentamycin, chloramphenicol*, and *streptomycin* were the drugs more active, while the majority of isolates were resistant to *amoxicillin, ampicillin,* and *penicillin.* The present study indicated that the existence

of a frightening level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents in the study area. This antibiotic resistance development among these bacteria may pose a problem of concern, a result in agreement with reports from earlier researches suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobial. Therefore, the use of antimicrobials in both treatment and prevention of intra-mammary infections must be based on in vitro antimicrobial susceptibility test.

Conflict of Interest

The authors declare no conflict of interest.

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