Journal of Animal and Veterinary Advances 10 (6): 745-749, 2011
ISSN: 1680-5593

Prevalence and Susceptibility Assay of *Staphylococcus aureus* Isolated from Bovine Mastitis in Dairy Farms of Jimma Town, South West Ethiopia

[1]Tariku Sori, [1]Jemal Hussien and [2]Molalegne Bitew
[1]College of Public Health and Medical Sciences, Jimma University,
P.O. Box 378 Jimma, Ethiopia
[2]College of Agriculture and Veterinary Medicine, Jimma University,
P.O. Box 307 Jimma, Ethiopia

Abstract: Cross sectional experimental study was conducted to assess the prevalence and susceptibility pattern of *Staphylococcus aureus* isolated from bovine mastitis to commonly used antibacterial agents in Jimma town dairy farms, South West Ethiopia from January-July, 2010. Milk samples were collected aseptically and California Mastitis Test (CMT) was carried out to identify subclinical mastitis from dairy cows. All CMT high scored and clinically positive samples were investigated microbiologically. Rate of isolation of *Staphylococcus aureus* was determined and susceptibility of 11 antibiotics against *S. aureus* was evaluated using the Kirby-Bauer disc diffusion method and the result was expressed as sensitive, intermediate and resistant. From total of 218 milk samples collected, 164 CMT high score milk samples were cultured of which 86 (52.4%) of pure strains of *S. aureus* were isolated. Out of 86 pure isolates of *S. aureus* resistance was detected for Penicillin (87.2%), Nalidixic acid (92%), Amoxicillin (46%), Chloramphenicol (16%), Clindamycin (4%) and Vancomycin (3%). The study also revealed that *S. aureus* was found to be sensitive for Norfloxacilin, Gentamycin, Tetracycline and Bacitracin. The present finding indicates that these isolates exhibited the highest degree of resistance to Nalidicic Acid, Penicillin, Amoxicillin and Chloramphenicol of among the tested anti microbial agents in comparison to previous studies reported. Furthermore, resistance follow-up, appropriate selection and use of antibiotic is recommended in the treatment of mastitis.

Key words: Bovine mastitis, Antimicrobial susceptibility, *Staphylococcus aureus*, mastitis, nalidixic acid, vancomycin

INTRODUCTION

Despite many years of research, mastitis remains the most economically damaging disease for dairy industry worldwide irrespective of species of animal (Owens *et al*., 1997). It is a disease that can cause devastating effects to a farmer because of serious economic losses (Ameh and Tari, 2000). In addition, there is a danger that the bacterial contamination of milk from affected cattle may render it unsuitable for human consumption by causing food poisoning or in rare cases provide a mechanism of spread of disease to humans (Bitew *et al*., 2010).

Pervious studies conducted in different countries indicate the distribution and economic importance of the disease. Contreras *et al*. (1997) from Spain; Moshi *et al*. (1998) from Tanzania; Ameh and Tari (2000) from Nigeria; Ndegwa *et al*. (2000) from Kenya and Kozacinski *et al*. (2002) from Croatia reported different prevalence rates of mastitis in dairy cattle. The impact of mastitis can be categorized as economic loss and zoonotic potential. Economic loss caused by mastitis includes reduction in milk production, calf mortality, culling of dairy cows, value of milk that has to be with held from sale, reduction in quality of milk and cost of treatment (Radostits *et al*., 2007). Bacterial contamination of milk from affected cows may render unsuitable for human consumption by causing food poisoning or interference with manufacturing process or in rare cases provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk includes brucellosis, caseous lymphadenitis, leptospirosis, listeriosis, melioidosis, Q-Fever, Staphylococcal food poisoning, toxoplasmosis and tuberculosis (Radostits *et al*., 2007).

The major obstacle in treating mastitis is antimicrobial resistance. Antimicrobial susceptibility testing is useful to carry out effective treatment because certain microorganisms are resistant to certain antimicrobials. It is found that *Staphylococcus* sp. is

Corresponding Author: Tariku Sori, College of Public Health and Medical Sciences, Jimma University, P.O. Box 378 Jimma, Ethiopia

resistant to penicillin (Smith and Sherman, 1994). However, there was no study carried out in Jimma and its surroundings about the prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in dairy farms. Therefore, this study is undertaken with the objectives to determine the prevalence of *Staphylococcus aureus* as a cause of mastitis in dairy cows to assess antimicrobial susceptibility, resistance and multi drug resistance pattern of *Staphylococcus aureus* to commonly used antimicrobials.

MATERIALS AND METHODS

Study design: Cross sectional and experimental studies were conducted. The study was conducted on dairy farms found in Jimma Town, South Western Ethiopia from January-July, 2010. The laboratory study was done in Veterinary Microbiology laboratory, Jimma University.

Sample size and sampling method: Simple random sampling technique was followed to select the study animal and the desire sample size was calculated according to the formula given by Thrusfield (2005). About 218 lactating and dry cows were considered to be as a sample.

Study methods

Physical examination of mastitis: Udder attachment, parity number, any physical abnormalities such as swelling of the udder, presence of lesions, anatomical malformations and tick infestation were recorded. The milk was examined for its color, odor, consistency and other abnormalities.

California Mastitis Test (CMT): The California Mastitis test was carried out as described by Schalm *et al.* (1971) and Quinn *et al.* (2004). A squirt of milk, about 2 mL from each half was placed in each of 2 shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 sec. The result was scored from 0-3.

Milk sample collection, handling and transportation: Aseptic procedures for collecting milk samples as described by Schalm *et al.* (1971), Sears *et al.* (1991) and Quinn *et al.* (2004) were followed. The time chosen for milk sample collection was before milking. Udders and especially teats were cleaned and dried before sample collection. Each teat end is scrubbed vigorously with a pledged of cotton moistened with 70% ethyl alcohol.

A separate pledged of cotton was used for each teat. The first few streams of milk were discarded and 10 mL of milk was collected into horizontally held vial. After collection, the sample was placed in an icebox and transported to the Veterinary Microbiology laboratory for analysis.

Bacterial isolation and identification: Bacteriological examination of the milk was carried out with respect to CMT high scores following standard procedures (Carter, 1984; Sears *et al.*, 1991; Quinn *et al.*, 2004). Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25°C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently (National Mastitis Council, 1990).

One standard loop (0.01 mL) of milk sample was streaked on 7% sheep blood agar (BBL®, Becton Dickinson, USA). The inoculated plates were incubated aerobically at 37°C. The plates were checked for growth after 24, 48 and 72 h to rule out slow growing bacteria. The plates were examined for growth, morphologic features such as colony size, shape, color and hemolytic characteristics. Suspected colonies were sub-cultured on Manitol Salt Agar (Oxoid, Hampshire, England) plates for further investigation.

For primary identification of bacteria, once a pure culture is obtained, the results from a few comparatively simple tests can often identify the bacterium to a genus level. A gram-stained smear from the culture will establish the gram reaction (gram-positive or gram-negative) and the cellular morphology (coccus or rod). The other primary tests include growth or absence of growth on MacConkey agar, Manitol Salt Agar, catalase and coagulase tests (Quinn *et al.*, 2004).

Antimicrobial susceptibility test: Susceptibility of bacteria to the commonly used antimicrobials was conducted using Kirby-Bauer method (Quinn *et al.*, 2004). About 11 antimicrobials such as Penicillin, Nalidixic Acid, Bacitracin, Tetracycline, Vancomycin, Gentamycin, Chloramphinicol, Cotrimoxazole, Clindamycin, Norfloxacilin and Amoxicillin (Oxoid, Hampshire, England) were selected from main class of antimicrobials and investigated for sensitivity testing.

The antibiotic disks were applied on to the surface of the inoculated agar plates using aseptic technique. Each disk was pressed down to ensure complete contact with the agar surface. The disks were deposited with centers at least 24 mm apart (Quinn *et al.*, 2004).

Statistical analysis: The data was compiled and analyzed with SPSS statistical package version 16. Percentage with confidence interval was used for determination of prevalence. Prevalence estimation of commonly isolated pathogens in Jimma town dairy farms was determined using standard formulae (i.e., the number of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percentages and frequency distributions was used to describe/present the nature and the characteristics of the data.

RESULTS AND DISCUSSION

From 218 mastitic milk samples, 164 (75.22%) were high CMT score and grew more than one type of organism while 54 (24.78%) of them grew 1 type of bacterium on blood agar. In this study from Staphylococcus species accounted for 39.44% of the CMT high scored milk samples (Table 1).

Regarding the drug susceptibility assay, out of 86 isolates of the *S. aureus* which is isolated from Jimma Town dairy farms, 46 (52.4%) were resistant to 2 or >2 antimicrobial agents tested. The antimicrobial activity of the tested agents were presented using zone of inhibition in mm and compared with the standard by Clinical and Laboratory Standards Institute (2005) (Table 2).

Resistance was detected for Nalidixic Acid, Penicillin G, Amoxicillin, Chloramphenicl, Clindamycin and Vancomycin. Out of these drugs, Nalidixic Acid (92%), Penicillin (87.2%) Amoxicillin (46%) were the most resisted agents by the organism. *S. auerus* was susceptible to Gentamyicn, Norfloxacilin and Tetracycline while Vancomycin (3%) and Clindamycin (4%) where exhibited slight resistance (Table 2).

The study also tried to show the multi drug resistance profile of *S. aureus* isolated from bovine mastitis. From 86 pure isolates (92), (25), (10.46) and (7%) of the isolates were resistant for 1-4 drugs, respectively (Table 3).

From the CMT high scored milk samples, 39.44% were *Staphylococcus* species. This is in agreement with findings of Lakew *et al.* (2009) in which the dominant bacterial isolates in their study animals were *Staphylococcus* species (41.4%), Contreras *et al.* (1997) and Ndegwa *et al.* (2000) research outputs indicate that *Staphylococcus aureus* is widely distributed in 43.8% of the animals and 70% of the herds. However, the result was lower than Bitew *et al.* (2010) who found 20.3% in dairy farms of Bahir Dar town and its environs. This is because *S. aureus* is contagious pathogen transmitted from one cow to another easily during unhygienic milking procedures (Rowe, 1999). Reports also show that most of the clinical cases of mastitis caused by *S. aureus* were affecting 50% of the gland and 50% of other part of the animals (Anyam and Adekeye, 1995). This finding is also in agreement with data reported by Ameh and Tari (2000) in Nigeria and Topolko and Benic (1997) in Croatia who found dominant number of *S. aureus* over CNS in dairy cow mastitis. The resistance detected for Nalidixic Acid, Penicillin G, Amoxicillin, Chloramphenicl, Clindamycin and Vancomycin in this study is in agreement with Hussein (1999) in bovine mastitis. In this study there was no resistance to Gentamyicn, Norfloxacilin and Tetracycline while Vancomycin (3%) and Clindamycin (4%) where exhibited slight resistance. Hussein (1999) found that Staphylococcus species was susceptible to chloramphenicol, Tetracycline, clindamycin and Gentamycin. Resistance was observed in all dairy farms selected which indicate that the problem is highly distributed and disseminated.

In this study 75 (87.2%) isolates of *S. aureus* were Penicillin G resistant. This result is similar to those reported from cows with mastitis in Tehran (57%) (Gooraninejad *et al.*, 2007), Argentina (40%) (Gentilini *et al.*, 2002) and Finland (50%) (Myllys *et al.*, 1998). But less reported for *S. aureus* strains isolated from

Table 1: Bacterial isolates and their frequency from subclinical mastitic cows

Type of bacteria	Frequency	Prevalence (%)
Staphylococcus aureus	86	39.44
Coagulase negative staphylococcus	41	18.80
Streptococcus agalactiae	20	9.17
Pseudomonas aeruginosa	15	6.88
Actinomyces pyogenes	20	9.17
Escherichia coli	20	9.17
Bacillus cereus	16	7.33
Total bacterial isolates	218	100.00

Table 2: Sensitivity pattern of *S. aureus* strains (n = 86) to selected antimicrobial agents

		Range of disk diffusion inhibition zone diameters (mm)		
Antimicrobial agent	Disc content	Susceptible	Intermediate	Resistance
Penicillin G	10 units	12.8%	0	87.2%
Tetracycline	30 µg	100.0%	0	0.0
Gentamycin	10 µg	100.0%	0	0.0
Norfloxacilin*	10 µg	100.0%	0	0.0
Vancomycin*	30 µg	97.0%	0	3.0%
Co-trimoxazole	25 µg	81.0%	19%	0.0
Clindamycin	2 µg	96.0%	0	4.0%
Bacitracin	10 unit	100.0%	0	0.0
Chloramphenicol	30 µg	75.0%	9%	16.0%
Nalidixic acid	30 µg	0.0%	8%	92.0%
Amoxicillin	10 µg	54.0%	0	46.0%

Table 3: Percentage and frequency of multi-drug resistance pattern of *S. aureus* strains (n = 86) for selected antimicrobials agents

Antibiotic	Frequency	Resistant (%)
One drug	79	92.00
Two drugs	22	25.00
Three drugs	9	10.46
Four and above	6	7.00

bovine mastitis milk in west India (23%) (Adesiyun, 1994). Similarly, it was also found that nalidixic acid suffers from serious resistance problem (92%). This finding is also similar with the reports in many countries Abdel-Gadir (2001) in camel reported that *S. aureus* were resistance (91.4%) to Penicillin G and Nalidixic acid.

The third drug with significant resistance profile in this study was amoxicillin (46%). The high resistance profile of amoxicillin similar to penicillin G could be associated with the same biochemical mechanism of the bacterial for resistance these class of agents. It was not possible to get report to compare this result with other reports. Moreover, it is worth noting that vancomycin (a toxic glycopeptides) and clindamycin (a potent lincomycin) are showing resistance to organism. This might indicate the improper and indiscriminate use of these agents.

The multi-drug resistance pattern found in this study is in agreement with that of Hussein (1999) who found that *S. auerus* was resistant to four antimicrobials, namely Ampicillin, Penicillin, Nalidicic acid and Bacitracin in bovine. Similar, result was found by Abdel-Gadir (2001) in camel that *S. aurus* were resistant to Nalidicic acid, Bacitracin and Penicillin G. Out of 86 isolates 100% of *S. aureus* were susceptible to Norfloxacilin, Gentamycin, Tetracycline and Bacitracin. This study revealed that Gentamycin, Norfloxacilin and Tetracycline were found to be effective drugs. This is in agreement with Almaw *et al*. (2008) who found that *S. aureus* susceptible to Norfloxacilin (100%) in bovine. Hussein (1999) in bovine also found that *S. aureus* was susceptible to Gentamycin. The second effective drug was Vancomycin (97%) of the tested isolates were susceptible. Where as Clindamycin (96%) and Co-trimoxazole (81%) were also the third effective drugs. Nalidixic acid was the weakest of all drugs used in the antimicrobial susceptibility test where only 0% of the isolates were susceptible and penicillin is the second weak drug of all drugs used and its susceptibility was only 12.8%.

This finding indicates that care has to be taken by the Veterinarian and farmers in selection of antimicrobials for treatment of mastitis. In this study it was found that the most effective drugs were Gentamycin, Norfloxacilin, Tetracyclin, Bacitracin, Vancomycin, Clindamycin and Co-trimoxazole, respectively.

CONCLUSION

In present study the prevalence of mastitis causing *S. auerus* at cow level was high (52.4%). This indicates that mastitis caused by *S. aureus* is one of the major problems of cross breed dairy cows in milk production.

The distribution of *S. aureus* and its resistance to antimicrobial in the herd of the selected dairy farms, indicate the economic impact of the disease. Moreover, the high isolation rate of *Staphylococcus aureus* in mastitis indicates the potential risk of consumption of raw milk.

It was found that the majority of the tested isolates were susceptible to the various antimicrobial agents especially Gentamycin, Vancomycin, Norfloxacilin, Tetracycline and Clindamycin. Penicillin, Nalidixic Acid, Amoxicillin and Chloramphenicol were observed to be less effective against *S. auerus*. The high resistance to Penicillin G found in this study emphasize as current susceptibility data are necessary to select appropriate antibiotics for a successful treatment.

RECOMMENDATIONS

Based on the results of present study the following recommendations are forwarded:

- In order to prevent the active transmission of mastitis in dairy farms pre milking and post milking udder washing and disinfections need to be practiced. Because of the economic and public health importance of mastitis in dairy cows. Hygienic milking procedures such as milking mastitic cows last have to be practiced by the farmers
- Care has to be taken in selection of drugs before instituting treatment against mastitis
- Further studies are necessary for assessing the importance of intramamary infection caused by other bacterial pathogens

ACKNOWLEDGEMENTS

The researchers would like to thank Jimma University, College of Public Health and Medical Sciences for Financial Support to execute this research study. It is also the pleasure to extend the gratitude to Veterinary Microbiology Laboratory for its technical and material support in the realization of this study.

REFERENCES

Abdel-Gadir, A.A., 2001. Cross sectional study of mastitis in camels (*Camelius dromedaries*) in selected sites of Ethiopia. M.Sc. Thesis, Adiss Ababa University School of Graduate Studies and Free University of Berlin.

Adesiyun, A.A., 1994. Characteristics of *Staphylococcus aureus* strains isolated from bovine mastitic milk: Bacteriophage and Antimicrobial agent susceptibility and enterotoxigenocity. J. Vet. Med., 42: 129-139.

Almaw, G., A. Zerihun and Y. Asfaw, 2008. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Trop. Anim. Health Prod., 40: 427-432.

Ameh, J.A. and L.S. Tari, 2000. Observation on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. Small Rum. Res., 35: 1-5.

Anyam, A.A. and J.O. Adekeye, 1995. Bacterial flora associated with mastitis in goats end sheep in Zaria (Nigeria) area. Bull. Anim. Health Prod., 43: 163-166.

Bitew, M., A. Tafere and T. Tolosa, 2010. Study on bovine mastitis in dairy farms of Bahir Dar town and its environs. J. Anim. Vet. Adv., 9: 2912-2917.

Carter, G.R., 1984. Diagnostic Procedures in Veterinary Bacteriology and Mycology. 4th Edn., C.C. Thomas Publisher, USA, ISBN-13: 978-0398048709.

Clinical and Laboratory Standards Institute, 2005. Performance Standards For Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, Pa.

Contreras, A., J.C. Corrales, A. Sanchez and D. Sierra, 1997. Persistence of subclinical intramammary pathogens in goats throughout lactation. J. Diary Sci., 80: 2815-2819.

Gentilini, E., G. Denamiel, A. Betancor, M. Rebuelto, M.R. Fermepin and R.A. De Torrest, 2002. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. J. Dairy Sci., 85: 1913-1917.

Gooraninejad, S., M. Ghorbanpoor and A.P. Salati, 2007. Antibiotic susceptibility of staphylococci isolated from bovine subclinical mastitis. Pak. J. Biol. Sci., 10: 2781-2783.

Hussein, N., 1999. Cross sectional and longitudinal study of bovine mastitis in urban and peri urban dairy systems in the Addis Ababa region, Ethiopia. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University School of Graduate Studies and Freie Universitat, Berlin.

Kozacinski, L., M. Iladziosmanovi, T. Majic, I.K. Jole and Z. Cvrtila, 2002. Relationships between the results of mastitis tests, somatic cell counts and the detection of mastitis agents in milk. Paraxis Vet., 57: 255-260.

Lakew, M., T. Tolosa and W. Tigrie, 2009. Prevalence and major bacterial causes of bovine mastitis in Asella, south eastern Ethiopia. Trop. Anim. Health Prod., 41: 1525-1530.

Moshi, N.G., G.C. Kitaro and U.M. Minga, 1998. Prevalence of mastitis in dairy goats in some selected farms in Morgoro and Arusha, Tanzania. Tanzania J. Agric. Sci., 1: 173-180.

Myllys, V., K. Asplund, E. Brofeldt, V. Hirvelä-Koski and T. Honkanen-Buzalski *et al*., 1998. Bovine mastitis in Finland in 1988 and 1995. Changes in prevalence and antimicrobial resistance. Acta Vet. Scand., 39: 119-126.

National Mastitis Council, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. 3rd Edn., National Mastitis Council Inc., Arlington, VA.

Ndegwa, E.N., C.M. Mulei and S.J. Munyna, 2000. The prevalence of subclinical mastitis in diary goats in Kenya. J. S. Afr. Vet. Assoc., 71: 25-27.

Owens, W.E., C.H. Ray, J.L. Watts and R.J. Yancey, 1997. Comparison of success and results of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J. Dairy Sci., 80: 313-317.

Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter, 2004. Clinical Veterinary Microbiology. Mosby Publishing, London, pp: 43-55, 327-344.

Radostits, O.R., D.C. Blood and C.C. Gay, 2007. Mastitis. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats and Horses. 9th Edn., Bailler Tindall, London, pp: 563-614.

Rowe, J.D., 1999. Milk quality and mastitis. Small Ruminant for the Mixed Practitioner. Western Vet. Conference. Lasvagas, pp: 152-156.

Schalm, O.W., E.J. Carrrlole and N.C. Jain, 1971. Bovine Mastitis. Lea and Febiger, Philadelphia, PA USA., pp: 1-21.

Sears, P.M., D.J. Wilson, R.N. Gonzalez and D.D. Hancock, 1991. Microbiological results from milk samples obtained pre-milking and post-milking for the diagnosis of bovine intramammary infection. J. Dairy Sci., 74: 4183-4188.

Smith, M. and D. Sherman, 1994. Goat Medicine. 1st Edn., Williams and Wilkins Waverly Company, USA., pp: 465-487.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd Edn., Blackwell Science Ltd., UK., pp: 233-250.

Topolko, S. and M. Benic, 1997. Problems and epidemiology of subclinical mastitis in small farm milk production. Praxis Veterinaira, 45: 69-76.