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Antibiotic Sensitivity of Mastitis Causing Bacteria Isolated From Dairy Cows in Welayta Soddo, Southern Ethiopia

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Abstract

In Welayta Soddo, mastitis is a highly prevalent disease of economic as well as public health importance and antibiotic therapy is the principal method of controlling the disease. Antibiotic sensitivity test using disc diffusion method was conducted on 43 strains of bacteria isolated from clinical and subclinical mastitis cows of local zebu and Jersey breeds in Welayta Soddo, southern Ethiopia. Tested organisms include *Staph. aureus*, *Staph.epidermidis*, *Streptococci* species, *Coryn. Pyogenes*, *Esch.coli*, *Coryn.bovis* and *Kleb. aurogenes.* The results indicated that the organisms showed varying degrees of sensitivity to antimicrobial agents tested. Of the total organism tested, 67% exhibited resistance to Penicillin, 58% to Polymyxin, 56% to Streptomycin and 35% to Trimethoprim. Almost all the population of *Staph. aureus*, the most frequently isolated pathogen, were resistant to 80% of the drugs tested where as only 24% of the population of *Staph. epidermidis*, were resistant to 80% of the antibiotics tested. Similarly, 26% of *Streptococci* species showed varying degrees of resistance to seven types of antibiotics. Penicillin exhibited poor activity against 87%, 45% and 40% of *Staph.aureus*, *Staph.epidermidis* and *Streptococci* species population respectively and to the whole population of the rest isolates. Likewise, streptomycin exhibited poor activity against 60% and 64% of *Staphylococci* and *Streptococci* species population, respectively. On the other hand, Gentamycin and Chloramphenicol were demonstrated to be the most active antibiotics (98% and 95% of the organisms exhibited sensitivity to theses antibiotics). Mishandling and irrational use of the drugs over a long time had led to the emergence of resistant bacterial population. Thus, proper handling and application of antibiotics together with routine drug sensitivity test and appropriate management and good hygienic

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condition of dairy environment is recommended for better control of mastitis in Welayta-Soddo.

Keywords: Ethiopia/ Welayta-Soddo/ dairy cows/ mastitis/ pathogenic bacteria/ antibiotic

Introduction

Antimicrobial therapy is the primary tool for controlling intramammary infection of dairy cows (Watt, *et al.*, 1995) if properly applied. Antimicrobial drugs widely used today are antibiotics which are produced mainly by microorganisms living in the soil where they may play a part in overcoming competing species in their natural habitat (Cruickshank, 1968). In areas where antibiotic therapy is the principal strategy towards the control of mastitis, the nature of interaction between the two vital factors, causal organisms and drugs, over a course of time has to be investigated in order to determine and cope with the problem of drug resistance.

The choice of a drug to be used for treatment of infectious diseases may have to be based not only on a species identification of the causal organisms isolated from the patient but also on the results of in-vitro sensitivity tests made with this particular strain (Cruickshank, 1968). Antimicrobial sensitivity tests provide important information for selecting the most effective antimicrobial agent for use in treatment of intramammary infection (Watt *et al,* 1995). Variations compelling on bacteria an increased resistance to the action of antibiotic drugs are of great importance in medicine because they may lead to failure in the drug therapy of infections (Cruickshank, 1968). Failure of therapy may be due to a variety of factors the most common of which are: incorrect identification of the causative pathogens, the emergence, of a drug-resistant variant of the originally sensitive infecting organisms, and primary infection with a drug- resistant variety of a species of organisms that is generally susceptible to the drug used (Cruickshank, 1968).

Despite an extensive use of different antibiotics separately or in combination for treatment of mastitis in dairy farms in Ethiopia, only very limited information is available on the pattern of sensitivity of mastitis causing microorganisms and the extent of drug resistance problem. And to what extent these factors are affecting the absolute incidence of the disease also remains unclear. Few studies conducted on antibiotic sensitivity of those

bacteria causing mastitis (Mengistu, 1986; Geressu, 1989;) revealed the presence of drug resistance problems.

In Welayta, mastitis is a highly prevalent disease and antibiotic therapy, over a long period of time, is the principal method towards control of the disease. However, to what extent this has led to the problem of drug resistance is yet to be investigated. This study, thus, investigates the pattern of antibiotic sensitivity of bacteria responsible for causing mastitis in local zebu and Jersey breeds in Welayta Soddo, southern Ethiopia and recommends the most appropriate drugs that could help control mastitis in the area.

Materials and Methods

Study area

The study was conducted in 1993 in Welayta Soddo, a province located at about 390km south of Addis Ababa. Altitude ranges from 1100-2500 meter above sea level. The area experiences a mean annual temperature of about 19^{0}C and the rainfall regime over much of the area is typically bimodal with the big rain season occurring from June to September and small rain season from February to April. The annual rainfall ranges from 450 to 1446mm. The prevailing agricultural system is typically mixed farming. Livestock population of the area is estimated to be 1.8 million, of which 53% are cattle,

9% sheep and goat, 3% equines and 35% poultry. Disease is the main constraint of animal production in the area. The subsistence needs of

increasing human population are causing a progressive expansion of the area under cultivation and subsequent limitation of the grazing lands.

Source of milk samples

Milk samples were collected from local zebu cows managed under traditional husbandry system and Jersey cows from Soddo dairy farm. Zebu cows are milked twice a day and are allowed to dry off by milking at gradually increasing interval. Hygienic milking techniques such as pre-milking and post milking udder washing and disinfecting and routine screening of milk for any abnormality are not practiced. The Jersey cows are managed relatively under good husbandry system. They are kept in exclusive stalls and provided with hay and concentrate and also allowed a grazing time of five hours a day on native pastures. Milking is made by hand twice a day inside the night barns and animals are allowed to dry off at late lactation period by abrupt cessation of milking. Pre-milking udder washing with warm water and drying using

clear towel is always practiced although post-milking disinfection is not. Despite the frequent use of several kinds of antibiotics separately or in combination, mastitis remains to be one of the most economically as well as disease of public health importance. Clinical and sub-clinical cases as well as blind quarters are common at any point in time.

Detection of mastitis

Mastitis was detected using bromothymol blue indicator test, strip cup method and physical examination of the mammary glands and milk samples for any abnormalities

Milk sampling

Milk samples were collected from mastitis cows following udder washing using clean and dry towel and disinfected by Benzalkonium chloride (2ml of the reagent was diluted in 1 liter of clean water). Hands were also washed with detergent (soap) and disinfected. The external sphincter of the teats was extruded by pressure to ensure that dirt and wax were removed from the orifices. About 15- 20ml of the milk sample from all affected quarters was collected in sterile test tube in an oblique manner to avoid contamination. During collection, the tubes were canted and well corked to avoid the entrance of dust, skin scales and other contaminants. These were transported to laboratory for detail microbiological analysis. Samples, which weren't processed immediately, were put in refrigerator at 4^0C for subsequent analysis within 12- 24hrs.

Isolation and identification of the organisms

Collected milk samples were analyzed microbiologically at Soddo veterinary diagnostic and research laboratory, department of bacteriology based on the methods described by Brown, *et al* (1969) and David and Peggy (1988) to isolate and identify organisms responsible for causing mastitis.

Antibiotic sensitivity test

Ten antibiotics including those frequently used for treatment of mastitis in the area were tested. These were: Penicillin (10U/IE), Streptomycin (10μg), Tetracycline (30μg), Trimethoprim (75μg), Polymyxin (300/IE), Chloramphenicol (30μg), Kanamicin (30μg), Methicilin (5μg), Gentamycin (30μg) and Cephalotin (30μg). The test involved disc diffusion method described by David and Peggy (1988) and the results compared with zone diameter interpretive standards set for each antibiotic. With a standard concentration of antibiotics in the disc and standard antibiotic sensitivity test

media and conditions, the concentration of the diffused antibiotics at any given distance from the disc was relatively predictable and constant (Woolcock and Mutimer, 1983). For each antibiotic minimal inhibitory concentration break points have been established above or below which an organism is classified as resistant (R), susceptible (S) or intermediate (I) sensitivity (David and Peggy, 1988).

Results

The isolates

The following genera of bacteria were isolated and identified in order of importance from microbiologically analyzed milk samples: *Staphylococcus*, *Streptococcus, Corynebacterium* and *Bacillus*. Of the total microorganisms isolated and identified (n=88) from 90 milk samples, 43 strains of isolates (15 *Staph. aureus,* 10 *Staph. epidermidis*, 11 *Streptococcus species* (*Strep.agalactiae*, *Strep. dysagalactiae* and *Strep.uberis*), 3 *Coryn. pyogenes*, 1 *Cory. bovis*, 2 *Esch. coli* and 1 *Kleb. aurogenes*) were tested for sensitivity to ten antibiotics including those frequently used in the area for treatment of mastitis. Selection of the bacteria was based mainly on frequency of isolation. The test was then carried out soon following isolation and identification of the organisms.

Antibiotic sensitivity test pattern

Responses of the organisms to antibiotics tested were summarized in table 2. There were wide ranges of variations in the sensitivity patterns of the isolates to antimicrobial agents tested. From the total isolates showing resistance to the drugs tested, 67% (29), 58% (25), 56% (24), 35% (15), 26% (11), 23%(10) and 19% (8) had shared resistance to Penicillin, Polymyxin, Streptomycin Trimethoprim, Tetracycline, Methicilin and Kanamycin and Chloramphenicol respectively.

Organisms showing multiple drug resistance of considerable concern had also been encountered. *Staph. aureus,* and *Staph. epidermidis*, the first and second most frequently isolated pathogens respectively from clinical and subclinical cases of mastitis in the area, showed resistance to more than three-fourth of the antibiotics tested. 26% of *Streptococcus* species showed resistance to seven types of antibiotics, though in all cases the proportion of resistant bacteria for each drug varied considerably.

Penicillin exhibited poor activity against 87%, 45% and 40% of *Staph. aureus*, *Staph.epidermidis* strains and *Streptococcus* species respectively and to the whole strains of the rest isolates. Similarly, streptomycin demonstrated poor activity against 60% of *Staphylococci* and 64% of S*treptococci species.*

In the present study, Gentamycin was the most active drug against all species of the organisms tested. With the exception of one resistant strain of *Streptococci species*, the overwhelming majorities of the organisms ((98%) were sensitive to the drug. Similarly Chloramphenicol showed good activity against most bacterial population tested (only 5% of the organisms showed resistance against Chloramphenicol). Trimethoprim, Polymyxin and Tetracycline demonstrated poor activity against 60%, 73% and 40%of *Staph.aureus* strains respectively. *Coryn pyogens* showed resistance to seven types of antibiotics namely; Penicillin; Streptomycin, Tetracycline, Trimethoprim, Polymyxin, Chloramphenicol and Kanamicin where as *Coryn. bovis* showed resistance to only Penicillin. Coliform organisms were 100% resistant to Penicillin and 50% resistant to Tetracycline. On the other hand, almost all *coliforms* were sensitive to most antibiotics tested.

Discussion

The relative high incidence of Penicillin and Streptomycin resistant bacterial population (67% and 56% respectively) reported in this study should be of great concern and it consolidates the observation that treatment of mastitis using these drugs is less valuable economically. In fact, Penicillin and Streptomycin, used separately or in combination towards the control of mastitis in Welayta Soddo, were the most common drugs constituting about 85% of the total antibiotics used (Figure.1).

Cruickshank (1968) described that one of the most common causes of failure of drug therapy is the emergence of a drug-resistant variant of the originally sensitive infecting organisms. The presence of many infected cases, with lesions capable of harboring pathogenic organisms subjected to continual treatment with the antibiotics, provides conditions that selectively favor the proliferation and dissemination of virulent strains of bacteria with multiple resistances to antibiotics in common use (Cruickshank, 1968). Mishandling and irrational application of antibiotics towards the control of mastitis (under-dosage, adulteration, inappropriate route and frequency of

application, use of expired drugs and mixing with other chemicals) seems to have favored development of drug resistance and hence failure.

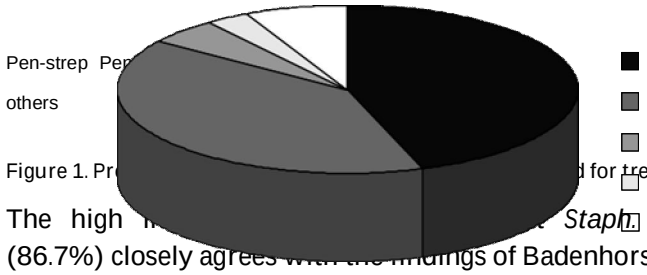

Figure 1. Pr d for treatment of Mastitis in Welayta

The high i *Staph. aureus* population in this work (86.7%) closely agrees with the findings of Badenhorst (1978) and Mengistu (1986) who reported 83% and 93.3% resistant population of the bacteria respectively. This finding, however, was surprising in relation to the work of Yao and Moellering, (1991) who reported that Penicillin, a β-lactamin antimicrobial agent, is believed to exhibit good activity against Staphylococcus and Streptococcus species. Supporting this opinion, Blood and Radostits (1989) also stated that Penicillin, especially Procaine Penicillin G, is universally used as an intramammary infusion towards the control of mastitis. Brander (1963) also suggested that in order to combat the problem of acquired bacterial drug resistance, synthetic Penicillin should be used among which Oxacillin is the most important one.

However, it was generally reported that drug resistant strains of Staphylococci appeared to be increasing from time to time with varying rate of incidence (Blood and Radostits, 1989). The high degree of Penicillin resistant population of *Staph. aureus* could be attributed to high proportion of strains that are naturally resistant to the drug by virtue of their capacity for producing Penicillinase, an enzyme that destroys Penicillin, or emergence of resistance strains as a result of wide and irrational use of the such drug since their survival and spread is selectively favored (Cruickshank, 1968). On the other hand, the high degree of Penicillin and Streptomycin resistance of these organisms could be explained from the fact that a drug sensitive pathogens is present in a part of the body, in which drug-resistance commensal bacteria may be present, thus it could acquire a drug- resistance character by the transfer of genes from one of the later organisms (Cruickshank, 1968). In this work too *Staph. epidermidis* and Streptococci

species showed considerable resistance to Penicillin (40% and 45% resistance respectively).

The findings of the present study revealed that Gentamycin and Chloramphenicol were found to be the most active and recommendable drugs in the treatment of mastitis. Rao *et al* (1989) and Chandra (1988) also reported that Gentamycin and Chloramphenicol were the most effective antibiotics against mastitis pathogens. Fred (1960) observed the importance of Chloramphenicol in the treatment of many staphylococcal infections that were resistant to Penicillin, Streptomycin and Tetracycline. However, the use of such drugs in the area is limited because of unaffordable cost and inadequate supply.

As little was known about the incidence of drug resistant population of mastitis causing bacteria, the findings of the present study could be of considerable value in formulating mastitis treatment strategies in Welayta Soddo. Mishandling and irrational use of antibiotics had, interalia, contributed much to the development of drug resistance problem particularly with those antibiotics commonly applied for treatment of mastitis.

In this study Penicillin and Streptomycin were shown to be ineffective drugs against the treatment of mastitis while Gentamycin and Chloramphenicol were the best. Because of the complexity of the disease they cause and their opportunistic nature and high capacity to develop adaptation with changing environment mastitis causing bacteria are widely spread and persistently present in dairy environment. Thus, control of mastitis by chemotherapy along seems less feasible unless integrated with factors like appropriate management and good hygienic condition of the dairy environment. However, proper handling and rational use of antibiotics is very important in areas where drug therapy is the principal method of controlling the disease. Furthermore, the application of routine drug sensitivity test is recommended as this technique assists not only proper selection of the drugs to be used but also reveals the extent of resistance problem in the area.

Table 1. Responses of the test organisms to various antibiotics

Test organism	No. Tested	Sensitivity of test organisms to antibiotics																														
		Penicillin (10U/IE)			Methicillin (5µg)			Streptomycin (10µg)			Gentamycin (10µg)			Tetracycline (30µg)			Trimethoprim (75µg)			Polymyxin (300/IE)			Cephalotin (30µg)			Chloramphenicol (30µg)			Kanamycin (30µg)		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staph.aureus	15	2	-	13	12	-	3	3		9	15	-	-	9	-	6	6	-	9	4	-	11	10	-	5	15	-	-	10	-	5
Staph. epidermidis	10	6	-	4	7	-	3	4	-	6	10	-	-	9	-	1	8	-	2	7	-	3	7	-	3	10	-	-	8	-	2
Strep.species [a]	11	4	2	5	9	-	2	4	-	7	10	-	1	10	-	1	9	-	2	3	-	8	11	-	-	11	-	-	11	-	-
Coryn.pyogens	3	0	-	3	3	-	-	2	-	1	3	-	-	2	-	1	1	-	2	0	-	3	3	-	0	2	-	1	2	-	1
Coryn.bovis	1	0	-	1	1	-	-	1	-	-	1	-		1	-	-	1	-		-	1	-	1	-	-	1	-		1	-	-
Esc.coli	2	0	-	2	0	-	2	2		-	2	-	-	1	-	1	2	-	-	2	-	-	2	-	-	2	-				-
Kleb. aurogen	1	0	-	1	1	-	-	0	-	1	1	-	-	0	-	1	1	-	-	1	-	-	1	-	-	-	-	1	1	-	-
Total	**43**	**12**	**2**	**29**	**33**	**-**	**10**	**16**	**3**	**24**	**42**	**-**	**1**	**32**	**-**	**11**	**28**	**-**	**15**	**17**	**1**	**25**	**35**	**-**	**8**	**41**	**-**	**2**	**35**	**-**	**8**

[a] Streptococci species tested include Strep. agalactiae, Strep. dysagalactiae, Strep.uberis.

Responses of the bacteria to antibiotics were expressed as resistance (R), sensitive (S) or Intermediate (I).

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