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Isolation and identification of pathogenic bacteria causing mastitis in sheep and Goats of Panjgur City

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Abstract

Mastitis is an important disease of sheep and a goat because it decreases the amount and quality of the milk produced by a dairy animal and reduces weight gain in lambs and meat kids. It can also affect animal wellbeing. Total 200 samples were collected and examined on various culture media. The 70 (35%) samples were found positive, while 130 (65%) showed no growth on culture media and were recorded as negative. The percentage incidence of each bacterial species isolated and recognized from mastitic milk samples of sheep and goats. Three bacterial species were recognized from mastitic milk samples of sheep and goats which were: *Staphylococcus aureus*, *Escherichia coli* and *Proteus spp*. The result data showed that the percentage of *Staphylococcus aureus* was high in mastitic milk samples.

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Introduction

In the economy of Pakistan livestock, especially buffaloes, cattle, sheep and goats, plays a vital role. Livestock contribution about 55.4% to agriculture and 11.9% to national GDP as reported by economic survey of Pakistan. The 8.5 million landless and small families raised Livestock in the rural areas of Pakistan and 35-40 million urban populations are independent on Livestock. In Pakistan livestock includes buffalo, cattle, sheep, asses, camels, goat, horses, and mules. Meat, wool, hair, milk, bones, blood, fat, hides, skins and eggs are the major livestock yield while milk and meat are taken as main products. Moreover this, for draught purposes these animals are used. Sheep and goats provides vast collection of harvest and services such as direct cash earnings, milk, skin, manure, meat, and social functions (Adane and Girma, 2008).

The milk supports the growth of pathogenic bacteria which comes from animal infections or milk pollution (Santos *et al.*, 2009). The cheese and fermented products were processed from milk, thus, things affect milk amount and excellence, such as mastitis, have an irresistible effect on financial losses to the farmer and human health. The inflammation of the udder is called Mastitis. Mastitis is an vital farm animals disease and under untreated circumstances, it constitutes a severe trouble in dairy herds with significant economic cost, largely due to decrease in milk manufacture, decreased milk excellence for dairy used and poor milk sanitation; especially main when unpasteurized milk is utilized for cheese making and use (Seegers *et al.,* 2003; Persson and Olofsson, 2011).

Milk from common uninfected quarters usually contains less 200,000 somatic cells/ml. Somatic cells counts of 300,000 or extra is well thought-out as a sign of inflammation in the udder (Hillerton, 1999).

Mastitis is cure by treatment with different antibiotics after recognition of the causative pathogens. Antibiograms sensitivity test will be performed to guarantee sufficient treatment. In sheep and goats, intramammary medicine or drugs therapies using a mixture of nafcillin, penicillin and, dihydro streptomycin have been establish to be effective in dropping the load of mastitis agents after lambing (Chaffer *et al.*, 2003). The principle of the current study was to inspect the virtual prevalence, incidence and to reading the biochemical profiles of different bacterial pathogens from sheep and goat suffering from mastitis.

Material and method

Sample collection

200 milk samples of sheep and goats from Panjgur were collected in clean bottles and were brought to the CASVAB, UoB. The tips of teats of sheep and goats were washed with antiseptic agent before gathering of milk**.** Milk samples were straightly collected in bottles. The bottles having milk samples were stored in refrigerator before additional analysis. The different culture media were used for identification of different bacteria from milk samples. Further that the morphological, staining and identification of the bacteria was made as described by Bergeys, (1992) and Bauer *et al.,* (1966).

Cultural and staining characteristics of bacteria

The milk samples were streaked on different media and were incubated at 37°C for 24 hs to get bacterial growth. The process was done till pure growths obtained. The morphological shape and cultural characteristics of the different bacterial was determined. After examining their growth, the stock cultures of diverse purified bacteria obtained was stored in the refrigerators at 4°C for further analysis.

Biochemical tests

The different biochemical tests like, urease test, catalase test, indole test, nitrate reduction test and VP/MR test, were used for recognition of different targeted bacteria.

Results

Total 200 samples were examined on various culture media. Total 70 (35%) samples were found positive, while 130 (65%) were negative and showed no growth on culture media. It is concluded that majority cases of the mastitis were caused by bacterial species as shown in Table 1.

Table 1. The overall percentage prevalence of mastitis caused by various bacterial species in sheep and goats.

Animal species	Total No. of samples examined	No. of positive milk samples	% of positive samples	No. of negative milk samples	% of negative samples
Sheep and goats	200	70	35%	130	65%

The number and percentage incidence of each bacterial species isolated and recognized from mastitic milk samples of sheep and goats are presented in Table 2. Three bacterial species were isolated and recognized. The bacterial species identified were: *Staphylococcus aureus*, *Escherichia coli*, *Proteus spp*, and their incidence in samples were: 55.71, 32.84, and 11.41 respectively as shown in Table 2. However, the incidence of *Staphylococcus aureus* was higher than other bacterial species. It is concluded from the present investigation that in sheep milk and goats' milk have *Staphylococcus aureus, Escherichia coli,* and *Proteus spp* as shown in Table 2.

Table 2. The number and percentage incidence of individual bacterial species in mastitic milk samples of sheep and goats.

Bacterial species	No. of samples occurring	Percentage (%)
Staphylococcus aureus	39	55.71
Escherichia coli	23	32.84
Proteus spp	8	11.41

Three bacterial species were isolated and recognized through their morphological, cultural and staining characteristics are presented in the Tables 3. However, individual bacterial species has been identified and their characteristics are described as under:

Staphylococcus aureus

The *Staphylococcus aureus* was determined as Gram-positive cocci in shape and possessed grape-like structure. The cells were arranged in pairs, tetrads, and irregular clusters. The size of cells varied from 1.0-1.4µm in diameter. Cells were non-motile, non-capsulated and non-flagellated. During culture of the species on Brain Heart Infusion agar it produced white to yellowish white and golden yellow colonies glistening, shiny with circular and entire margin. It produced β hemolysis of red blood cells on blood agar medium, whereas it did not grow on MacConkey's agar at all. In broth medium, uniform turbidity was recorded (Tables 3 and 4).

Escherichia coli

The *Escherichia coli* was Gram-negative; the species was observed as coccobacillary, with short rods or straight rods with 0.5x1.0 to 3.0µ in size The cells of the organism were non-motile and arranged in singles or in pairs and sometimes in short rods. The cultural characteristics of the species exhibited on Brain Heart Infusion agar showed grey-white, large round, mucoid, shiny, convex with entire margin colonies. The size of the colonies varied from 1-3mm in diameter. When the species was cultured on blood agar medium, it did not break down (hemolysis) red blood cells of sheep and produced non-hemolytic colonies. Whereas on MacConkey's agar, it produced lactose fermentative pink colonies (Tables 3 and 4).

Proteus spp

The *Proteus* spp was Gram-negative, rods in shape. The cells of the species were motile with the help of peritrichous and arranged singly, pairs or in short chains in fresh culture whereas long filamentous were common. On Brain Heart Infusion agar medium, it produced yellowish, spreading colonies with slightly raised layer of growth and an earthy smell. However, swarming and spreading colonies were noted on both, blood and Brain Heart Infusion agar media. On blood agar medium, it also produced white and non-hemolytic colonies. While on MacConkey's agar medium, produced lactose fermentative colonies (Tables 3 and 4).

Table 3. Morphological and staining characteristics of bacterial species from mastitis samples of sheep and goats.

Bacterial species	Shape	Arrangement	Staining	Motility
Staphylococcus aureus	Cocci in shape	Grapes like structure pairs, and irregular clusters	G +ve	Non-motile
Escherichia coli	Cocco bacilli, short rods	Single and pairs	G –ve	Non-motile
Proteus spp	Rods shape	Occurred in singles, pairs or in short chains	G –ve	Motile

Table 4. Cultural characteristics of bacterial species from mastitis samples of sheep and goats.

Bacterial species	Colony characteristics on solid media	Colour of bacterial colonies
Staphylococcus aureus	Colonies were circular, entire margin in shape irregular, convex and shiny on BHI/A. whereas on B/A, they were entire convex, and β hemolytic. Whereas it did not grow on M/A.	White to yellowish and golden yellow colonies
Escherichia coli	Colonies were grey-white, large round, shiny, convex with entire margin colonies on BHI/A. While on B/A, dew-drop like with narrow zone of β hemolysis were noted. Whereas on M/A, fermentative pink colonies produced.	Grey to white, and shiny surface
Proteus spp	Colonies were circular, spreading colonies with swarming growth on BHI/A. Whereas on B/A colonies were white, and non-hemolytic. On MacConkey's agar medium, it produced pink colonies.	Yellowish and shiny surface.

BHI/A = Brain Heart Infusion agar B/A = Blood agar
M/A = MacConkey's agar

Biochemical tests were performed to isolate and identify *Staphylococcus aureus, Escherichia coli* and *Proteus* spp as shown in Table 5.

Table 5. Biochemical properties of bacteria from mastitic milk samples of sheep and goats.

Bacterial species	Cat	Coag	Oxid	Ind	TSI	Ure	M.R	V.P	G.L	S. citr	H_2S	Aesc
Staphylococcus aureus	+ve	+ve	-ve	-ve	A/A	+ve	+ve	-ve	+ve	-ve	-ve	-ve
Escherichia coli	+ve	-ve	+ve	+ve	A/A	-ve	+ve	-ve	-	-ve	-ve	-ve
Proteus spp	+ve	-ve	+ve	-ve	K/A	+ve	-ve	-ve	+ve	+ve	+ve	-ve

Whereas:

- = not tested

-ve = negative + ve = Positive
Cat = Catalase GL = Gelatin liquefaction
VP = Voges-Proskauer Coag = Coagulase
Asc = Aesculin H2S = Hydrogensulphide Gas
MR = Methyl red S.citr = Simmons's citrate
Ure = Urease Oxid = Oxidase
Ind = Indole TSI = Triple sugar iron agar
A/A =acidic slant and acidic butt
K/K=alkaline slant and alkaline butt
K/A=alkaline slant and acidic butt
N/N = normal slant and normal butt

Discussion

The 200 samples were collected and examined on various culture media. Total 70(35%) samples were found positive, while 130(65%) showed no growth on culture media and were recorded as negative. Gebrewahid *et al.* (2012) analysed 390 lactating animals comprising 255 goats and 135 sheep were randomly selected from population and screened for evidence of subclinical mastitis. The overall prevalence of subclinical mastitis was found to be 18.03% (46/255) and 28.14% (38/135) in goats and sheep respectively.

The percentage incidence of each bacterial species isolated and recognized from mastitic milk samples of sheep and goats. Three bacterial species were recognized from mastitic milk samples of sheep and goats which were: *Staphylococcus aureus*,

Escherichia coli and *Proteus* spp Gebrewahid *et al.* (2012) studied the incidence of individual bacterial species in subclinical mastitis milk samples of sheep and found coagulase negative *Staphylococcus* (44.7%), *Staphylococcus aureus* (27.7%), *Escherichia coli* (17.0%) and *Streptococci* (10.63%). The results regarding percentage incidence of individual bacterial species recorded in the present study are similar to the findings of the above worker. *Staphylococcus aureus* had higher incidence in mastitic milk samples which has also been reported by above worker.

All related biochemical properties tested and recognized for the species are the similar as observed by Khan and Rind, (2001) and Fazlani *et al.* (2008).

Conclusions

The 35% cases of mastitis in sheep and goats are caused by bacterial organisms. *Staphylococcus aureus* is the most dominant pathogen and caused mastitis in sheep and goats either alone or in association with other bacterial species. The mastitis caused by bacterial species in sheep and goats can be treated through use of proper antibiotics.

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