

Bio-Morphological Characteristics of Bacterial Species Identified from Mastitic Milk Samples of Camel

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Abstract

Eight different bacterial species were identified from mastitic milk samples of camels. The species were *Bacillus cereus*, *Corynebacterium pyogenes*, *Escherichia coli*, *Micrococcus luteus*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Morphologically, they varied from cocci to rod shape and were gram-positive and negative. The bacterial species produced a variety of colonies on different culture media. Some were spherical, swarming and spreading colonies on agar media while in broth granular turbidity with powdery deposits were also seen during investigation. A few of them produced α and β haemolysis of red blood cells in blood agar.

INTRODUCTION

Mastitis is recognized world wide as the most important and costly disease of dairy animals. Field surveys of major livestock diseases in Pakistan have indicated that mastitis is one of the most important health hazards in the country (Ajmal, 1990). Mastitis is caused by interaction of various factors associated with host, pathogens and the environment. Infectious agents like bacteria, viruses, fungi, and algae are mostly the primary causes of disease. The etiology of mastitis is very complex because a large number of microorganisms are known to cause inflammation of the udder. Generally, well-recognized organisms responsible for mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus hovis*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* etc. (Radostits et al., 2000).

Bio-morphological variation in the characteristics of bacterial species that cause mastitis is described by Dewani (2000). In addition, several workers have observed the bio-morphological characteristics of bacterial species throughout the world (Gabbar, 1992; Bergey's 1992; and Khan & Rind, 2001). So in the following investigation, bio-morphological characteristics from isolation and identification of species, independent of their bio-morphological and antigenic characteristics, were studied and described.

MATERIAL AND METHODS

Seventy eight clinical mastitic milk samples from different herds of camels were collected in sterilized bijoux bottles (completely wrapped/ covered with aluminum foil) and brought to Central Veterinary Diagnostic Laboratory (CVDL), Tando Jam, Sindh, Pakistan for isolation and identification of bacterial species. Before collection of samples, the tips of mastitic teats were cleaned with cotton wool moistened with 70% alcohol and few strips of milk were discarded to avoid contamination as much as possible.

Before processing the samples, all preparations were made as described by Gabbar (1992). The media to be needed for proper cultivation and inoculation of bacterial organisms were prepared, inoculation and identification characteristics whether of physical, cultural, biochemical and morphological were recorded as adopted by Khan and Rind (2001).

The biochemical tests were conducted to confirm the identification of bacterial organisms. For this purpose, oxidase, catalase, coagulase, indole, Voges Proskauer, Urease, methyl red, gelatin liquefaction, Simmon's Citrate, H₂S production, asculin hydrolysis and TSI teats were carried-out (Difco, 1960) while for sugar fermentation properties, nine different sugars of 1% were prepared and used for each bacterium as described by Cruickshank (1970). The sugars were: Mannose, Xylose, Inositol, Galactose, Mannitol, Glucose, Maltose, Creatinin and Dulcitol.

RESULTS AND DISCUSSION

During present investigation, morphological, cultural, staining and chemical characteristics of various bacterial species of camel mastitis were recorded and presented in different tables.

BACILLUS CEREUS

The cells of the species were observed as straight rods with rounded ends, arranged singly or in chains and motile. Stained positively in young cultures and gram-negatively in old cultures. Colonies on blood agar were seen dull-white with undulated margin and produced β haemolysis, uniform turbidity also recorded in nutrient broth medium, it did not grow on MacConkey's agar. Biochemically species was observed that possessed catalase, oxidase, citrate utilization and aesculin hydrolysis properties and considered to be positive. It fermented glucose, mannose, xylose, galactose, creatinin, dulcitol, maltose and mannitol, but did not ferment inositol (Tables 1, 2, 3 and 4). Gabbar (1992) also described almost similar morphological, staining and cultural characteristics, whereas Dewani (2000) reported that *Bacillus cereus* produced a variety of colonies from small shiny compact to large feathery and spreading types. They observed positive interaction with Voges-Proskauer and negative to methyl red and urease. It fermented glucose, mannose, xylose, galactose, creatinin, dulcitol, maltose and mannitol, but did not ferment inositol.

CORYNEBACTERIUM PYOGENES

The organisms of this specie were straight, slightly curved short rods, arranged singly, in pairs, V forms and small clusters. Non-motile, gram-positive but also stained irregularly and sometimes considered to be acid-fast. The colonies on blood agar were seen gray-white, convex, entire and produced β haemolysis. It produced granular and pellicle growth in nutrient broth. It did not grow on MacConkey's medium. *Corynebacterium pyogenes* did not show catalase, aesculin, urease and nitrate reduction properties when tested for different chemicals. It produced K/A that means alkaline slant and acidic butt and did not produce H_2S in TSI agar. It fermented glucose, mannose, xylose, galactose, dulcitol and maltose, but did fail to ferment mannitol, creatinin and inositol (Tables 1, 2, 3 and 4). Gabbar (1992) and Shaikh (1999) both described the similar morphological and staining properties of *Corynebacterium pyogenes* as recorded in this investigation. They observed the morphological and staining characters as small coccoid, pleomorphic bacilli varying from 0.9-1.0 μ in diameter. On serum agar, it formed minute

dewdrop like colonies (Khan and Rind, 2001 and Shaikh, 1999). They observed that the species interacted positively to gelatin and negatively to catalase, aesculin, urea and nitrate. It produced alkaline slant and acidic butt and did not produce H_2S in TSI agar. It ferment glucose, mannose, xylose, galactose, dulcitol and maltose, but did fail to ferment mannitol, creatinin and inositol.

Figure 1

Table 1: Morphological and staining characteristics of bacterial species identified from mastitic milk samples of camels

Bacterial species	Shape	Arrangement	Staining reaction	Motility
<i>Bacillus cereus</i>	Straight rods with rounded ends	Singles / chains	G+ve	Motile
<i>Corynebacterium pyogenes</i>	Straight, slightly curved short rods	Singles, pairs and small clusters	G+ve	Motile
<i>Escherichia coli</i>	Coccobacillary, straight rods	Singles / pairs	G-ve	Non-motile
<i>Micrococcus luteus</i>	Cocci	Tetrads / pairs	G+ve	Non-motile
<i>Pasteurella haemolytica</i>	Rods, slightly curved	Singles	G-ve, bipolar	Non-motile
<i>Pasteurella multocida</i>	Coccobacillary, short rods	Singles	G-ve, bipolar	Non-motile
<i>Pseudomonas aeruginosa</i>	Straight and slightly curved rods	Singles	G-ve	Motile
<i>Staphylococcus aureus</i>	Cocci	Singles, pairs and irregular clusters.	G+ve	Non-motile

ESCHERICHIA COLI

Escherichia coli cells were seen as coccobacillary, short rods, arranged singly and in pairs, stained negatively and non-motile. The cultural characteristics recorded were moist, gray, shiny and convex with entire margin colonies, non-haemolytic and easily dispersible in normal saline solution. Uniform turbidity was observed in the nutrient broth. It produced lactose fermentative colonies on MacConkey's agar (Tables 1, 2, 3 and 4). Khan and Rind (2001) described *Escherichia coli* as short rods of 0.5-3.0 μ with a variable shapes from coccoid bipolar to long rods. Usually occurred singly but short chains were uncommon. The cells were motile and non-spore forming, gram-negative and non-acid fast. On blood agar, non-haemolytic while on MacConkey's agar, *Escherichia coli* gave circular, smooth and convex colonies with pink colour. Dewani (2000), Shaikh (1999), Khan and Rind (2001) also demonstrated that *Escherichia coli* showed positive characteristics of catalase but not oxidase. It exhibited A/A and produced gas in TSI agar. It interacted negatively to indole, urea, Voges-Proskauer,

gelatin and citrate, while positively to methyl red and nitrate. The species fermented glucose, mannose, maltose, mannitol, xylose and inositol but did not ferment galactose, creatinin and dulcitol.

MICROCOCOCCUS LUTEUS

Micrococcus luteus was found gram-positive cocci, non-spore forming, non-haemolytic, non-motile aerobic or facultative anaerobes, arranged in pairs, irregular clusters and tetrads. It grew in circular, entire, convex and creamy yellow pigmented colonies heaving 0.5-2.5 μ diameter. On MacConkey's agar, it formed round, opaque and colourless colonies, while on blood agar, it formed cubical packets usually produced colonies with a granular surface and matt appearance. It produced uniform turbidity in nutrient broth medium (Tables 1 to 4). The species showed cubical packets like colonies on blood agar and also produced uniform turbidity in nutrient broth medium were also encountered by Gabbar (1992). Khan and Rind (2001) also recorded similar biochemical properties of *Micrococcus luteus* as recorded in the present study.

PASTEURELLA HAEMOLYTICA

The organism was observed as gram-negative, bipolar and slightly pleomorphic short rods, arranged singly and non-motile. Colonies were mucoid, shiny and convex with irregular margin and β haemolytic with distinctive smell on blood agar. Turbidity growth was observed in nutrient broth medium. Showed its positive interaction with catalase, oxidase, gelatin and nitrate reduction, but did not interact with indole, urea, methyl red, Voges Proskauer and citrate. It produced A/A when cultured on TSI but no H₂S production occurred in TSI agar (Table 1 to 4). *Pasteurella haemolytica* positively interacted with catalase, oxidase, gelatin and nitrate, but negatively to indole, urea, methyl red, Voges-Proskauer and citrate. Obied (1983), Ramadan et al. (1987) and Dewani (2000) who recorded similar biochemical properties as demonstrated in the present investigation.

Figure 2

Table 2: Cultural characteristics of bacterial species isolated from mastitic milk samples of camels

Bacteria species	Colony characteristics		
	Solid medium	Colour	Broth medium
<i>Bacillus cereus</i>	On blood agar, β haemolytic with undulate margin colonies produced but it did not grow on MacConkey's agar.	Dull white or yellowish.	Uniform turbidity
<i>Coryne. pyogenes</i>	Gray-white, convex, entire, β haemolytic. It did not grow on MacConkey's agar, difficult to disperse in normal saline solution.	Grayish – white.	Turbidity with pellicle growth colonies were observed.
<i>Escherichia coli</i>	Shiny surface, convex, entire, non-haemolytic colonies on blood agar, Lactose fermented colonies on MacConkey's agar, easily dispersible in normal saline.	Moist gray on nutrient agar plates, while rose pink on MacConkey's agar plates.	Organism produced uniform turbidity.
<i>Micrococcus luteus</i>	Circular, entire, convex with regular edges on nutrient agar. Cubical packets and non-haemolytic on blood agar. Colonies were opaque and round on MacConkey's agar.	Yellow-pigmented.	Uniform turbidity growth seen.
<i>Paste. haemolytica</i>	Moist, shiny, mucoids, convex with irregular margin colonies were seen on nutrient agar. On blood agar, organism produced β haemolytic colonies with distinctive smell. While on MacConkey's agar, it produced lactose fermented colonies.	White colonies on nutrient agar while on MacConkey's agar it produced pink colonies.	Turbidity growth
<i>Paste. Multocida</i>	Shiny, moist, mucoid non haemolytic and convex on blood agar. On MacConkey's agar no growth occurred.	Brownish.	Granular powdery deposits in the bottom of tube.
<i>Pseudo. aeruginosa</i>	On nutrient agar, colonies were white, large wrinkled, β haemolytic with musty odour, low convex, irregular margin on blood agar, while on MacConkey's agar produced lactose positive colonies.	Gray-white on nutrient agar. On MacConkey's pale coloured colonies were observed.	Thick pellicle, dense turbidity and heavy sediment in the bottom of the tube.
<i>Staph. aureus</i>	Smooth raised, glistening colonies with circular and entire margin seen on nutrient agar. On blood agar plates, organism produced β haemolysis. No growth was observed on MacConkey's agar.	White to Yellowish-white.	In broth uniform turbidity was recorded

PASTEURELLA MULTOCIDA

Organisms showed gram-negative, bipolar and coccobacillary short rods, arranged singly and non-motile. On the blood agar, it exhibited moist, mucoid and shiny growth. The colonies were convex with regular margin and distinctive smell, but not grew on MacConkey's agar. While in nutrient broth medium, granular deposits were formed in the bottom of the tube (Tables 1 and 2). It interacted positively to catalase, oxidase, indole, gelatin liquefaction and nitrate reduction, but negatively to urea, methyl red, Voges-Proskauer and citrate (Tables 3 and 4). The findings regarding general characteristics of *Pasteurella multocida* are in agreement to that of Gabbar (1992), Dewani (2000) and Shaikh (1999) who described the cultural characteristics of *Pasteurella multocida* as non-haemolytic, but some strains produced brownish discolouration on blood agar medium.

Figure 3

Table 3: Biochemical properties of bacterial species identified from mastitic milk samples of camels

Bacterial species	Catalase	Oxidase	Coagulase	Indole	TSI	H ₂ S	Ure	MR	VP	GL	Citrate	Nitrate reduction	Aesculin
<i>B. cereus</i>	+ve	+ve	-	-ve	K/A	-	-ve	-ve	+ve	-	+ve	-	+ve
<i>C. pyogenes</i>	-ve	-	-	-ve	K/A	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
<i>E. coli</i>	+ve	-ve	-	-ve	A/A	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-
<i>M. luteus</i>	+ve	+ve	-ve	-	K/K	-	+ve	-	-	-	-	-	-ve
<i>P. haemolytica</i>	+ve	+ve	-	-ve	A/A	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-
<i>P. multocida</i>	+ve	+ve	-	+ve	A/A	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-
<i>P. aeruginosa</i>	+ve	+ve	-	-ve	K/N	-ve	+ve w	-ve	-ve	+ve	+ve	+ve	-
<i>S. aureus</i>	+ve	-ve	+ve	-	A/A	-ve	-	+ve	-ve	-	-	-	-ve
Whereas: -ve = negative - = not done + = positive +ve = weak positive K/A = alkaline slant and acidic butt K/K = alkaline slant and alkaline butt A/A = acidic slant and acidic butt K/N = alkaline slant and neutral butt GL = gelatin liquefaction MR = methyl red VP = Voges proskauer TSI = triple sugar iron. Ure = Urease													

Figure 4

Table 4: Sugar fermentation properties of bacterial species identified from mastitic milk samples of camels

Bacterial species	Mannose	Xylose	Inositol	Galactose	Mannitol	Glucose	Maltose	Creatinin	Dulcitol
<i>Bacillus cereus</i>	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
<i>Corynebacterium pyogenes</i>	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve
<i>Escherichia coli</i>	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
<i>Micrococcus luteus</i>	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve
<i>Pasteurella haemolytica</i>	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
<i>Pasteurella multocida</i>	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
<i>Pseudomonas aeruginosa</i>	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve
<i>Staphylococcus aureus</i>	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
+ve = Positive -ve = Negative									

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa was recorded as gram-negative, straight or slightly curved rods, motile and arranged singly.

Culturally, it produced gray-white, large wrinkled, non-haemolytic, low convex with irregular margin colonies. On blood agar, it produced a peculiar musty odour, while on MacConkey's agar, it produced lactose fermentative colonies. Other characters of this species on different media (solid media) as well as in nutrient broth are presented in the same Tables (Tables 1 to 4). Same findings were observed by Khan and Rind (2001), Gabbar (1992) and Dewani (2000). It produced K/N and did not produce H₂S in TSI medium. The organism utilized glucose, mannose, and mannitol, but not galactose, xylose, maltose and inositol and dulcitol sugars.

STAPHYLOCOCCUS AUREUS

The cells were gram-positive, cocci, arranged singly/pairs or in irregular clusters. The species was non-motile and non-spore forming. Cultural characteristics observed was a white to yellowish white smooth raised and glistening colonies with circular and entire margin. It produced non-haemolysis of red blood cells on blood agar. It produced uniform turbidity in nutrient broth, whereas it did not grow on MacConkey's agar medium. (Tables 1 to 4). It fermented glucose, mannose, galactose, maltose, inositol, xylose, dulcitol and mannitol sugars, but not utilized creatinin. Gabbar (1992), Khan and Rind (2001), Obied (1983) and Shaikh (1999) also recorded similar characteristics regarding *Staphylococcus aureus*.

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