Incidence and Phenotypic Characterization of Staphylococcus Aureus Isolated from Mastitic Cows

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Abstract

A total of 412 milk samples were collected from clinically and subclinical mastitic cows (188, 224) respectively and examined bacteriologically for Staphylococcus aureus for the isolation rate and studying of the phenotypic characterization of the isolates. The results revealed that S. aureus was isolated in an incidence of 50% and 17.5% from clinically mastitic and subclinically mastitic cows respectively. All Staphylococcus isolates showed symmetrically in their phenotypic characterization including cultural characters on different media. Vitek2 system succeeded in providing definitive identification results for gram positive bacterial by identification card (GP) were used for rapid and easy identification of Staphylococcus spp.

Keywords: Saureus, Clinical, Subclinical, Mastitis-Vitek2.

1.Introduction

Mastitis (inflammation of mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally [1]; because of reduced milk production, treatment costs, increased labor, milk withholding following treatment, death and premature culling. Due to multiple etiologies, it always remained a challenge to veterinarian worldwide. Approximately, 140 species of microorganisms have been identified as etiological agents of bovine mastitis. Of these various etiological agents, Staphylococcus aureus is a major pathogen associated with bovine clinical and subclinical mastitis [2,3,4,5].

The mastitis caused by S. aureus is characterized by significantly lower cure rates compared with infections caused by other microorganisms, which may be either as a result of unusually frequent acquisition of antibiotic resistance mechanisms among this group of bacteria or also their ability to form biofilm (slime) [6]. Considering the potential of the area and the economic significance of dairy production to the local community.

Almost any microbe that can opportunistically invade tissue and cause infection can cause mastitis. About 150 species of microorganisms mostly bacterial is able to cause mastitis [7]. However, staphylococci, streptococci and other related gram-positive, catalase-negative cocci represent the most important causative agents [8].

Staphylococcus species are aerobically growing Gram-positive cocci. Isolation of Staphylococcus species is usually not difficult since Staphylococci not fastidious organism and will grow on commonly media and under variety of conditions [9].

Mastitis is recognized as the most important dairy herd problem worldwide. Economic losses of mastitis include decrease in milk quantity & quality and high cost of treatment Staphylococcus aureus is one of the most common etiological pathogens, causing intramammary infections in dairy herds leading to severe economic losses in worldwide industry. Accurate identification of the Staphylococcus aureus is therefor of great importance in bacteriological laboratory. The vitek2 system used with gram positive (GP) identification card [10] is an automated machine designed to provide rapid and accurate phenotypic identification for most clinical staphylococcus. [11] the present study was carried out to detect the incidence of Staphylococcus aureus infection in mastitis cases and their phenotypic characterization.

Recently a new automated identification system such as Vitek2, accompanied by identification cards that give reliable and rapid identification, was internationally reported by [12]. In addition, identification of bacteria by VITEK2 system has revealed prominent inter laboratory reproducibility and is quickly being included as a routine method for animal laboratory microbiology.

2. Material and methods

2.1 Animals

A total of 103 cows were examined in this study were classified into clinical and subclinical mastitic cases as (47) clinically mastitic cows and (56) subclinically mastitic cows.

2.2 Samples

A total of 412 milk samples of which 188 from clinically infected cases and collected according to [13]. The udder of each animal was palpated before sampling for presence of clinical signs of mastitis, the examined udders were thoroughly washed and carefully dried with clean dry towel. Then the teat was swabbed with 70% ethyl alcohol. After that the first few jets of milk were discarded and milk samples were collected in 50ml sterile falcon tube from clinically affected quarter. As well as 224 milk samples were collected from subclinically infected cows (Normal milk) after...
application of California mastitis test (CMT) according to [14].

Clinical examination: The cases of Clinical Mastitis (CM) were diagnosed on the basis of history, clinical signs, physical examination of udder (swelling and pain) and milk (colour-yellow or blood tinged and consistency-watery, etc.), while subclinical mastitis (SCM) was diagnosed on the basis of California Mastitis Test (CMT) [15].

Bacterial isolation and identification: Each of the thoroughly mixed milk sampler (Mastitis/subclinical mastitis) was transferred to 10 mL of nutrient broth and incubated at 37°C for 15-18 h to resuscitate the organisms. Thereafter, a loopful of inoculum from the nutrient broth was streaked on to nutrient agar plates and incubated at 37°C for 24 h. Presumptive Staphylococcus colonies (golden/white, round, smooth, glistening, opaque) were picked up and characterized biochemically as [16]. Identification by Vitek2 compact system and gram positive test (GP card) were done according to the manufacture’s instruction [12].

3. Results
3.1 Isolation and identification of microbe
3.1.1 Morphological identification
Out of 188 milk samples of clinically bovine mastitis 94 isolates with incidence (50%) and out of 224 subclinical mastitis 39 isolates with incidence (17.5%) were positive for Staphylococcus.

3.1.2 Cultural characteristics
After aerobic incubation on nutrient agar, mannitol salt agar for 24-48 h at 37°C, colonies suspected as Staphylococcus were large, 1-3 mm in diameter, and well isolated colonies reached 4 mm in diameter. The suspected colonies were round, convex, smooth with glistening surface. After aerobic incubation of 33 isolates on Baird-Parker’s agar media for 24-48 hours at 37°C, 10 isolates (30.3%) produced black, shiny, convex colonies with entire margins and clear zone surrounding the colonies with or without an opaque zone. This result confirmed by biochemical identification of 33 suspected Staphylococcus isolates that they were 10 isolates (30.3%) were positive for Catalase test (slide technique), Oxidase test, Oxidation - fermentation of glucose (O-F test), Urease test, Gelatin liquefaction test, Mannitol fermentation test, Coagulase test and showed B-hemolysis on Nutrient agar containing 7.5% NaCl and 5% (V/V) defibrinated sheep blood was used.

4. Discussion
Among 34 samples, 12 (32.29%) showed B-hemolysis on 5% cattle blood agar with circular, small, smooth raised whitish colony [17] reported that 89.3% S. aureus from bovine origin were hemolytic. This variation was due to the difference in sample origin indicating that raw milk contained less association with S. aureus as compared with feces of cattle from where the bacteria were isolated by them. After overnight incubation on MS agar media, some plates showed yellow colony and some plates showed whitish colony. All the suspected S. aureus which produced B-hemolysis on 5% blood agar were able to ferment mannitol salt agar characterized by the formation of yellow colony and white/transparent colony indicated other Staphylococcus spp., as indicated by [18] and [19].

In Gram staining, the organism revealed as Gram positive, violet colored, cocci shaped and arranged in grapes like cluster under light microscope.

In this study, Staphylococcus cultural characteristics on nutrient agar, white, yellow or orange water insoluble pigments were formed. And on mannitol salt agar, yellow or golden yellow water insoluble pigments were formed. Also they were aerobic and facultative, liquefied gelatin and fermented a number of carbohydrates to acid. These results were agreed with [20]. Staphylococcus cultural characteristics on Baird-Parker’s agar media for 24-48 hours at 37°C, produced black, shiny, convex colonies with entire margins and clear zones surrounding the colonies with or without an opaque zone. These results were agreed with that of [21] and [22].

The incidence of Staphylococcus aureus in clinical and subclinical mastitis were shown in Table (1). Overall incidence of Staphylococcus aureus in clinical as well as sub clinical mastitis, was 94% isolates out of 188 and 39% out of 224 respectively. The incidence of Staphylococcus aureus was higher (50.00%) in clinical mastitis in comparison to that of subclinical mastitis (17.50%) and the incidences of Staphylococcus aureus in clinical as well as sub clinical mastitis were higher. These results are almost in the concurrence of previous study conducted in the region in 2010, which revealed S. aureus as a major pathogen in the cases of mastitis in Mathura and its surroundings. The incidence of S. aureus was 37.03% and 31.70% in cattle (1) It clearly indicated the presence of S. aureus as most prevailing pathogen in the cases of mastitis in dairy animals. Moreover, it is persisting in the similar pattern not only in clinical cases but also in subclinical cases. Various studies have been conducted in different parts of country to assess the prevalence status of bacterial pathogens in mastitis of dairy animals. Similar to the present findings, [23] also reported the staphyloccocal mastitis in cows to be 31.94% while [24], [25] and [26], reported the incidence to be comparatively as 27.37% in Jharkhand, 27.1% and 21.0%, respectively. However, higher incidence of staphyloccocal mastitis was reported by [27, 28, 29] who reported the incidence of staphylococcal mastitis in cows to be 45%, 44% and 47.06% respectively. The high
prevalence of staphylococci has been reported by several researchers [30,31] and [32, 33, 34]. All Staphylococcus aureus isolates Table(1) were found catalase positive, oxidase negative urease positive, failed to grow on Macconkey agar, Voges Proskauer (VP) positive and coagulase positive on being subjected to above mentioned biochemical tests.

Similarly, previous studies conducted by[35] and [29] also reported high percentage positivity of S. aureus for coagulase production i.e. 100.00%, where as lower percent positivity of S. aureus for coagulase production were also reported earlier by[36]34.50%,[37] 50.00% and[28] 51.11%. The presence of 100% coagulase positive isolates in this study further suggests the increase in the number of pathogenic S. aureus in dairy animals. This is an alarming condition as in general S. aureus are supposed to be non pathogenic commensal organisms.

Staphylococcus aureus is the most important bacterial microorganism in bovines causing contagious mastitis and highly economic losses in dairy herds [38].

In the present study bacteriological examination and identification of Staphylococcus aureus were depend on gram stain, culturing on Bairded parker medium, catalase test, Coagulase tube test and DNase test.

Table (1) Incidence of Staphylococcus aureus in the mastitis cows

<table>
<thead>
<tr>
<th>Animal case</th>
<th>no.</th>
<th>no. of quarter</th>
<th>S.aureus isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically mastitic</td>
<td>47</td>
<td>188</td>
<td>94</td>
</tr>
<tr>
<td>Subclinical mastitic</td>
<td>56</td>
<td>224</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>412</td>
<td>133</td>
</tr>
</tbody>
</table>

Identification of staphylococcus aureus using vitek2 compact system

Identification information Card: GP Lot number:242381940 Expires:May29,201713:CDT

Completed Apr 19,2016 17:40CDT Status :final Analysis

Time:4.75 hours

Selected Organism 99%probability Staphylococcus aureus
Bionumber:10402062763231 Confidence: Excellent identification

References
Epidemiology of Staphylococcus aureus and Agalactiae Isolated from Bovine Mastitis in Ethiopia. Mensch und Buch, pp.139, 2003