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## Isolation and identification of bacteria from lesions of contagious ecthyma in goats

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### Abstract

Contagious ecthyma is an acute, highly contagious, debilitating and economically important viral skin disease of sheep and goat. The morbidity of disease can be as high as 100% and subsequent bacterial infections can cause deaths in approximately 15% cases. Secondary bacterial infection or co-infection at lesions in contagious ecthyma causes severe complications and also delays the healing process. This research work was aimed for isolation and identification of bacteria from secondary bacterial infection at the lesions in goats suffering from contagious ecthyma. A total of 30 scab samples from 30 affected goats were collected aseptically. In the present study, mono-infections were found in 27 (96.43%) samples and mixed infection with two different bacteria were found in only one (3.57%) sample. Out of these all the isolated bacteria *Staphylococcus* sp. 65.52% (19/29) was most dominant followed by *Proteus* sp. 27.59% (8/29) and *Klebsiella* sp. 6.89% (2/29). This research provides valuable insights into the microbial ecology associated with contagious ecthyma lesions, which can aid in developing effective treatment options.

**Keywords:** Contagious ecthyma, ORF, bacterial isolation

### 1. Introduction

Goats have the broadest ecological range and have traditionally been the most consistent source of income for poor people. India had 148.88 million goats overall (20th Livestock census, 2019). According to Nath *et al.* (2014) <sup>[1]</sup>, the most common ailments affecting goats include peste des petits ruminants, goat pox, infectious ecthyma, enterotoxaemia, tetanus, brucellosis, mastitis, and contagious caprine pleuropneumonia.

The different terms used to describe the contagious ecthyma are orf, contagious pustular dermatitis, infectious labial dermatitis, scabby mouth and sore mouth. The disease is found throughout the world <sup>[2]</sup>. It is an acute, highly contagious, debilitating and economically important viral skin disease, commonly occurs in sheep and goat. The disease is zoonotic in nature, so it can be transmitted to humans through contact with infected animal or fomites <sup>[3]</sup>. The disease is more severe in goats than sheep. The contagious ecthyma is characterised by proliferative lesions on the lips and mouth, which normally disappear in 1-2 months <sup>[4]</sup>. The morbidity of disease can be as high as 100% and subsequent bacterial infections can cause deaths in approximately 15% cases <sup>[5]</sup>. Primary orf lesions progress from erythematous macule, papule, vesicle, pustule to scab formation in 4-6 weeks and young animals are at high risk <sup>[6]</sup>.

Contagious ecthyma is caused by dsDNA virus species *Orf virus* (ORFV) which belongs to genus *Parapoxvirus* and family *Poxviridae*. The members of the genus *Parapoxvirus* have an oval shape (250-300 nm in length and 160-190 nm in diameter) and are covered with long thread-like surface tubules that appear to be arranged in criss-crossed pattern, resembling a ball of yarn. Virions are made up of a dumbbell-shaped core and two lateral bodies surrounded by one or two lipid membranes <sup>[7]</sup>. *Orf virus* is a large enveloped virus that replicates in the cytoplasm of the infected host cell. The ORFV has a double-stranded DNA genome (134-139 kbp). The linear genome is linear and flanked by inverted terminal repeat sequences. The average G + C content of the genome is 64 percent <sup>[8]</sup>.

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Since most of the research work on contagious ecthyma focuses on the virus, very less information is available for bacteria isolated from lesions of contagious ecthyma. Secondary bacterial infection at lesions in contagious ecthyma causes severe complications and also delays the healing process. The results of isolation of bacteria can help in the treatment of contagious ecthyma. Considering the importance of secondary bacterial infection in contagious ecthyma in goats this research work was aimed for isolation and identification of bacteria from secondary bacterial infection at the lesions in goats suffering from contagious ecthyma.

## 2. Materials and Methods

The disease contagious ecthyma was categorized based on the location of lesions on the body of the goat i.e., labial form, genital form and generalized form. A total of 30 scab samples from 30 affected goats were collected aseptically in sterile 5 ml microcentrifuge tube. For bacterial isolation samples were collected by gentle rotating of sterile swabs around the scab. All the precautionary measures were undertaken during sample collection. The goats from which samples were collected have labial form of disease and all were non-descript breed.

The identification of bacterial isolates was done as per method described by Barrow (2004) and Markey *et al.* (2013) [9]. The collected swab samples were streaked on 6% defibrinated sheep blood agar (SBA) and incubated at 37°C for 24-48 hours. The identification of bacterial organisms was made on the basis of colony characteristics on agar, Gram's staining, microscopic features viewed under the microscope and biochemical tests (Potassium hydroxide test, catalase test, oxidase test, urease test, TSI reaction).

## 3. Results and Discussion

A total of 30 samples were collected from lesions of contagious ecthyma in goats. Bacteria were isolated from 28 (93.33%) samples, while 2 (6.67%) samples were found negative for bacterial isolation. From these 28 samples, a total of 29 bacterial isolates were recovered having 3 different genera viz. *Staphylococcus* sp. (19/29), *Proteus* sp. (8/29) and *Klebsiella* sp. (2/29).

In the present study, monoinfections were found in 27 (96.43%) samples and mixed infection with two different bacteria were found in only one (3.57%) sample. Out of these all the isolated bacteria *Staphylococcus* sp. 65.52% (19/29) was most dominant followed by *Proteus* sp. 27.59% (8/29) and *Klebsiella* sp. 6.89% (2/29) (Table 1, Figure 1).

**Table 1:** Overall percentage of bacterial isolates

Sr. No.	Bacteria species	No. of isolates	Percentage
1	<i>Staphylococcus</i> sp.	19	65.62%
2	<i>Proteus</i> sp.	8	27.59%
3	<i>Klebsiella</i> sp.	2	6.89%
Total isolates		29	-

The most predominant bacteria found at lesions of contagious ecthyma in goats was *Staphylococcus* sp. followed by *Proteus* sp. and *Klebsiella* sp.

### 3.1 Cultural and Biochemical Characterization of *Staphylococcus* sp. Isolates

Out of total 29 bacterial isolates obtained from lesions of contagious ecthyma, 19 bacterial isolates were confirmed as *Staphylococcus* sp. based on morphological and cultural features. All the isolates of *Staphylococcus* sp. were appeared

as gram positive cocci arranged in clusters just like a bunch of grapes. All the isolates of *Staphylococcus* sp. were found negative for KOH test, positive for catalase test and negative for oxidase test.

#### 3.1.1 Pigment production on Nutrient agar

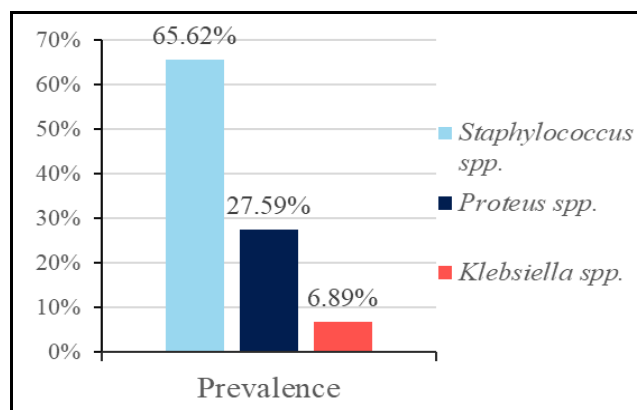
Only 5.26% (1/19) *Staphylococcus* sp. isolates produced a yellow colour pigmentation and remaining (94.74%) isolates produced a white colour pigmentation on nutrient.

#### 3.1.2 Detection of hemolysin production on Sheep blood agar (SBA)

In the present study, on the 6% SBA, 57.9% (11/19) isolates showed  $\beta$  haemolysis (partial haemolysis), 36.84% (7/19) isolates showed  $\alpha$  hemolysis (complete haemolysis) and 5.26% (1/19) isolates showed  $\gamma$  hemolysis (no haemolysis).

#### 3.1.3 Mannitol fermentation on Mannitol salt agar (MSA)

In the present study, out of 19 *Staphylococci* isolates, 78.95% (15) isolates showed fermentation on MSA (Figure 2), whereas 21.05% (4) isolates were mannitol non-fermentative.



**Fig 1:** Overall prevalence of bacterial isolates



**Fig 2:** Mannitol fermenting *Staphylococcus* spp. on MSA

### 3.2 Cultural and biochemical characterization of *Proteus* sp.

Total eight of 19 bacterial isolates obtained from lesions of contagious ecthyma were confirmed as *Proteus* sp. based on morphological, cultural and biochemical features. All the isolates of *Proteus* sp. were appeared as gram negative rods.

All the isolates of *Proteus* sp. produced a swarming growth on nutrient agar (Figure 3), non-lactose fermenting colonies on MacConkey agar. The isolates were found positive for KOH, catalase and urease test (Figure 5), but negative for the oxidase test. In TSI slant isolates of *Proteus* sp. were produced red slant, yellow butt and positive for H<sub>2</sub>S production (Figure 4).

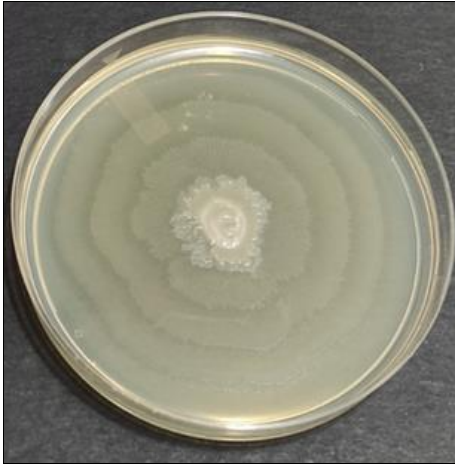


Fig 3: Swarming growth of *Proteus* spp. on Nutrient agar

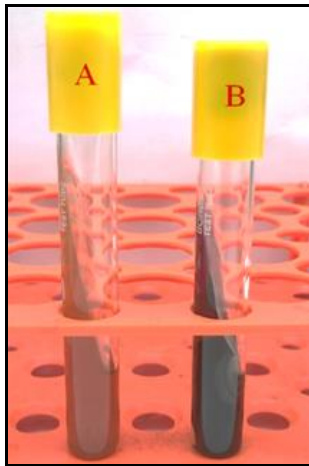


Fig 4: TSI test for *Proteus* spp. (A- Control, B- R/Y/ H<sub>2</sub>S +ve)

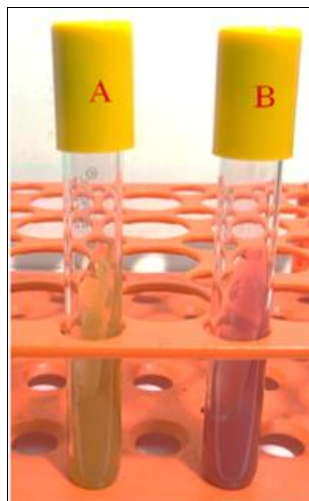


Fig 5: Urease test (A-control, B- positive)

### 3.3 Cultural and biochemical characterization of *Klebsiella* sp.

Only two bacterial isolates obtained from lesions of contagious ecthyma were confirmed as *Klebsiella* sp. based on morphological, cultural and biochemical features. All the isolates of *Klebsiella* sp. were appeared as gram negative rods.

All the isolates of *Klebsiella* sp. produced lactose fermenting bright pink colour mucoid colonies on MacConkey agar (Figure 6). The isolates were found positive for KOH, catalase and urease test, but negative for the oxidase test. In

TSI slant isolates of *Klebsiella* sp. were produced red slant, yellow butt and negative for H<sub>2</sub>S production (Figure 7).



Fig 6: *Klebsiella* spp. on MA

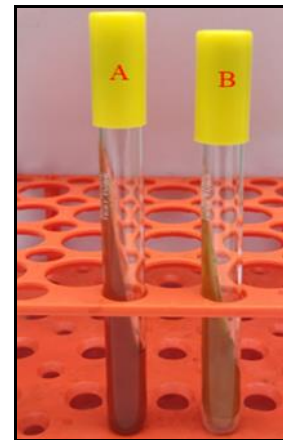


Fig 7: TSI test for *Klebsiella* spp. (A- Control, B- Y/Y/-ve H<sub>2</sub>S)

The *Staphylococcus* sp., common bacteria with a high degree of environmental adaptability, placed first in terms of secondary infections, as predicted and based on its features by Chi *et al.* (2017) [10]. Our results differ from PASTOR *et al.* (2020) [11], who isolated *Pseudomonas* sp., *Bacillus* sp. and *Corynebacterium* sp. along with *Staphylococcus* sp. as a most predominant bacterium.

### 4. Conclusion

A total of 30 samples were collected from lesions of contagious ecthyma in goats. A total 93.33% (28/30) samples were found positive for bacterial isolation. From these 28 samples, a total of 29 bacterial isolates were recovered. Out of these *Staphylococcus* sp. 65.52% (19/29) was most dominant followed by *Proteus* sp. 27.59% (8/29) and *Klebsiella* sp. 6.89% (2/29). The isolation of bacteria from lesion of contagious ecthyma will be helpful regarding secondary infection causing bacteria at site of lesions.

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