



# Isolation and Identification of Bacterial Flora from Foot Lesions of Asian Elephants (*Elephas maximus*)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The present study was undertaken for the isolation and identification of the bacterial agents present in the foot lesions of captive elephants. A total of 78 samples were collected from various foot lesions of 46 captive elephants and subjected to bacterial culture. Sixteen of these collected samples did not show any bacterial growth in any type of media. The remaining 62 samples had 155 isolates; 94 (60.6%) of them were Gram negative bacteria, and the rest 61 (39.3%) isolates were Gram positive bacteria. Among the 155 isolates, they comprised of *E. coli* 63 (40.6%), *Bacillus* species 25 (16.1%), *Pseudomonas* species 18 (11.6%), 36 (23.2%), *Staphylococcus* species, *Citrobacter* species 3 (1.9%) and *Enterobacter* species 14 (9.03%). The study highlights the microbiological fauna of foot lesions, emphasizing the prevalence of Gram-negative bacteria and underscoring the need for periodic health screening and targeted interventions for captive elephants' welfare.

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**Keywords:** Asian elephant; foot lesions; gram-positive bacteria; gram-negative bacteria.

## 1. INTRODUCTION

“India has a long fascinating history of domesticating wild elephants. Captive Asian elephants have been very closely associated and deeply entwined with the religion, myths and cultural heritage of India for centuries” [1]. “Elephants have adapted to a wide variety of environments in captive conditions. The health status of captive elephants depends on various factors including body mass index, foot health, husbandry management, nutrition and infectious and non-infectious diseases” [2-4]. “Research on the health and diseases of captive elephants is limited, making the development of effective measures to promote their health and welfare a challenging endeavor. Understanding the health-related parameters and evidence of diseases in captive-reared elephants will be significantly helpful towards enriching the management as well as their healthcare. This study addresses significant knowledge gaps regarding foot health management of elephants in captive situation” [2 -4].

Foot problems constituted the most important ailment in captive elephants and prevention of these problems is of great importance for maintaining elephant welfare [5]. “Elephants kept in captive enclosures with hard surfaces for four hours each day were more likely to develop the foot disorders including lameness, joint stiffness and osteoarthritis etc” [6]. “Preventing foot problems requires knowledge of prevalent etiologies and risk factors. The occurrence of foot ailments and disorders are often linked to inadequate daily husbandry practices, poor hygiene in the elephant housing area, improper use of tools, inadequate exercise and lack of appropriate preventative measures and suboptimal health issues” [7]. “Further, prolonged period of contact with concrete flooring was one of the primary causal agents of elephant foot problems and further they reported the hard, unyielding surface caused the sole to crack and abscesses to form in the nails or on the pad of the foot” [8].

Infectious foot problems in captive elephants were found to be relatively common and were more prevalent and severe in Asian elephants [9]. Subramaniyan et al. [10] isolated bacterial organisms such as *Streptococcus agalactiae*, *Bacillus cereus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* from

the foot lesions of captive Asian elephants. Kumar et al. [1] isolated *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *Fusobacterium* spp., *Bacteroides* spp. and *Pseudomonas* spp. from sole abscesses in Asian elephants. This study was undertaken to find out the prevalence of microbiota in foot lesions of captive Asian elephants rearing in various environmental conditions. The results obtained in this study will help to better understand the bacterial organisms associated with foot lesions in captive elephants and enable the veterinarians to ensure the proper treatment and control therapy and to take adequate measures to prevent the severity of lesions.

## 2. MATERIALS AND METHODS

### a. Study Population and collection of samples

The study was carried out in 46 captive elephants maintained in different parts of Tamil Nadu. Out of this, 24 captive Asian elephants were assessed during a health and welfare campaign for Tamil Nadu temple elephants. Remaining 22 captive elephants were privately maintained elephants and were assessed during their periodical health and veterinary examinations. The age of elephants in this study ranged from 11 years to 62 years. The study was carried out for 2 years period from October 2019 to September 2021. All these captive animals were under captive feeding programme and fed with green grass, coconut leaves, seasonal fruits and vegetables, sugar cane and food bolus mixed with ragi, horse gram, rice, jiggery and salt.

A total number of 78 swab samples were collected from 37 captive elephants presence with foot disorders during their clinical examinations from various foot lesions located in nails, cuticles, sole and pad (Fig. 1). Sterile swabs (Hi-Media container) were used for collection of samples. The swabs were placed under 4°C subsequently after collection, till they were cultured and examined.

**b. Bacterial culture and identification** “The samples were inoculated into nutrient broths and were incubated at 37 °C for 24 hrs; they were then streaked onto different bacteriological media such as Nutrient agar (NA), MacConkey Agar (MAC), Brilliant Green Agar (BGA), Methylene

**Table 1. Cultural characteristics of isolated bacterial organisms**

Bacterial organisms	Agar	Cultural characteristics
<i>E. coli</i> (Figs. 2&3)	MacConkey agar Eosin methylene blue (EMB) agar	Pink colour, smooth and circular Green metallic sheen colour colony
<i>Bacillus</i> spp. (Fig. 6)	Nutrient agar	Gry-white granular colonies
<i>Pseudomonas</i> spp.(Fig. 4)	Nutrient agar	Green colour colony
<i>Staphylococcus</i> spp. (Fig. 6)	Nutrient agar	Pink colour round colonies with mucoid consistency
	Mannitol agar	Yellow colour colonies
<i>Citrobacter</i> spp. (Fig. 8)	MacConkey agar	Pale-pink coloured colonies
<i>Enterobacter</i> spp.(Fig. 7)	MacConkey agar	Lactose fermented mucoid pink colour colonies

Blue Agar (EMB), Mannitol Salt Agar (MSA) and Bismuth Sulphite Agar (BSA) (Himedia, India) and incubated at 37 °C for 24 hrs to obtain pure culture of the bacteria. Identification of bacteria was performed on the basis of colour, size, shape, texture and edge elevation of colony growth”.

Identification of bacterial organisms were also performed by cultural method and the cultural characteristics were presented in Table 1.

### 3. RESULTS

#### 3.1 Sample Demographics

A total of 78 samples were collected from various foot lesions of elephants. Sixteen of these

collected samples did not show any bacterial growth in any type of media. The remaining 62 samples had 155 isolates; 94 (60.6%) of them were Gram negative bacteria, and the rest 61 (39.3%) isolates were Gram positive bacteria.

#### 3.2 Bacterial Flora

Among the 155 isolates, they comprised of *E. coli* 63 (40.6%), *Bacillus* species 25 (16.1%), *Pseudomonas* species 18 (11.6%), 36 (23.2%), *Staphylococcus* species, *Citrobacter* species 3 (1.9%) and *Enterobacter* species 14 (9.03%) (Table 2).

**Table 2. Bacterial floral isolated from foot lesions of elephants**

No. of isolates	Types of Microbes isolated					
	<i>E.coli</i>	<i>Bacillus</i> spp.	<i>Pseudomonas</i> spp.	<i>Staphylococcus</i> spp.	<i>Citrobacter</i> spp.	<i>Enterobacter</i> spp.
155	63	25	18	36	3	14



**Fig. 1. Collection of swabs from foot lesion**



**Fig. 2. Green metallic sheen colour colony in EMB agar shows the positive growth of *E. coli***



Fig. 3. *E. Coli* in MacConkey Agar- Dry, dark pink in colour



Fig. 4. Smooth, large and translucent colonies in Nutrient agar shows the positive growth of *Pseudomonas* spp.



Fig. 5. Yellow colour colony in Mannitol salt agar shows the positive growth of *Staphylococcus* spp.



Fig. 6. Gram's staining of *Bacillus* spp.

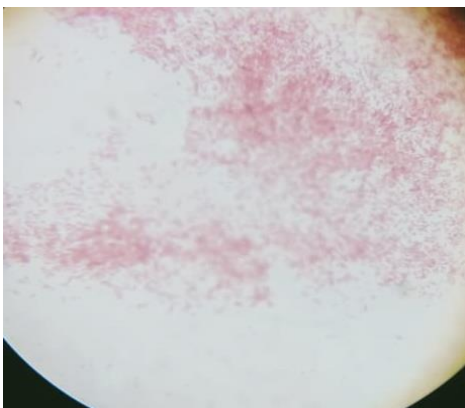


Fig. 7. Gram's staining of *Enterobacter* spp. Showing Gram-negative rods

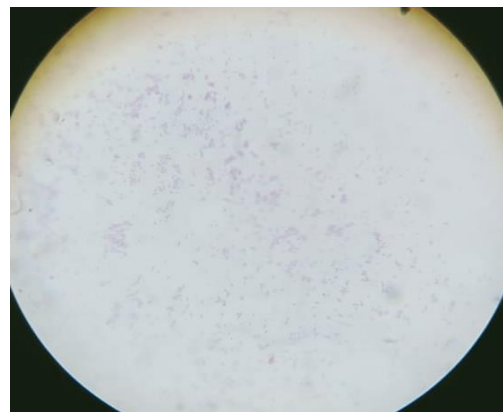


Fig. 8. Gram's staining of *Citrobacter* spp. Showing Gram negative bacilli



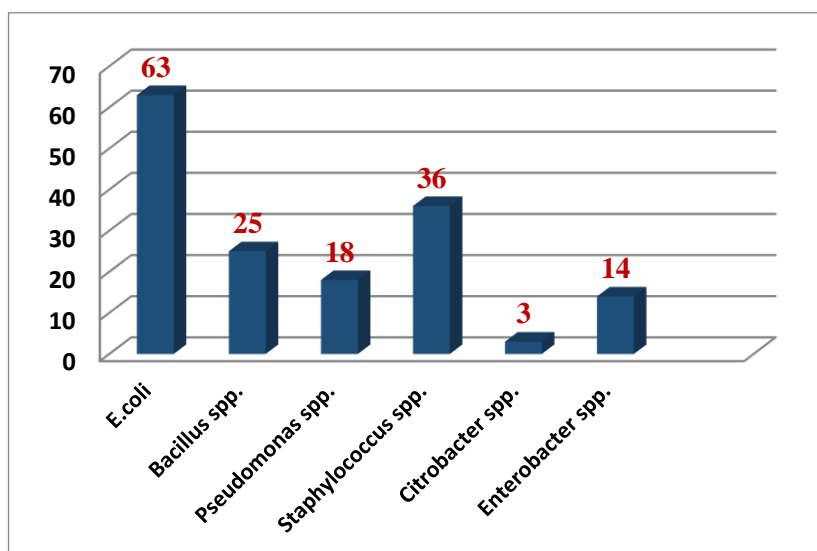


Fig. 9. Prevalence of bacteria isolated from foot lesions of elephants

#### 4. DISCUSSION

“Foot disorders constitute the single most important ailment of captive elephants (Fowler and Mikota, 2006). Various bacterial and other microorganisms are associated with lesions of the elephant feet. Most of organisms are contaminants or opportunistic pathogen found in unsanitary environments contaminated with faeces and urine” [11]. In this study, six different genera of bacteria were isolated from the foot lesions of elephants. The isolated bacteria were *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Citrobacter* spp. and *Enterobacter* spp. These findings were in agreement with similar findings reported by [10,12]. This was also supported by [11] who reported of distribution of *Streptococcus agalactiae*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Clostridium* spp. in the foot lesions of captive elephants.

In this study *E. coli* was isolated from sole abscess, cuticular follicles, wounds and infected nails. Similar findings were described by [13] and [14]. The more common pathogen isolated in this study was *E. coli* (40.6%). This was in agreement with the report of [10] who reported that *E. coli* was the most frequently observed in the foot infections and abscesses. Maintenance of animals in insanitary enclosures, improper cleaning of floor and excessive moisture were the possible factors involved here. Foot lesions may be infected through dung, pastures and bedding, since *E. coli* is common enteric isolates

form the digestive tract of healthy elephants. So, this enteric genus may be pathogenic, opportunistic, or simply contaminants. This was supported by [13] who reported in his study that the *E. coli* was a common microflora in the floor of elephant enclosures.

*Pseudomonas* species were isolated from 11.6% cases with foot infections and abscess. Similar finding was also reported earlier. Kumar et al. [1] reported an incidence of 12.5% of *Pseudomonas* spp. from the foot lesions of elephants. *Pseudomonas* being considered to be an opportunistic organism it caused skin abscess and wound [15]. *Bacillus* spp. was also isolated from the wounds and foot lesions of elephants (16.1%) in this study. This concurred with the previous findings; [16] and [11] had also isolated *Bacillus* spp. in foot lesions and abscess of captive elephants. *Bacillus cereus* has been isolated from the wounds, peritonitis, ascites, pustules and abscesses in various parts of the body including the skin and foot of animals [17]. Akesson et al. [18] also opined that those members of the genus *Bacillus* spp., especially *B. cereus* and *B. subtilis* were the commonest species reported in wounds. Observations of *Bacillus* spp. from the foot lesions in this study concurred with these reports.

In this study 23.2% had *Staphylococcus* species and these were isolated from foot lesions and abscess. Subramaniyan et al. [10] and Senthilkumar et al. [12] had earlier isolated *Staphylococcus* species from the foot lesions of elephants under captive conditions. Devrajani et

al. [19] reported that the *Staphylococcus intermedius* was the most common organism present in wounds. In this study too the *Staphylococcus* spp. were observed as second the most common species in the foot lesions of elephants next to *E. coli*. *Citrobacter* spp. and *Enterobacter* spp. were also isolated from the foot lesions of studied elephants. Keet et al. [20] isolated the *Citrobacter* spp. in ulcerative pododermatitis of elephants. Subramaniyan et al. [10] reported of the presence of *Enterobacter* spp. from sole abscess. Further, [21] isolated *Enterobacter* spp., *Citrobacter* spp., *Bacteroides* spp. and *Proteus mirabilis* pathogens from the nail and sole abscess in captive elephants. *Citrobacter* spp. and *Enterobacter* spp. were considered opportunistic pathogens and were responsible for a wide range of infections associated with severe septicemia, pneumonia and urinary tract infections in animals [22]. In this study, *Citrobacter* spp. encountered in low occurrence (1.9%) in the foot lesions of elephants. Hence lesser the bacterial affections of foot, better will be the foot health [23].

## 5. CONCLUSION

Foot health assessment has scope for further improvement, both in terms of management and health care of these elephants. In this study, many of the bacterial organisms were isolated and they included, the common bacterial agents such as *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Citrobacter* spp. and *Enterobacter* spp. Among them *E. coli* was predominant one. As foot health issues are always a major challenge for treating veterinarians, this study on foot health assessment by screening the bacterial organisms in foot lesions will certainly help in the maintenance of optimal foot health of captive elephants.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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