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Oral and cloacal aerobic bacterial and fungal flora of Indian rock python (*Python molurus*) from Agra, Uttar Pradesh

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Abstract
In order to provide effective veterinary care and management, the present study aimed to understand the aerobic microflora yielding from oral and cloacal cavities of the Indian rock python (*Python molurus*). Oral and cloacal samples collected from the rescued *P. molurus* (n=10) involved in human-snake conflict situations at Agra, Uttar Pradesh were subjected to microbiological analysis. Bacterial isolates were evaluated using Gram staining and IMViC biochemical tests. Whereas the fungal isolates were evaluated through Lactophenol cotton blue staining. Eleven bacterial and three fungal strains were isolated from both oral and cloacal cultures. Compared to other strains, the incidence of *Pseudomonas* Sp. was found to be higher (15%) in both oral and cloacal cultures. Among the three isolated fungal strains isolated, 2 from oral and 3 from cloacal, majorly, *Candida glabrata* was represented with a rate of incidence of 8% and 7% in oral and cloacal cavities respectively. All the isolated aerobic microflora were opportunistic pathogens and capable of causing healthcare-associated infections in humans. The prevalence of various zoonotic microbial strains in *P. molurus* reveals that the snake could be potentially transferring pathogens to other animals, snake handlers, general public, and snake-bite victims. Further studies need to be conducted to understand the antibiotic resistance of these pathogens in order to prevent infections.

Keywords: Indian rock python, oral and cloacal cavity, microbiological analysis, aerobic microflora, opportunistic infection

Introduction

The rhizosphere holds a high content of nutrients, and the habitat is enhanced with bacterial abundance. Microorganisms present in the healthy animals may aid or harm the host or may exist as commensals (Davis, 1996) [5]. Reptiles are potential reservoirs of microorganism-mediated diseases for humans and animals (Berg *et al.*, 2005) [2]. While engulfing, the prey might often defecates in the oral cavity being ingested. Thus resulting in colonization of microorganisms in the mouth (Goldstein *et al.*, 1981) [10]. Bacteria, parasites, and protozoa have been known to be zoonotic agents for both reptiles and humans (Mendoza-Roldan *et al.*, 2020) [18]. Identification of microflora in snakes not only helps to understand the bacteria that cohabit but also the etiological agents of secondary infections (Padhi *et al.*, 2020) [19]. Several authors have studied oral bacterial microflora of venomous snakes and the complications associated with snakebite (Jho *et al.*, 2011; Liu *et al.*, 2012; M. Lukac *et al.*, 2017) [13, 16, 17]. Information about microflora of non-venomous snakes should not be neglected as it harbours a wide range of bacteria (Dipineto *et al.*, 2014; Yak *et al.*, 2015) [6, 23]. Complications associated with venomous or non-venomous snakes may include subcutaneous abscesses or tetanus (Habib, 2002; Garg *et al.*, 2009) [8, 11]. Microbial infections in snakes with *Shigella spp.*, *Klebsiella*, and *Pseudomonas aeruginosa*, have the potential to transmit these infections to humans (Blaylock, 2001) [3]. In India, the incidence of snakebite is high in rural and sub-urban areas. So, secondary microbial infection from snakebite may be considered during the treatment process.

Python molurus (Linnaeus, 1758), commonly recognized as Indian rock python (IRP), is a nocturnal snake and widely distributed throughtout the Indian subcontinent. It is categorized as Schedule I reptile in the Indian Wildlife Protection Act, 1972 and assessed as Near threatened for the IUCN Red List (Aengals *et al.*, 2021) [1].

Information regarding microflora and potential pathogens from the oral and cloacal cavities of *P. molurus* are largely unknown. Hence, this study was undertaken to get an insight into physiological microflora and opportunistic organisms in *P. molurus* for definitive diagnosis and therapeutic success in case of snakebites.

Materials and Methods

For over 15 years, in and around Agra, Wildlife SOS operates a rapid response unit for wildlife rescue that are injured or caught in conflict situations in collaboration with the Uttar Pradesh Forest Department (Prerna *et al.*, 2021) [20]. The ailing snakes are brought to hospital for medical treatment and observation before being released into suitable natural habitats. For study purposes, manual restraint method was adopted by an experienced snake handler. All sterile cotton swabs, culture media, and microbial strain identification kits were purchased from Hi-Media Laboratories, Mumbai, India and all reagents, chemicals used in the study were Analytical Reagent (AR) grade. Aseptic swab samples were collected from the oral and cloacal cavities of *P. molurus* (n=10). Morphometry of all pythons were recorded and described in Table 1. Samples were inoculated on culture plates with Eosin methylene blue agar (EMB), Pseudomonas agar (PIA), MacConkey sorbitol agar (MAC), Salmonella shigella agar (MAC), Enterococcus differential agar (EDA), Candida differential agar (CDA), Mannitol salt agar (MSA), and Clostridial agar (CA) and incubated at 37ºC for 18-24 hours. To identify bacterial species, isolated colonies were examined microscopically using gram staining, and IMViC test (HiMedia, India). Lactophenol cotton blue staining method was adopted for microscopic examination and identification of fungi.

Table 1: Morphometric information of rescued *P. molurus* (*n*=10)

Snake ID	Length (cm)	Weight (kg)
IRP-1	262.1	9
IRP-2	243.8	7.5
IRP-3	225.5	6
IRP-4	222.5	5.7
IRP-5	176.7	5.1
IRP-6	170.6	4.7
IRP-7	158.4	4.2
IRP-8	152.4	3.3
IRP-9	137.1	3
IRP-10	48.7	1

Results and Discussion

Eleven bacterial and three fungal strains were isolated from both oral and cloacal cultures. The percent abundance of *Pseudomonas* Sp. (15%) in both oral and cloacal cultures were higher than that of the other strains. Oral bacteriae with incidence rates over 10% included *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*., and *Pseudomonas* Sp. (Fig. 1). The incidence rate of *E. faecium* in oral samples (15%) was higher than in cloacal samples (12%). The incidence rates of most of the bacterial flora were higher in cloacal samples than in oral samples. In cloacal culture, *S. aureus*, *C. perfringens*, *E. coli* were isolated with incidence of 14, 14, and 10 percent respectively. *Candida glabrata*, *Candida tropicalis*, and *Candida krusei* were isolated from cloacal culture with a rate of incidence of 7, 3, and 1 percent respectively (Fig. 2). Among the two fungal strains isolated from the oral cavity, the incidence of *C. glabrata* was highest (8%) followed by *C. tropicalis* (5%).

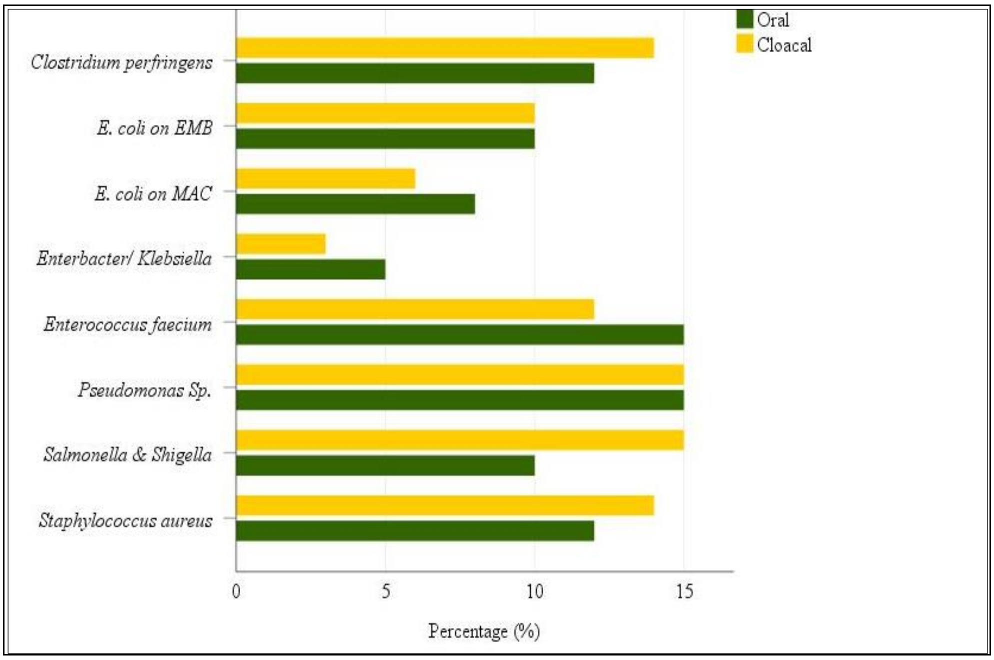

Fig 1: Percent occurrence and comparison of bacterial flora among isolates from oral and cloacal culture

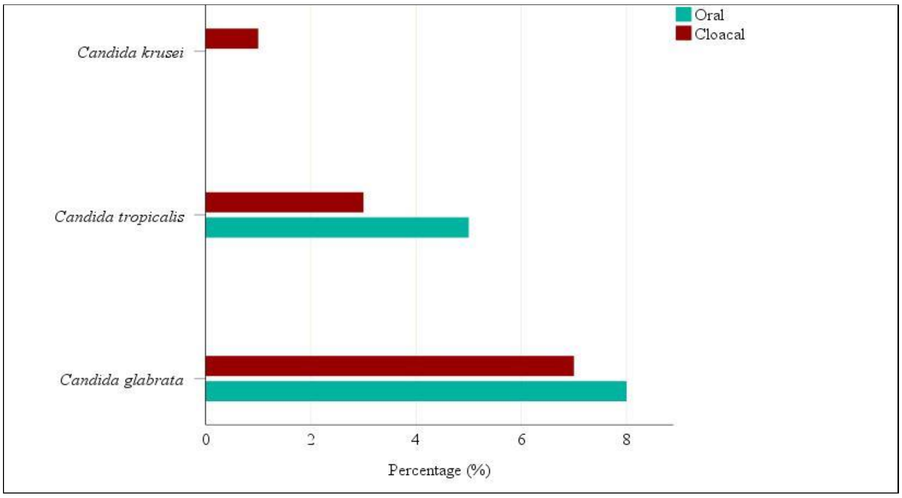

Fig 2: Percent occurrence and comparison of fungal flora among isolates from oral and cloacal culture

Snakes don't appear to have fixed oral bacterial flora, either they are transient or the occurrence is environmental linked (Blaylock *et al.*, 2014; Soveri *et al.*, 1986) [4, 22]. Generalized microbial infections in *P. molurus* may be influenced by environmental bacteria or their predation upon other animals on the ground. Among the snakes sampled, the authors observed, *Pseudomonas spp.* was the most commonly isolated bacterial species. Consistent with the observations of Jho *et al.* (2011) [13], in captive Burmese pythons, the oral culture yields *Pseudomonas spp.* majorly. In *P. molurus*, Singh *et al.* (2018) [21] revealed salmonella as a causative agent for infectious stomatitis thus may lead to systematic disease and possible death (Divers, 2016) [7]. Bacteria, including *Staphylococcus spp.*, *Enterobacter spp.*, *Escherichia coli*, *Proteus spp.*, *Pseudomonas pseudo alcaligenes*, and *Salmonella arizonae*, can infect humans via snake bites (Kerrigan, 1992; Liao *et al.*, 2000) [14, 15]. *Enterococcus faecalis* generally found in the gastrointestinal tract of snakes but, Gilmore and Ferretti (2003) [9] found under certain pathological conditions this pathogen can gain resistance to antibiotics. Some strains of *Enterobacter* may cause opportunistic infections in humans such as urinary and respiratory diseases (Iglewsk, 1996) [12]. All of the identified strains in this study are opportunistic pathogens, and most of them can cause infections in humans. For comparison, Table 2 provides a summary of the microbial isolates identified in previous studies analyzing different python species. The outcome of the present study established a database of microbial taxa in the oral and cloacal cavities of *P. molurus* for diagnosis and medical treatment.

Table 2: Microbial isolates from the oral and cloacal cavities of pythons reported from different locations worldwide

Scientific name	Status	Number of individuals (n)	Cavity	Predominant organisms	Location	Reference
Malayopython reticulatus	Captive	3	Oral	*Neisseria* sp., *E. coli*, *Staphylococcus* sp., *Klebsiella* sp., and *Burkholderia cepacia*	Indonesia	Govendan *et. al.*, 2022
Python bivittatus		1				
Python regius		4				
Python molurus	Wild	1	Oral	*Salmonella*	India	Singh *et al.*, 2018 [21]
Python reticulatus	Wild	10	Oral	*Pseudomonas* sp., *Enterobacter* sp., *Klebsiella pneumonia*, and *Salmonella enterica diarizonae*	Singapore	Yak *et al.*, 2015 [23]
Python regius	Captive	60	Oral	*Staphylococcus* sp. *Pseudomonas* sp. *Morganella morganii*	Italy	Dipineto *et al.*, 2014 [6]
Python bivittatus	Captive	18	Oral & Cloacal	*Pseudomonas aeruginosa Acinetobacter* sp. *Escherichia coli*	Vietnam	Jho *et al.*, 2011 [13]

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