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Comparative study on checklist of aerobic microflora of oral and cloacal sample of Spectacled cobra; rescued from snake charmers & wild cobra rescued from the conflict situations in Agra, Uttar Pradesh, India

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Abstract
Snakebites are a great public health concern in tropical and subtropical countries. It cannot only cause poisoning but also sometimes yield some infections in victims. There are some pathogenic agents in the snake's oral and cloacal cavity. This study was carried out to determine bacterial agents present in the oral and cloacal cavity of Spectacled cobra (*Naja naja*) which were rescued from snake charmers and human-snake conflict situations at Agra, Uttar Pradesh. Oral and cloacal samples were collected in sterile condition from 20 rescued cobras (10 from each group). Later the samples were subjected to microbial culture to understand the aerobic microflora present in oral and cloacal cavity. The results revealed both the oral and cloacal swabs found positive bacterial microflora with a majority of swabs yielding multiple organisms. The most common organisms isolated were *Pseudomonas* Spp., *Staphylococcus aureus*, *E. coli*, *Salmonella*, *Shigella*, *Clostridia* Spp., *Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Enterococcus faecium* are known human pathogens and also affected snake health.

Keywords: Human-snake conflict, spectacled cobra, oral & cloacal microflora

1. Introduction
The Spectacled cobra (*Naja naja*), also known as the Asian cobra, or Binocellate cobra, is a species of the genus *Naja* found in India, Pakistan, Bangladesh, Sri Lanka, Nepal, and Bhutan, which a member of the "big four" species that inflict the most snakebites on humans in India. The Spectacled cobra is revered in Indian mythology and culture and is often seen with snake charmers. Based on Indian mythology, Naga Panchami is a day in which traditional worship of snakes especially cobras happens by snake charmers where Hindu adherents live. In India, Spectacled cobras are listed under Schedule II of the Wildlife Protection Act (1972). Fifty percent of snakebite deaths in India is due to cobra bite and has later complication like local necrosis and sloughing of skin which takes several months to recover. This extensive necrosis may be due to both venom and the contaminated microflora (Sujogya Kumar Panda, 2018) [18]. The interaction between microbes and humans can result in the outcomes such as disease, transient colonization, and prolonged colonization. Microorganisms of the healthy flora may aid the host (by competing for microenvironments more effectively than such pathogens as *Salmonella* Spp or by producing nutrients the host can use), may harm the host (by causing dental caries, abscesses, or other infectious diseases), or may exist as commensals (inhabiting the host for long periods without causing detectable harm or benefit). The transient and prolonged colonization implies a distinction based on the duration of the interaction, which may extend to weeks, months, or years. Microbes found in and on the human body can cause serious diseases. Not the recovery of a specific organism, but the recovery of the organism in a normally sterile site is the hallmark of the pathogenesis of microbial infections. For example, *Escherichia coli* is a normal resident confined to the gastrointestinal tract. If it is demonstrated in the stool, it may be considered normal, but is found in the abdominal cavity or the patient's bloodstream, this would be considered abnormal.

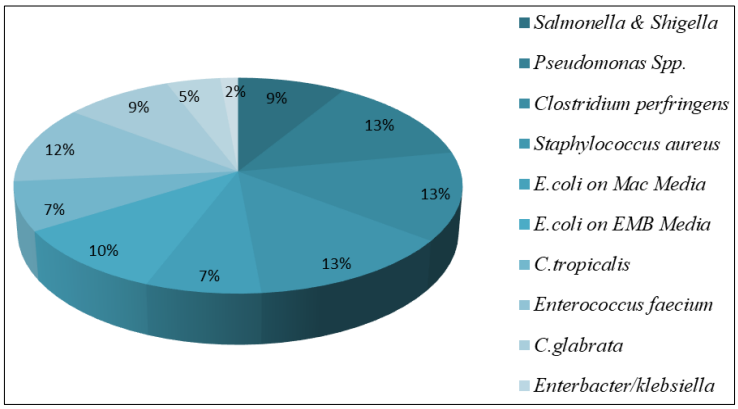
Similarly, certain organisms are never present as part of the normal microbial flora in humans; hence their recovery in humans is always associated with clinically significant diseases (e.g., *Bacillus anthracis*, *Brucella* Spp., *Francisella tularensis*, and *Histoplasma capsulatum*, etc.). According to Sahoo, 2018, the buccal swabs from Spectacled cobra were subjected to microbial culture and isolated 74 noticeable colony characters. Snake bite victims especially immune deficiency should be intensively monitored for presence of any potential pathogenic fungal flora as the oral cavities of venomous and nonvenomous snake represent portions of fungi, yeast and penicillium (Dehghani, 2016) [13]. During reptile collections *A. hydrophila*, *E. coli*, *S. marcescens* and *S. maltophilia*, *C. albicans*, *A. flavus* and *Cladosporium* Spp. found to be secondary causative disease agents (Lukac, 2017) [17]. Considering human pathogens, oral bacterial microflora of free-living Reticulated pythons isolated both gram-positive and gram-negative organisms. Ten snake species comprising the families Boidae, Colubridae, Elapidae, and Viperidae, indicated occurance of Gram-negative bacilli and cocci, gram-positive bacilli and cocci (MG, 2009) [20]. The highest rate of infection belonged to coagulase negative *Staphylococcus* (34.5%) and the lowest rate was for Pseudomonas (3.1%). *Salmonella* (18.8%); Escherichia and Providencia (each 12.5%); and Proteus, Enterococcus, and *Bacillus* (each 6.2%) were other contributing pathogens found in snakes' oral cavity. Hence, the present study aimed to examine the associated bacteria from the oral and cloacal cavity of Spectacled cobra rescued from human-snake conflict situations (wild) and from snake charmers to provide quality care and management.

2. Material and methods

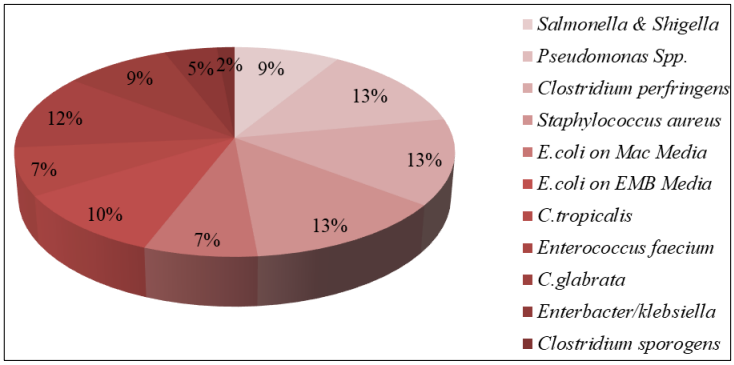
Spectacled cobras were rescued from both human-snake conflict situation and snake charmers at Agra, Uttar Pradesh by rapid response team of Wildlife SOS. During the health evaluation of these snakes by a veterinarian, aseptic samples from oral and cloacal cavity of spectacled cobra was collected by a microbiologist. A total of 20 nos. samples collected in which 10 were from wild and others are from snake charmer. The samples were subjected to microbial culture and IMVIC biochemical test to determine presence of microflora as a part of health evalution to provide quality care and treatment. Staining done by using Lactophenol cotton blue (Hi-media) and the morphological characteristics observed under microscope to identify the fungi. Once the snakes found fit, they were released back to suitable natural habitat.

3. Results and discussion

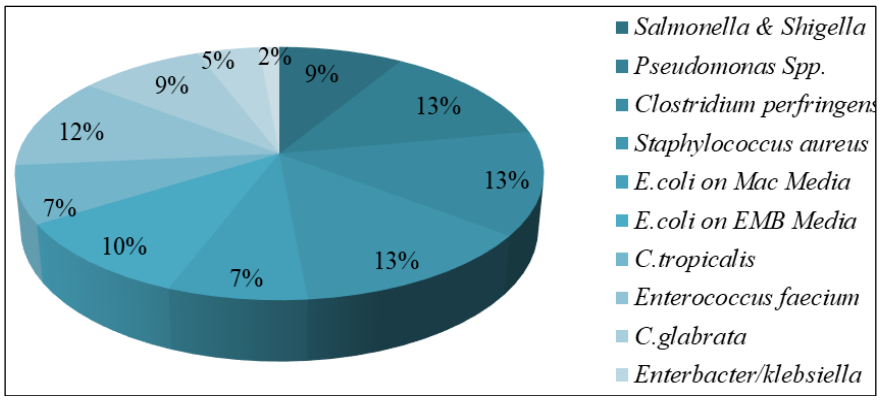
The aerobic microorganisms from oral and cloacal cavities of *N. naja* were successfully isolated (Graph 1 & 2). The cloacal cavity of wild *N. naja* was shown to harbor diverse and abundant microflora. A total of 11 bacterial and 3 fungal strains isolated from oral and cloacal cavity which was known human pathogens. Majorly in wild *N. naja*, *Clostridium perfringens*, *E. coli and Pseudomonas* Spp. have proportionated distribution whereas, *Enterobacter/ klebsiella and C. glabrata* were the minor components (Graph 1). The culture results of *N. naja* rescued from snake charmers demonstrated abundant diversity of microflora in oral than cloacal cavity may be due to the brutal human handling such as stitching of mouth, destroying of venom gland, fangs, force-feeding contamination, recurrent infection from baskets, etc. The most dominant organisms in *N. Naja* rescued from charmers were *Pseudomonas* Spp., *Clostridium perfringens*, *Staphylococcus aureus* followed by *Candida tropicalis*, *E. coli and Enterococcus faecium* (Graph 3).



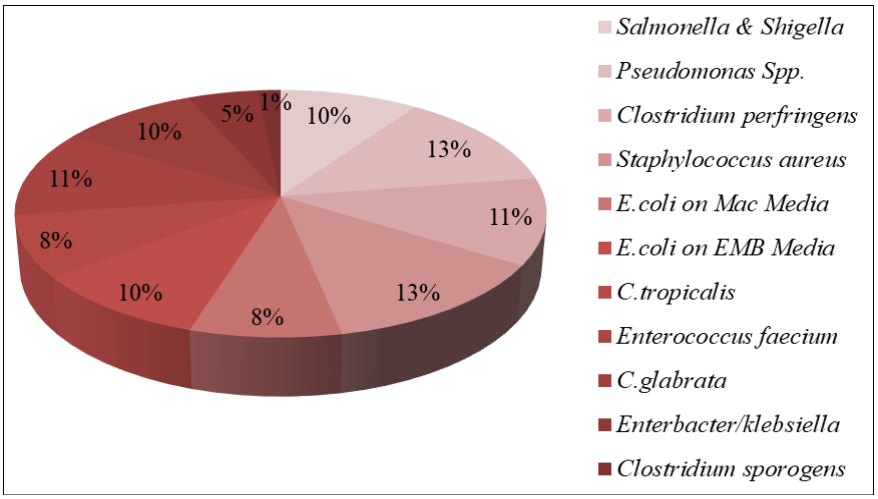
Graph 1: Representation of aerobic microflora found in the oral cavity of rescued wild cobra from conflict situations



Graph 2: Representation of aerobic microflora found in the cloacal cavity of rescued wild cobra from conflict situations



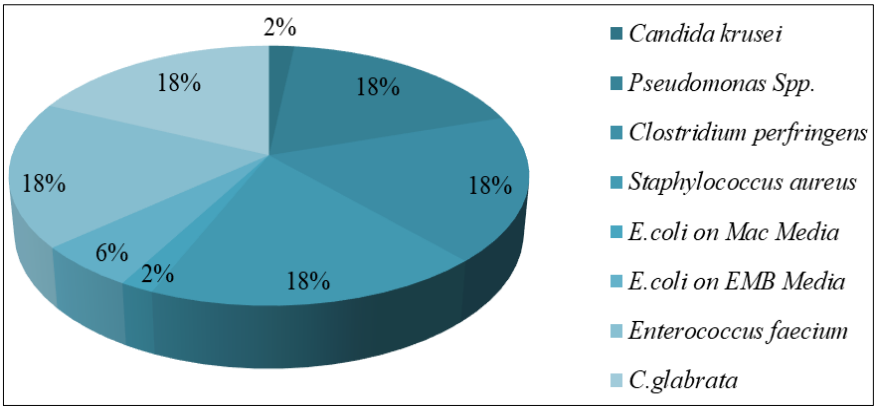
Graph 3: Representation of aerobic microflora found in the oral and cloacal cavity of cobras that were rescued from snake charmers



Graph 4: Representation of aerobic microflora found in the oral and cloacal cavity of cobras that were rescued from snake charmers

In addition to this, the swab samples collected from the snake charmer's wooden wooden box were subjected to microbial culture (Graph 5). The results reveled equivalent diversity of *Pseudomonas* Spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Candida*, *glabrata* and *Enterococcus faecium*. Minor diversity of *E. coli* and *C. krusei* were also observed on different media cultures.

Differences in habitat, predation strategies, and the type of prey can explain the high variation in bacterial flora. Snakebites have a high rate of infection because of Gram-negative bacteria may be due to their eating habits where the prey head is ingested first, leaving colonization of fecal flora on the oral cavity. This may also explain the higher amount of enterobacterial isolates found in the mouth of the individuals sampled. Bacteria isolated from snakes Oral and Cloacal Cavity, such as *Enterobacter* sp., *Escherichia coli*, *Clostridial* Spp., *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Pseudomonas* sp., could be opportunistic pathogens and generate nosocomial infections. Besides, *Enterobacter* Spp. and Fungal Spp. has been associated with infections of the oral mucosa of humans. On the other hand, pathogens like *Enterococcus faecalis*, *Salmonella*, *Shigella*, and *Staphylococcus aureus* can generate zoonosis. Similar bacterial genera were found compared to our results, where predominantly *Staphylococcus*, *Pseudomonas*, and *Enterobacter* match our findings. Since the microbial diversity associated with snakebite is concerned, handlers should maintain personal hygiene.



Graph 5: Representation of aerobic microflora found in snake charmers' wooden wooden basket

Fig 1: Spectacled cobra (*Naja naja*) rescued from human-snake conflict by Wildlife SOS

Fig 2: N. naja seized from snake charmers during Naga panchami at Agra, Uttar Pradesh

Fig 3: Wooden basket being used by snake charmer to keep N. naja

Fig 4: Asptic oral swab sample collection

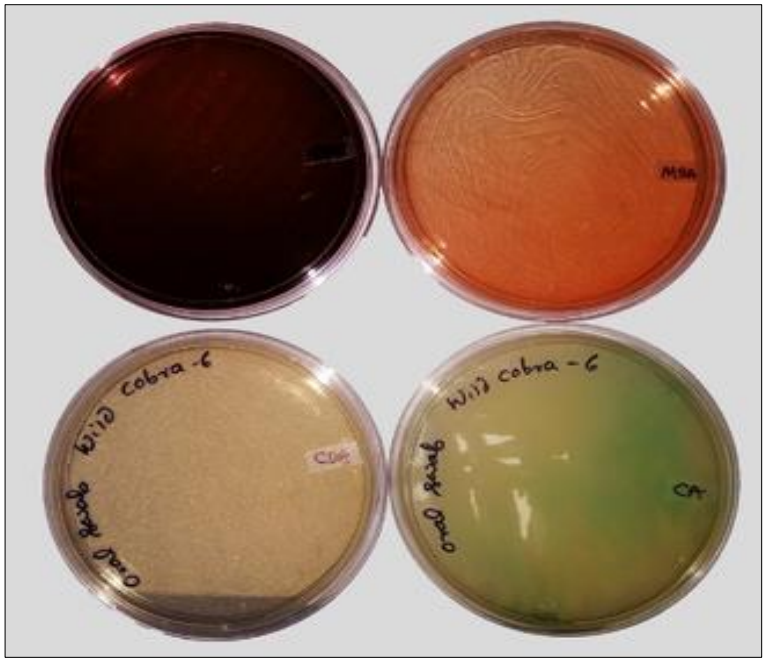


Fig 5: Oral swab culture of wild N. naja showing colonies of different aerobic microbes

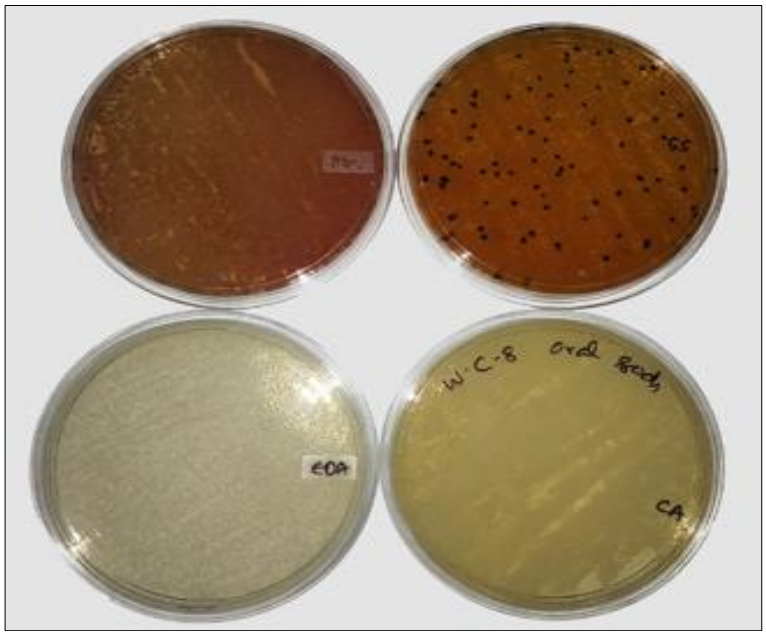


Fig 6: Colonies of different aerobic microbes from the oral swab culture of N. naja rescued from the snake charmers

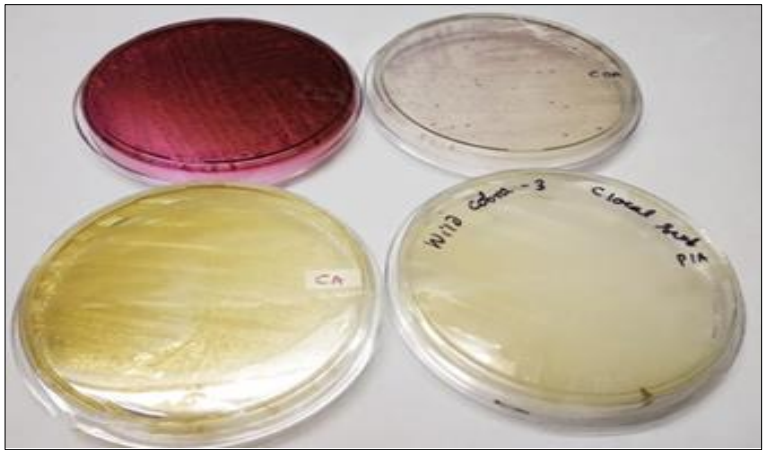


Fig 7: Cloacal swab culture of wild N. naja showing colonies of different aerobic microbes

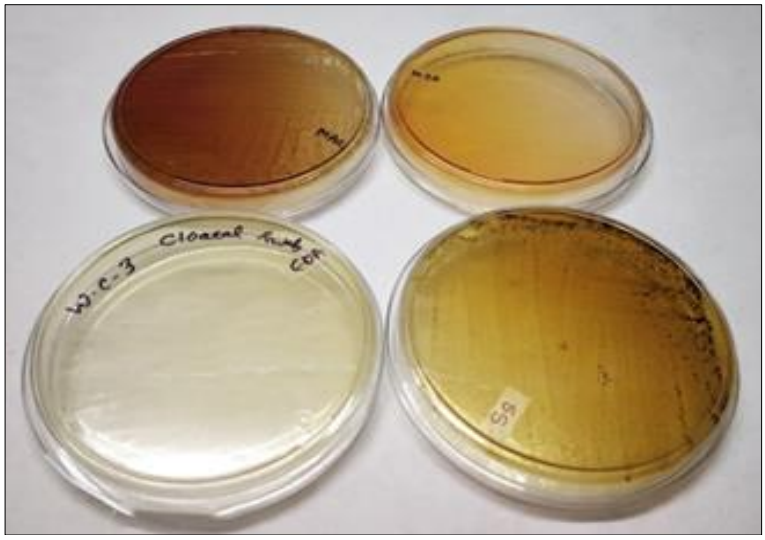


Fig 8: Cloacal swab culture of N. naja rescued from snake charmers showing microbes

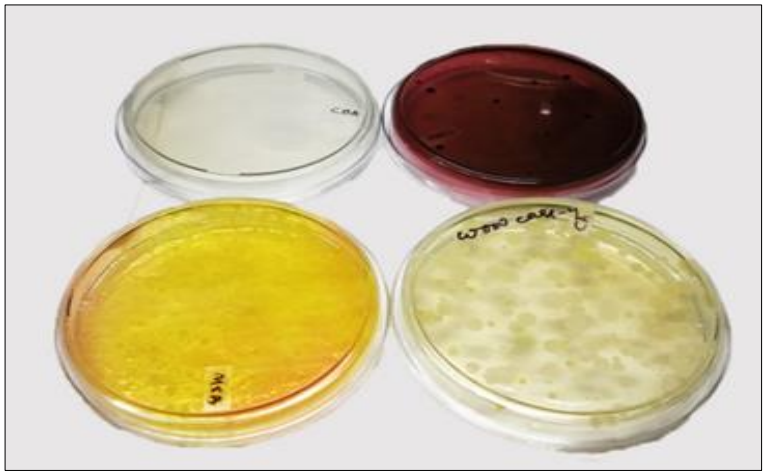


Fig 9: Culture results of swab collected from wooden basket of snake charmers showing microbes

Fig 10: IMViC test for identification of bacterial species

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