

Isolation and Identification of Bacteria of Normal Flora in Handfed Exotic Psittacine Birds at Tamil Nadu, India

R. Legadevi¹, V. Dillibabu², N. Karunakaran³, K.Nagarajan^{4*} and C.Soundararajan⁵

Cavin Estates Private Limited,
Bharathi Avenue, Injambakkam, Chennai, Tamil Nadu

ABSTRACT

Bacterial normal flora were identified in handfed psittacine birds (lory, amazons, lorikeet, cockatoo, conure, grey parrot, macaw, eclectus and African love birds) at Cavin estate exotic bird farm, Chennai, Tamil Nadu. A total of 54 cloacal swabs were collected from different species of psittacine birds for bacteriological screening to identify the microorganisms by morphological and biochemical characteristics. In the present study, four Gram positive organisms such as *Staphylococcus*, *Enterococcus*, *Bacillus* and *Corynebacterium* and ten Gram negative bacteria such as *Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *Yersinia* sp., *Providencia* sp., *Erwinia* sp., *Citrobacter* sp., *Serratia* sp., *Acinetobacter* sp. and *Buttiauxella* sp., were identified as the normal flora in apparently healthy handfed psittacine birds. The frequency of occurrence of *Staphylococcus* sp., and *E.coli* was higher when compared to others.

Key Words: Psittacines, Normal flora, Cloacal swab, hand feeding

INTRODUCTION

Parrots are found all over the world and popular as pets due to their sociable and lovable nature, intelligence, beauty and ability to imitate human voices.

Economically also avian pets are very much beneficial to communities as a

source of income through pet trade. Mostly parrots are kept in a cage or aviary. Species of parrot vary in their temperament, noise level, cuddliness with people and talking ability (Akhter et al., 2010).

Cavin estates exotic birds farm, Chennai is located on the south-eastern coast of India in the north-eastern part of Tamil Nadu on a flat coastal plain known as the Eastern Coastal Plains. Here, so many exotic bird varieties like speaking amazons, beautiful macaws are available. Other than this, lories, lorikeets, cockatoos, conures, monk, grey parrot, eclectus are also available.

Maintaining a healthy environment for birds is very important to prevent

¹Research Associate, Cavin Estates Private Limited, Bharathi Avenue, Injambakkam, Chennai, Tamil Nadu.

²Research Associate, Cavin Estates Private Limited, Bharathi Avenue, Injambakkam, Chennai, Tamil Nadu. Chief Veterinarian, Cavin Estates Private Limited, Bharathi Avenue, Injambakkam, Chennai, Tamil Nadu.

Corresponding author and Assistant Professor, Department of Veterinary Pathology, Madras veterinary college, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu.

³Department of Veterinary Parasitology, Madras veterinary college, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu

spreading of infectious diseases which are the major threat for avian species. Routine screening of birds for bacterial infection is also necessary for proper treatment and to understand the epidemiology of the disease (Machado et al., 2018).

Isolation, identification and characterisation of microorganisms at the time of disease outbreaks are the key issues in determining whether the organism is pathogenic or non-pathogenic. Based on that, therapeutic and preventive measures are to be standardised to get the highest success rate in establishing a healthy farm. Keeping all these in view, microbiological investigation was carried out to know the normal flora of bacteria in exotic psittacine birds at Cavin estates exotic bird farm, Chennai, Tamil Nadu.

MATERIALS AND METHODS

Sample collection:

A total of 54 cloacal swab samples were collected from apparently healthy handfed psittacine birds of lorys (green napped lory, red lory, yellow bipped lory, violet necked lory, chattering lory and olive swainson lory), amazons (red lord amazons, Cuban amazon, vinaceous breasted amazon and blue fronted amazon), cockatoos (major mitchell cockatoo, galah cockatoo and umbrella cockatoo), conures (jandey conure), parrots (gray parrot, African gray parrot, rupella parrot, timney grey parrot, caique), macaws (hyacinth macaw, blue gold macaw and green wing macaw), eclectus and African love birds at Cavin estate exotic bird farm, Chennai,

Tamil Nadu. This exotic bird farm consists of more than 400 psittacine birds and is reared in highly sophisticated cages with maximum possible flight space than the recommended standards, sprinklers to create suitable microenvironment, natural perches and sufficient numbers of breeding nest boxes of different size and shape to mimic the natural nest. The birds are provided with a highly nutritious and balanced feed consisted of seeds, grains, nuts, fresh fruits, vegetables, greens and fresh fruit juices as per the standard recommended requirements for each species of birds. The birds are regularly and periodically dewormed with ivermectin suspension (ivermectin 2.5% w/v) or alzonon (albendazole 3% w/v + niclosamide 10% w/v) at the dose of 5ml per litre in drinking water to whole night water deprived exotic pet birds.

Isolation of bacteria and morphological characterization:

Collected cloacal swab samples were inoculated in nutrient broth and incubated at 37°C overnight. The bacterial culture obtained was plated onto nutrient agar plate and incubated at 37°C for 24 hrs. Based on the colony morphology, individual colonies were subcultured and stained by Gram's method to study the morphology and staining characters (Gram, 1884).

Biochemical characteristics:

Oxidase and catalase test:

Oxidase test was carried out by Himedia oxidase disc and catalase test was performed using three per cent hydrogen peroxide.

Fermentation test for bacterial identification

Pure bacterial colonies were plated on Mannitol salt agar (MSA), MacConkey agar (MA) and Eosin methylene blue agar (EMB) to identify the fermentation of glucose, sucrose, lactose, dextrose and mannitol (Abbas et al., 2016).

Hydrolysis test:

Hydrolysis test was carried out for further identification of bacteria. Pure colonies were plated over bile esculin and urease media to identify the hydrolysis of bile esculin and urea.

IMVic

Biochemical tests such as indole, methyl red, Voges-Proskauer and citrate were carried out as a secondary bacterial

identification method and interpretation of the results was carried out (Bergey's manual of bacteriology and ABIS online tool, George et al., 2004).

RESULTS AND DISCUSSION

In this present study, 14 bacteria were isolated which included Gram positive (four) and Gram negative (10) micro organisms. Four Gram positive organisms such as *Staphylococcus* sp., *Enterococcus* sp., *Bacillus* sp., and *Corynebacterium* sp., and ten Gram negative microorganisms such as *Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *Yersinia* sp., *Providencia* sp., *Erwinia* sp., *Citrobacter* sp., *Serratia* sp., *Acinetobacter* and *Buttiauxella* sp. were identified as the normal flora in apparently healthy psittacine birds (Table 1). Birds are appeared healthy, there was no clinical symptoms observed in the birds.

Table 1 Organisms isolated from handfed birds

Sl. No.	Species of birde	No.of Birds	Organism Isolated
1.	Amazon		
	Red lord amazon	4	<i>Enterobacter aerogen</i> , <i>Klebsiella</i> , <i>Enterococcus</i> , <i>Staphylococcus aureus</i> and <i>Serratia</i>
	Cuban amazon	4	<i>Staphylococcus</i> , <i>E.coli</i> , <i>Serratia</i> , <i>Buttiauxella</i> and <i>Acinetobacter</i>
	Vinaceous breasted amazon	2	<i>Bacillus</i>
	Double yellow headed amazon	1	<i>Staphylococcus</i>
	Blue fronted amazons	1	<i>E.coli</i>
2.	Macaw		
	Hyacinth macaw	5	<i>Staphylococcus</i> and <i>E.coli</i>
	Blue gold macaw	2	<i>Corynebacterium</i> , <i>Citrobacter</i> and <i>Klebsiella</i>
	Green wing macaw	1	<i>Staphylococcus</i> and <i>E.coli</i>

3.	Lories and Lorikeets		
	Green napped lory	1	<i>Klebsiella</i> and <i>Buttiauxella</i>
	Red lory	1	<i>Serratia</i> , <i>Staphylococcus</i> and , <i>Enterococcus</i>
	Yellow bippedlory	2	<i>Serratia</i> and <i>E.coli</i>
	Violet necked lory	1	<i>E.coli</i>
	Chattering lory	2	<i>E.coli</i>
	Olive Swainsonlory	2	<i>Erwinia</i> , <i>Yersinia</i> , and <i>Staphylococcus</i>
4.	Cockatoo		
	Medium sulphur cockatoo	1	<i>E.coli</i>
	Galah cockatoo	3	<i>E.coli</i> , <i>Serratia</i> and <i>Providencia</i>
	Triton cockatoo	1	<i>Citrobacter</i>
	Major Mitchell cockatoo	2	<i>Acinetobacter</i> and <i>Serratia</i>
	Umberlla cockatoo	1	<i>E.coli</i> and <i>Bacillus</i>
5.	Eclectus	8	<i>Staphylococcus</i> , <i>Yersinia</i> , <i>Proteus</i> , <i>Enterobacter</i> , <i>Enterococcus</i> , <i>E.coli</i> and <i>Serratia</i> .
6	Parrots(Timney grey pactor)	9	<i>Staphylococcus</i> , <i>Yersinia</i> , <i>Enterobacter</i> , <i>Enterococcus</i> , <i>Acinetobacter</i> and <i>Klebsiella</i>

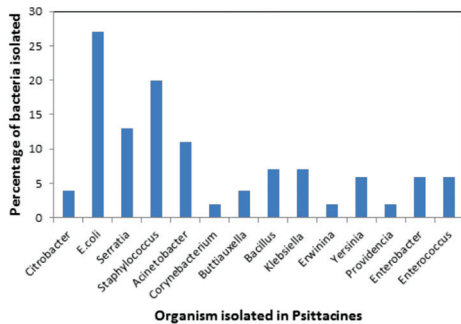
Most of the bacteria can be identified by primary screening like growth on selective and differential media. Full MSA is a selective medium for *Staphylococcus aureus*. It produced yellow colony surrounded by a yellow zone. *Escherichia coli*, *Klebsiella* and *Enterobacter* were identified using a EMB agar. *Escherichia coli* showed metallic sheen, *Enterobacter* sp. revealed pink colour colonies without sheen and *Klebsiella* sp., presented pink mucoid colonies on eosin methylene blue agar.

Citrobacter was identified by acid butt and acid slant with gas and H₂S production on TSI slant. Swarming growth in nutrient

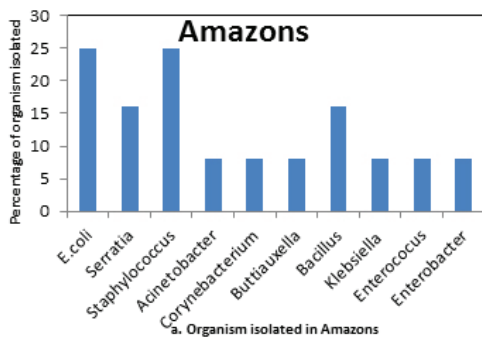
agar was observed for *Proteus* sp. Other bacteria such as *Serratia* sp., *Yersinia* sp., *Acinetobacter* sp., *Buttiauxella* sp., *Erwinia* sp., *Enterococcus* sp., and *Corynebacterium* sp. were identified by their biochemical characteristics.

Out of 14 bacteria isolated from psittacine birds, *E. coli* (27%) was found to be more followed by *Staphylococcus* sp. (20%), *Acinetobacter* sp. (11%), *Buttiauxella* sp. (7%), *Bacillus* sp. (7%), *Klebsiella* sp. (7%), *Serratia* sp. (7%), *Enterobacter* sp. (6%), *Enterococcus* sp. (6%), *Yersinia* sp. (6%), *Citrobacter* sp. (4%), *Providencia* sp. (2%), *Erwinia* sp. (2%), and *Corynebacterium* sp. (2%) were

isolated (Table. 2). Among these organisms, *E. coli* and *Staphylococcus* sp. was isolated at highest percentage from all the species of psittacine birds. *Corynebacterium* sp. was isolated only from Macaw, *Providentia* sp. from cockatoo and *Erwinia* sp. from lory (Table.1 and Fig.1).

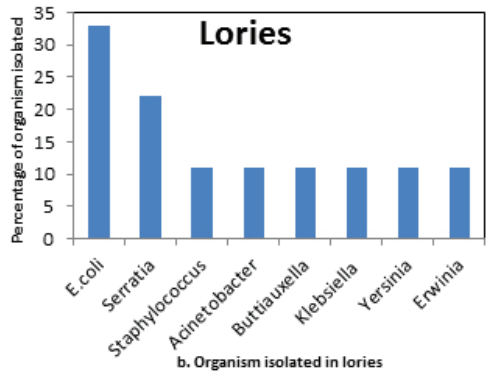


Out of the 54 psittacine birds, 12 amazon species were screened for bacterial isolation. It includes red lord amazon, Cuban amazon, vinaceous breasted amazon and double yellow headed amazon. Among all bacteria, *E.coli* and *Staphylococcus* sp. were isolated at a higher percentage (25%) followed by *Serratia* sp. and *Bacillus* sp. (16%) and other organisms were isolated at a lower percentage (8%) (Fig. 2a).

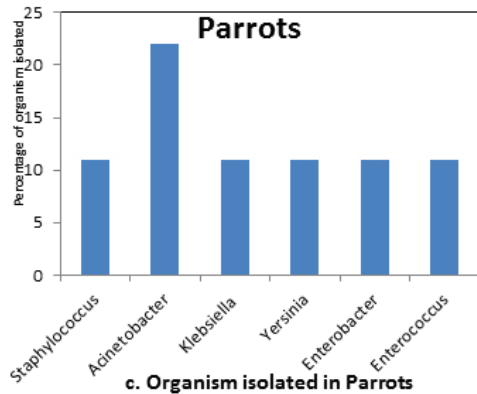


Out of 54 psittacine birds, nine lory species were analysed in this study which includes, green napped lored lory, yellow bipped lory, violet necked lory, chattering

lory, olive swainson lory. *Escherichia coli* was found to be more (33%) followed by *Serratia* sp. (22%) and other organisms (11%). In comparison with other species, *Erwinia* sp. was isolated from lorry only (Fig. 2b).

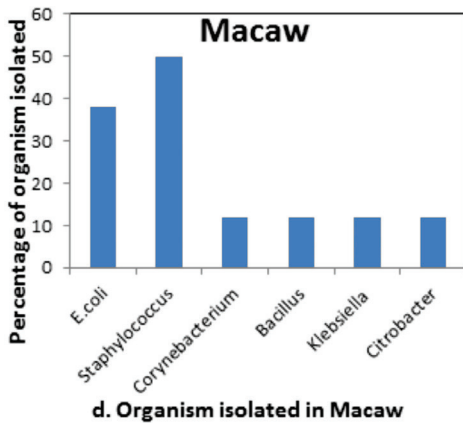


In parrots (9/54), *Acinetobacter* sp. was isolated at a higher percentage (22 %) followed by *Staphylococcus* sp., *Klebsiella* sp., *Yersinia* sp., *Enterobacter* sp. and *Enterococcus* sp. (11%) (Fig. 2c).

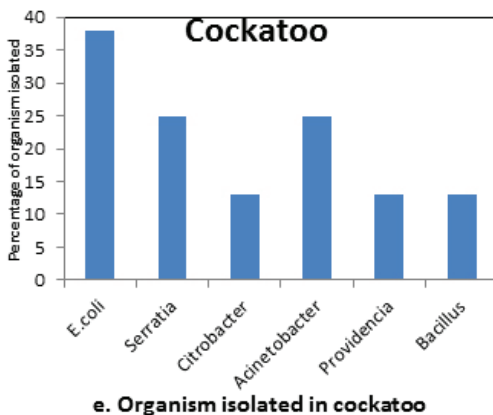


Among Macaws (8/54), hyacinth macaws, blue gold macaws and green wing macaws were included in this study. *Staphylococcus* sp. (50%) and *E. coli* (38%) were isolated at a higher percentage followed by *Corynebacterium* sp., *Bacillus*

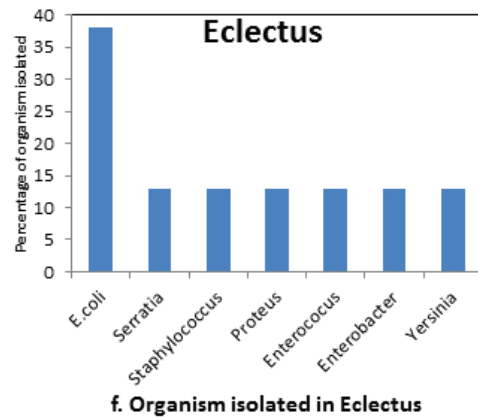
sp., *Klebsiella* sp. and *Citrobacter* sp. (12%). In comparison with other species, *Corynebacterium* was isolated only from macaw (Fig. 2d).



In cockatoos (8/54), medium sulphur cockatoo, galah cockatoo, Triton cockatoo, major mitchell cockatoo, umberlla cockatoo used for this study. Organism isolated form cockatoos were *E. coli*, *Serratia* sp., *Citrobacter* sp., *Acinetobacter* sp., *Providencia* sp. and *Bacillus* sp. *Escherichia coli* was isolated at a highest percentage (38) followed by, *Serratia* sp. and *Acinetobacter* sp. (25 each) and other organisms (13%) (Fig. 2e).



From eclectus, *E. coli* was isolated at a highest percentage of 38 and other organisms were isolated as shown in fig. 2f. It was reported that the presence of Gram-negative bacteria, including those belonging to the Enterobacteriaceae in their intestinal microbiota has been considered as an indication of potential diseases (Bangert et al., 1988; Mattes et al., 2005). Microbiological studies in psittacine have increasingly reported Enterobacteria in healthy birds (Serafini et al., 2015; Lopes et al., 2015; Machado et al., 2016). In the present study, we have isolated more numbers of negative microorganisms in healthy birds. Birds look apparently healthy and there were no clinical symptoms of illness.



The isolation of microorganisms from the birds in this study could be explained by the fact that young birds were colonised by microbes immediately after hatch, and acquired a microbial biomass by contact with environmental microorganisms. This could be the main sources of microbial transmission to growing birds (Mills et al., 1999). Moreover, the young birds might have low immunity and acquired this harmful microbial load by direct contact

with other animals that might have visited the artificial nests, such as mice, bats, and other avian species, or by indirect contact with their secretions (Allgayer et al., 2009; Serafini et al., 2015).

The absence of *Salmonella* sp. was also observed in apparently healthy handfed birds maintained in incubator at 37°C. Mostly psittacines are very sensitive to avian salmonellosis, particularly at young ages (Marietto-Gonçalves et al., 2010). The absence of *Salmonella* sp. in the present study suggests that the birds were not exposed to this pathogen (Godoy et al., 2007). This study gives the general knowledge on the normal microbial flora of apparently healthy handfed exotic pet birds and it is very essential to differentiate them from the pathogenic microorganisms isolated at the time of disease outbreaks.

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