

ISOLATION AND IDENTIFICATION OF MICROFLORA FROM APPARENTLY HEALTHY CAGED PARROTS OF DHAKA ZOO OF BANGLADESH

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ABSTRACT

The research work was conducted to isolate and identify the microflora from apparently healthy caged parrots. A total of 45 samples (oral swabs, cloacal swabs and feces) were collected from five types of caged parrots (Gray cockatiels, Rose ringed parakeet, Alexandrine parakeet, Red breast parakeet and Blossom headed parakeet) of Dhaka Zoo during the period from April to August 2009. The samples were cultured on different bacteriological media and the bacteria were identified by their cultural and biochemical properties. All the isolates were allowed for antibiogram study. The bacteria isolated in this study from different types of caged parrots were *E. coli* (64.44%), *Salmonella* spp. (46.67%), *Staphylococcus* spp. (46.67%), *Pasteurella* spp. (33.33%), *Proteus* spp. (6.67%) and some unidentified Gram-positive and Gram-negative bacteria. Of these isolates, *E. coli* was the most frequent isolate. The frequency of Gram-negative bacteria was higher in this study. The percentage of bacterial isolates recovered from each type of parrots was almost similar. Irrespective of types of parrots, the higher percentage of different bacteria was isolated from cloacal swab (77.78%) followed by feces (75.56%). The 68.89% isolates were recovered from oral swab. All the suspected isolates of *Salmonella* spp. were confirmed by slide agglutination test using *Salmonella* polyvalent 'O' antiserum. Among the 21 *Salmonella* spp. isolated in this study, 4 (19.05%) isolates were identified as *S. Pullorum* when tested with specific antisera against *S. Pullorum*. The results of antibiotic sensitivity tests revealed that ampicillin and amoxicillin were completely resistant to *E. coli* and *Pasteurella* spp.; ampicillin to *Proteus* spp.; and furazolidone to *Salmonella* spp. and *Pasteurella* spp. However, the antibiotics of fluoroquinolone group such as ciprofloxacin, norfloxacin and enrofloxacin showed moderate to high sensitivity against almost all the bacterial isolates. Of these, ciprofloxacin was found to be consistently highly sensitive to all the bacterial isolates.

Key words : Isolation, identification, microflora, caged parrot

INTRODUCTION

Parrots are found all over the world from a long time. They are popular as pets due to their sociable and affectionate nature, intelligence, bright colors, and ability to imitate with human voices. Economically they can be beneficial to communities as sources of income from the pet trade. The domesticated Budgerigar, a small parrot, is the most popular of all pet bird species. Pet birds are the source of recreation for human especially children. Pet parrots are kept in a cage or aviary; though generally, tame parrots should be allowed to be taken out regularly. Species of parrot vary in their temperament, noise level, talking ability, cuddliness with people. Parrots are excellent companion animals, and can form close, affectionate bonds with their owners. However they invariably require an enormous amount of attention, care and intellectual stimulation to thrive (<http://www.parrotsanctuary.co.uk>). Depending on locality, parrots may be either wild caught or be captive bred, though in most areas without native parrots, pet parrots are captive bred. Among a larger number of species of parrots, Alexandrine Parakeet, Cockatiel, Rose-ringed Parakeet, Red breast parakeet, Blossom Headed parakeet, Common Hill Myna, Macaw, Lovebird etc species are available in Bangladesh and widely reared in cage particularly in Dhaka Zoo as ornamental birds.

Parrots are often suffered from many bacterial diseases with often involvement of normal flora or environmental pathogens in response to stress and immunosuppression. Bacterial enteritis is often a spontaneous stress associated disease caused mainly by *E. coli*, *Klebsiella*, *Salmonella*, *Pasteurella*, *Pseudomonas*, *Aeromonas* and *Citrobacter* (Altman and Robert, 1997). Most of the enteric *Salmonellae* (*Salmonella typhimurium*, *Salmonella enteritidis*) are motile and classified as paratyphoid organisms and the diseases they produce are termed paratyphoid infections (Kirk *et al.*, 2002). As with bacterial enteritis, bacterial respiratory disease is also often a stress associated phenomenon where *Klebsiella*, *Escherichia coli*, *Enterobacter*, *Pseudomonas*, *Pasteurella* and *Mycoplasma* are commonly involved (Friend and Franson, 1999).

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Many zoonotic diseases are transferred from cage or pet birds to human through direct or indirect contact of the diseased or carrier birds. Visitors are more susceptible to acquire zoonotic diseases from cage birds in zoo. Bacteria are one of the most common causes of zoonotic diseases. For this, proper isolation, identification and characterization of the bacteria are essential to control zoonotic diseases. Outbreaks of zoonoses have been traced to human interaction with and exposure to animals at fairs, petting zoos, and in other settings. In 2005, the Center for Disease Control and Prevention (CDC) issued an updated list of recommendations for preventing zoonoses transmission in public settings. The CDC recommendations, which were developed in conjunction with the National Association of State Public Health Veterinarians, include sections on the educational responsibilities of venue operators, managing public and animal contact, and animal care and management (CDC, 2005). In 2002, seven people became ill with *E. coli*: 0157117 infections after visiting a large agricultural fair in Ontario, Canada. Investigators of outbreak conducted a case-control study, which indicated that goats and sheep from a petting zoo were the source of the *E. coli* among fair visitors. Other indications were that the fencing and environment surrounding the petting zoo that could have been a source of transmission (Warshawsky, 2002).

Very few works have been studied on the isolation and identification of bacteria from caged birds in Bangladesh and the present study, therefore, was undertaken to isolate and identify important species of bacteria from apparently healthy caged parrots, and to determine antibiotic sensitivity pattern of the isolated bacteria.

MATERIALS AND METHODS

A total of 45 cloacal swab, feces and oral swab samples, 15 of each, were carefully collected from five types (Gray cockatiel, Rose ringed parakeet, Alexandriane parakeet, Red breast parakeet and Blossom headed parakeet) of apparently healthy caged parrots of Dhaka Zoo, Mirpur, Dhaka. Immediately after collection, each sample was inoculated into sterile nutrient both (NB) and kept in ice box and transported to the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. The inoculated nutrient broths were incubated at 37°C for 24 hrs and then streaked onto different bacteriological media such as NA, EMB, MC, SS, BGA, BA, TSI (Himedia, India) to obtain pure culture of the bacteria. Gram's staining was performed to study the morphology of bacterial isolates (Merchant and Packer, 1967) and motility test was performed to differentiate motile bacteria from non-motile one (Cowan, 1985). Leishmann's staining was performed to identify bipolar organisms such as *Pasteurella multocida* according to the procedures described by Buxton and Fraser (1977). Isolated bacteria from each sample was biochemically identified by sugar fermentation test, indole test, MR-VP test, catalase and coagulase tests as per methods described by Cheesbrough (1984). Besides, triple sugar iron agar (TSI agar) slant was used to identify the lactose, saccharose and dextrose fermenters. The medium also helped to determine the ability of the organisms to produce hydrogen sulphide. The test organisms were heavily seeded with a platinum loop over the surface of the slants and stabbed into the butt of the TSI agar (Himedia, India). After incubation at 37°C for 24 hours, the tubes were examined for any change in the slant or butt (Cheesbrough, 1984).

Hemolytic activity

Hemolytic activities of the isolated bacteria were studied as per the method described by Chatterjee *et al.* (1990). All the isolates were tested for the production of hemolysis by growing them on bovine BA plate and were then incubated at 37°C for 24 hours. The colony developed on the BA plate was examined for various types of hemolysis. Hemolytic patterns of the bacteria were categorized according to the types of hemolytic zone they produced on BA plates and were listed as follows: (i) Alpha (α) hemolysis: a zone of greenish discoloration around the colony manifested by partial hemolysis and (ii) Beta (β) hemolysis: complete clear zone of hemolysis around the colony.

Sero-grouping of *Salmonella*

Sero-grouping of *Salmonella* isolates was performed by slide agglutination test using polyvalent 'O' as well as *Salmonella* Pullorum antisera. The test was performed according to the protocol of Buxton and Fraser (1977). Briefly, an amount of 20 μ l of antisera was taken with the micropipette on a glass slide placed on dark background. A small amount of bacterial mass from a single colony was taken with a bacteriological loop and placed on slide and mixed properly. The slide was agitated gently for 5 to 10 seconds. The reaction was read by the naked eye holding the slide in front of a light source against a black background. Positive reaction was recorded by a visible agglutination.

Antibiotic sensitivity test

Antibiotic sensitivity test was done using disc diffusion test following the method described by Bauer *et al.* (1966). One milliliter of fresh broth culture was poured on nutrient agar media and spreaded uniformly. Antibiotic discs (Mast Group Ltd, Merseyside, UK) were placed apart onto the surface of the inoculated plates aseptically with the help of a sterile forceps and incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the zone of inhibition were measured. Individual antibiotic was recorded as highly sensitive, moderately sensitive, less sensitive or resistant depending on the area of inhibition of bacterial growth as per the method described by Bauer *et al.* (1966).

RESULTS AND DISCUSSION

The bacteria isolated in this study were *E. coli*, *Salmonella* spp., *Pasteurella* spp., *Staphylococcus* spp., *Proteus* spp. and some other unidentified Gram-positive and Gram-negative bacteria. This finding is in consistent with the findings of Sandra *et al.* (1998) and Doneley (2009). In addition to these organisms, they also isolated *Streptococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp., *Corynebacterium* spp., *Lactobacillus* spp. etc. The specific media and biochemical tests were used for the identification of the bacterial isolates which was previously suggested by a number of authors (Buxton and Fraser, 1977 and Freeman, 1985). The cultural and biochemical properties revealed by the isolates in this study are in agreement with the reports of Buxton and Fraser (1977) and Cheesbrough (1984).

Among 45 different types of samples collected from parrots, a total of 29 (64.44%) samples were found positive for *E. coli*, of which 6 (40%) were isolated from oral swabs, 12 (80%) from cloacal swabs and 11 (73.33%) from feces of different types of parrots; 21 (46.67%) samples were found positive for *Salmonella* spp., of which 5 (33.33%) were isolated from oral swabs, 9 (60%) from cloacal swabs and 7 (46.67%) from feces; 21 (46.67%) samples were found positive for *Staphylococcus* spp., of which 8 (53.33%) were isolated from oral swabs, 7 (46.67%) from cloacal swabs and 6 (40%) from feces; 15 (33.33%) samples were found positive for *Pasteurella* spp., of which 6 (40%) were isolated from oral swabs, 4 (26.67%) from cloacal swabs and 5 (33.33%) from feces; 3 (20%) samples were found positive for *Proteus* spp., which were isolated from oral swabs only (Table 1). The bacteria most frequently isolated from all types of caged parrots was *E. coli* (64.44%) followed by *Salmonella* spp. (46.67%), *Staphylococcus* spp. (46.67%), *Proteus* spp. (6.67%) and *Pasteurella* spp. (33.33%). Bowman and Jacobson (1980) and Bangert *et al.* (1988) also reported that *E. coli* was the most frequent isolate from clinically healthy psittacine birds.

Table 1. Overall percentages of different types of bacteria isolated from different types of samples collected from caged parrots

Name of bacteria	Number of each type of samples			
	Oral swab (n = 15)	Cloacal swab (n = 15)	Feces (n = 15)	Total
<i>E. coli</i>	6 (40 %)	12 (80 %)	11 (73.33 %)	29 (64.44 %)
<i>Salmonella</i> spp.	5 (33.33 %)	9 (60%)	7 (46.67 %)	21 (46.67 %)
<i>Pasteurella</i> spp.	6 (40 %)	4 (26.67 %)	5 (33.33 %)	15 (33.33 %)
<i>Staphylococcus</i> spp.	8 (53.33 %)	7 (46.67 %)	6 (40 %)	21 (46.67 %)
<i>Proteus</i> spp.	3 (20 %)	0 (0 %)	0 (0 %)	3 (6.67%)
Unidentified Gram positive Rod	1 (6.67 %)	1 (6.67 %)	1 (6.67 %)	3 (6.67 %)
Unidentified Gram negative Rod	1 (6.67 %)	1 (6.67 %)	1 (6.67 %)	3 (6.67 %)
Unidentified Gram negative coccobacilli	1 (6.67 %)	1 (6.67 %)	3 (20 %)	5 (13.33 %)
Total	31 (68.89%)	35 (77.78%)	34 (75.56%)	

n = Number of examined samples.

It is interesting to note that only 24 isolates were Gram-positive among 100 isolates. This finding differ from earlier observation of Bangert *et al.* (1988) and Flammer and Drewes (1988) who reported higher incidence of Gram-positive bacteria in various species of psittacine birds. This variation could not be explained exactly, however, it may be due to differences in the site of sampling and sample size. All the suspected *Salmonella* were identified by using *Salmonella* polyvalent 'O' antiserum. Of the 21 *Salmonella* spp. isolated in this study, 4 (19.05%) isolates were identified as *Salmonella* Pullorum when tested with specific antisera against *Salmonella* Pullorum. The occurrence of *Salmonella* Pullorum in psittacine birds is not common (Fowler, 1986; Allgayer *et al.*, 2008). However, the present finding is in agreement with the findings of Shimakura *et al.* (1985) and Deem *et al.* (2005) who also reported the occurrence of *Salmonella* Pullorum in psittacine birds.

Irrespective of type of samples, 6 (66.6%) samples from each type of parrots were positive for *E. coli* except Red breast parakeet from which 5 (55.56%) samples were found positive; 5 (55.56%) samples each from Rose ringed parakeet and Red breast parakeet; 4 (44.44%) samples each from Gray cockatiel and Blossom headed parakeet, and 3 (33.33%) samples from Alexandriane parakeet were found positive for *Salmonella* spp.; 3 (33.33%) samples each from Rose ringed parakeet and Red breast parakeet, 4 (44.44%) from Alexandriane parakeet, 5 (55.56%) from Blossom headed parakeet, and 6 (66.67%) from Gray cockatiel were found positive for *Staphylococcus* spp.; 2 (22.22%) samples each from Gray cockatiel and Rose ringed parakeet, 4 (44.44%) samples each from Red breast parakeet and Blossom headed parakeet, and 3 (33.33%) samples from Alexandriane parakeet were found positive for *Pasteurella* spp.; 1 (11.11%) sample each from Gray cockatiel, Alexandriane parakeet and Red breast parakeet was found positive for *Proteus* spp. Samples from Rose ringed parakeet and Blossom headed parakeet were found negative for *Proteus* spp. (Table 2). Among the five types of parrots, no remarkable variation was found in the percentage of bacterial isolates. Almost similar number of isolates of different bacteria was recovered from each type of parrots.

Table 2. Overall percentages of different types of bacteria isolated from each type of parrots

Name of bacteria	Number of different types of bacteria isolated from each type of parrots (n = 9)				
	Gray cockatiel	Rose ringed parakeet	Alexandriane parakeet	Red breast parakeet	Blossom headed parakeet
<i>E. coli</i>	6 (66.67%)	6 (66.67%)	6 (66.67%)	5 (55.56%)	6 (66.67%)
<i>Salmonella</i> spp.	4 (44.44%)	5 (55.56%)	3 (33.33%)	5 (55.56%)	4 (44.44%)
<i>Pasteurella</i> spp.	2 (22.22%)	2 (22.22%)	3 (33.33%)	4 (44.44%)	4 (44.44%)
<i>Staphylococcus</i> spp.	6 (66.67%)	3 (33.33%)	4 (44.44%)	3 (33.33%)	5 (55.56%)
<i>Proteus</i> spp.	1 (11.11%)	0 (0%)	1 (11.11%)	1 (11.11%)	0 (0%)
Unidentified Gram positive Rod	1 (11.11%)	2 (22.22%)	0 (0%)	0 (0%)	0 (0%)
Unidentified Gram negative Rod	0 (0%)	0 (0%)	1 (11.11%)	1 (11.11%)	1 (11.11%)
Unidentified Gram negative coccobacilli	1 (11.11%)	1 (11.11%)	1 (11.11%)	2 (22.22%)	0 (0%)
Total	21 (46.67 %)	19 (42.22%)	19 (42.22%)	21 (46.67 %)	20 (44.44%)

n = No. of samples examined from each type of parrots.

Irrespective of types of parrots, the higher percentage of different bacteria was isolated from cloacal swabs (77.78%) followed by feces (75.56%). The 68.89% isolates were recovered from oral swabs (Table 1). This finding could not be compared due to unavailability of specific literatures. However, it is imperative to note that the bacterial load is usually higher in cloaca and feces than oral cavity of healthy birds (Pettrak, 1982).

Isolation and identification of microflora from healthy parrots

The sensitivity patterns of different bacteria to various antibiotics were so variable that it was difficult to interpret. Ampicillin and amoxicillin were found to be completely resistant to *E. coli* and *Pasteurella* spp.; and furazolidone to *Salmonella* spp. and *Pasteurella* spp. (Table 3). On the other hand, the antibiotics of fluoroquinolone group such as ciprofloxacin, norfloxacin and enrofloxacin showed moderate to high sensitivity against almost all the bacterial isolates (Table 3). Of these, ciprofloxacin was found to be consistently highly sensitive to all the bacterial isolates which is consistent with the findings of Brahmabhatt and Anjaria (1991), Morishita *et al.* (1996) and Mukhopadhyay *et al.* (1998).

Table 3. Antibiotic sensitivity pattern of the bacteria isolated from caged parrots

Name of bacteria	Sensitivity pattern	Sensitivity (%) of the bacterial isolates to various antibiotics							
		AMP	AML	CN	CIP	NOR	PEF	FR	ENR
<i>Escherichia coli</i>	Resistant	100	100	00	00	00	00	00	00
	Less	00	00	40	00	00	20	60	00
	Moderate	00	00	60	20	20	80	40	20
	High	00	00	00	80	80	00	00	80
<i>Salmonella</i> spp.	Resistant	00	00	00	00	00	00	100	00
	Less	20	00	20	00	00	80	00	10
	Moderate	80	20	80	20	40	20	00	00
	High	00	80	00	80	60	00	00	00
<i>Pasteurella</i> spp.	Resistant	100	100	00	00	00	00	100	00
	Less	00	00	20	00	00	40	00	00
	Moderate	00	00	80	40	40	60	00	20
	High	00	00	00	60	60	00	00	80
<i>Staphylococcus</i> spp.	Resistant	00	00	00	00	00	00	00	00
	Less	00	80	20	00	20	00	20	00
	Moderate	20	20	80	00	80	20	80	00
	High	80	00	00	100	00	80	00	100
<i>Proteus</i> spp.	Resistant	100	00	00	00	00	00	00	00
	Less	00	00	33.33	00	00	00	00	00
	Moderate	00	100	66.67	00	00	100	100	00
	High	00	00	00	100	100	00	00	100

AMP = Ampicillin; AML = Amoxicillin; PEF = Pefloxacin; CN = Gentamycin; FR = Furazolidone; CIP = Ciprofloxacin; NOR = Norfloxacin and ENR = Enrofloxacin.

Although, primary bacterial infections are not only a common cause of disease in parrots; a better understanding of normal microbial flora may help in interpreting the significance of bacterial isolates in sick birds (Bangert *et al.*, 1988; Lamberski *et al.*, 2003). A knowledge of normal bacterial flora is also important for identifying potential pathogens that can cause disease during times of stress or immune suppression (Petrak, 1982). For example, *Pasteurella multocida* can cause septicemia and cutaneous lesions in parrots and their relatives (Morishita *et al.*, 1996). Thus, the results of this study may help pet clinicians to interpret microbiological culture and sensitivity results in parrots and other psittacine birds as well.

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