Global Animal Science Journal-GASJ Vol: 2(1): pages: 139-144; 2015. DOI:10.5281/zenodo.27223
A. Khafagy et al 2015; Research Article

Prevalence and characterization of *Escherichia coli* isolated from apparently healthy and diseased Cockatiles and budgerigars

A. A. R. Khafagy[1], M. A. Moustafa[2], I. S. Ibrahim[3], Shabana, I.I.[1] and I. M. F. Khalifa[1]

[1] *Bacteriology, Mycology and Immunology Dept. Faculty of Vet. Medicine, Suez Canal University, Egypt*
[2] *Wildlife Management and Zoo Medicine Faculty of Vet. Medicine, Suez Canal University, Egypt*
3 Bacteriology Veterinary serum and Vaccine Research institute Abbassia, Cairo, Egypt

Submitted on 11th May; Accepted on 17th August 2014; Published on 1st January 2015

ABSTRACT

A total of 258 fecal samples from Cockatiles and budgerigars (230 from apparently healthy birds and 28 from clinically diseased) were collected. The bacteriological examination revealed the isolation of *E.coli* (51.28%), Salmonella (10.26%), *Klebsiella* (17.95%), Proteus (17.95%) and *Enterobacter* (2.56%) respectively in relation to total number of isolates. They were infected with percentage of *E.coli* (7.75%), *Salmonella* (1.55%), *Klebsiella* (2.71%), proteus (2.71%) and *Enterobacter* (0.39%) respectively in relation to total number of collected samples. The isolated *E.coli* serovars were belonging to O_1 (13.3%), O_2 (20%), O_{26} (6.7%) and $O_{untypable}$ (60%). *E.coli* strains were then examined for enterotoxin production, *E.coli* O_{26} and $O_{untypable}$ was heat labile toxin producer [LT]. While O_1, O_2, $O_{untypable}$ were verotoxin producer. On the other hand, no heat stable toxin producer strains [ST] were detected. RAPD PCR profile was used for differentiation between *E.coli* different serotypes and revealed a significant difference among the revealed serotypes. The antibiotic sensitivity tests revealed that, ciprofloxacin and gentamycin were the most effective drugs against the isolated *E.coli*.

Key words: Cockatiles, budgerigars, *E.coli*

INTRODUCTION

Cockatiels and budgerigars are usually cage birds. Belonging to family psittacidae, order psittaciforms. These birds as household pets are a hobby and give much pleasure **(Forshaw, 1973)**. There is a much progress in their diseases studies; the alimentary system was the most concerned system in these studies because of large number of its bacterial isolates **(Baker, 1996).** Few surveys were established to detect the normal gastrointestinal tract flora of psittacine birds **(Flammer and Drewes, 1988).** Bacterial enteritis is an Important disease in psittacine birds either a primary intestinal problem or a systemic disease manifestation **(Minsky and Petrak, 1982).** Cloacal swabs and faecal samples is a common practice for bacteriological culture used in the routine avian examination **(Flammer and Drewes, 1988).**

MATERIAL AND METHODS

Samples collection

Each sample was collected in sterile test tube containing peptone water and then transferred aseptically to the lab in Ice box.

Bacteriological examination

The swabs from fecal dropping were collected aseptically and inoculated into a tube of nutrient broth. The

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

inoculated media were incubated at 37 °C for 24 hours.

A loopfull from the incubated nutrient broth culture was streaked onto the following media, MacConkey's agar, xylose lysine deoxycholate agar and Eosin methylene blue (EMB) medium.

Microscopic examination

Smears from suspected isolated colonies were prepared, fixed and stained with Gram's stain for differentiation of isolates into gram positive and gram negative, and for identification of other morphological characters for the organisms.

Biochemical identification of isolates

Different biochemical reactions were carried out for identifying the gram negative isolates and differentiation between members of Enterobacteriaceae family according to standard procedures given by (**Finegold and Martin, 1982**) and (**Krieg and Holt, 1984**).

Antibiotic sensitivity

Media used for sensitivity test were:

-Mueller- Hinton broth (Oxoid).

-Mueller- Hinton agar (Oxoid).

Antibiotic sensitivity discs:

Tetracycline	30 Mg
Ampicillin	10 Mg
Kanamycin	30 Mg
Sulfamethoxazole/ trimethoprim	25 Mg
Ciprofloxacin	5 Mg
Cefotaxime CTX	30 Mg
Erythromycin	15 Mg
Rifamycin	5 Mg
Gentamycin	10 Mg
Chloramphenicol	30 Mg
Lincomycin	2 Mg
Amoxicillin	10 Mg
Streptomycin	10 Mg
Doxycycline	30 Mg

***E.coli* Serotyping**

The technique recommended by **Sojka (1965)** using slide agglutination. Twenty four hours culture was used in serotyping of the isolated *Escherichia coli* strains.

Monovalent and polyvalent anti-sera were locally prepared against standard *Escherichia coli* serogroups. The antisera was diluted in normal saline solution starting with 1/50 to 1/200, equal volumes of suspected *Escherichia coli* O-antigens were added. Negative control was prepared using saline and antigen suspension. The tubes were incubated at 56 °C for overnight in a water bath. If agglutination occurs within one of the polyvalent O-antisera, the O-bacterial suspension was tested against the individual constituent O-sera.

Enterotoxogenic and verotoxogenic *Escherichia coli* toxins:

Escherichia coli isolates were grown in culture medium prepared specifically for production of toxins according to **Emery et al. (1992).** Detection of heat stable enterotoxin produced by *E.coli* isolates using the suckling mouse assay according to **Giannella, R. A. (1976).**

RAPD PCR

The PCR reaction mix consisted of 0.25 mg/ml bovine serum albumin (BSA), 3mM MgC12, 50 mM Tris (pH 8.3), 0.2 mM nucleotides, 0.1 mM primers and 0.5 unite Taq DNA polymerase, one microliters of sample DNA template containing 100 ng was added to 10 µl of the PCR reaction mix. The reaction mixture was overlaid with mineral oil, and was incubated in a thermal cycler as follows:

1) 94 °C for 2 minutes, 94 °C for 30 seconds and 42 °C for 30 seconds.

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

2) 72 °C for 1 second, 42 °C for 7 seconds and 72 °C for 70 seconds 38 cycles and 72 °C for 10 minutes.

Arbitrary primers:

Five 10-mers oligonucleotides primers as mentioned in following table were obtained from (MWG-Biotech AG) and were used as pooling primers for RAPD amplification. **Williams et al., 1990.**

Table A: List of primers

Primers sequence (5\-3\)
5\AAG AGC CCG T 3\
5\AAC GCG CAA C 3\
5\ GCG ATC CCC A 3\
5\ GTG GAT GCG A 3\
5\AAA CGG TTG GGT GAG 3\

RESULTS & DISCUSSION

The data concerned with gastrointestinal bacterial infection in lovebirds, are very limited. The present study was concerned with some bacterial pathogens affecting budgerijars and cockatiels, their incidence, distribution, the important pathogens and its susceptibility to different antibiotic.

The prevalence of bacterial isolates from apparently healthy birds was (34/230; 14.8%), While its prevalence in diseased birds was (21/28; 75%).

The bacterial isolates were *identified as E.coli, salmonella*, *Klebsiella*, *Proteus* and *Enterobacter* was (51.28%), (10.26%), (17.95%), (17.95%) and (2.56%) respectively. Our results varied in accordance with previous studies **Darrel et al., 1991, Graham and Graham, 1978** and **Salehi and Ghanbarpour, 2010.**

Table (1): Antibiotic sensitivity test of isolated *E.coli* to different antibiotics.

Antimicrobial agent	Disc potency	Sensitive		Intermediate		resistant	
	µg	No	%	No	%	No.	%
Tetracycline	30	2	10	0	0	18	90
Ampicillin	10	0	0	1	0	19	95
Kanamycin	30	1	5	0	0	19	95
SXT	25	0	0	1	5	19	95
Ciprofloxacin	5	5	25	1	5	14	70
Cefotaxime	30	1	5	0	0	19	95
Erythromycin	15	0	0	0	0	20	100
Rifamycin	5	2	10	1	5	17	85
Gentamycin	10	4	20	8	40	8	40
Chloramphenicol	30	2	10	0	0	18	90
Lincomycin	2	0	5	1	5	18	90
Amoxicillin	10	2	10	1	5	17	85
Streptomycin	10	1	5	4	20	15	75
Doxycycline	30	1	5	2	10	17	85

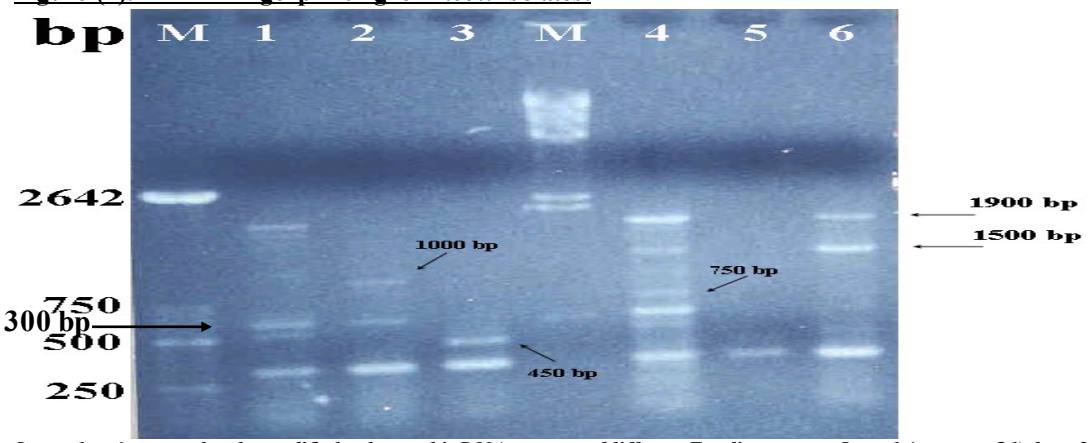
Table (2): Relationship between E. coli serotypes and types of toxins produced.

Serotype	No.	Source	ST	LT	ST+LT	VT
O1 : K1	2	Diseased	-ve	-ve	-ve	+ve
O2 : K1	3	Diseased	-ve	-ve	-ve	+ve
O26 : K1	1	Diseased	-ve	+ve	-ve	-ve
untypable	14	Diseased and apparent healthy	-ve	-ve	-ve	+ve
			-ve	+ve	-ve	-ve
			-ve	-ve	-ve	-ve

ST = heat stable Lt = heat labile VT= verotoxin

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

Figure (1): RAPD Fingerprinting of *E.coli* isolates.



Lanes 1 to 6 are randomly amplified polymorphic DNA patterns of different E.coli serotypes. Lane 1 (serotype O1), lane 2 (serotype O2), lane 3 (untypable), M = molecular size DNA ladder, lane 4 (serotype O26), lanes 5 and 6 (untypable). The polymorphic fragments (marked with arrows) of approximate molecular sizes of 1900 bp, 1500 bp, 1000 bp, 750 bp, and 450 bp are characteristic for each fingerprint.

E.coli serovars obtained from diseased samples (15 samples) were O_1 (2; 13.3%), O_2 (3; 20%), O_{26} (1; 6.7%) and untypable (9; 60%) respectively The *E.coli* serovars obtained from healthy birds (5 samples) were unypable, table (4) in parallel with **Char and Rao, 1991**. Table (3) revealed that all isolated *E.coli* serovars were not heat stable toxin (st) producers.* the ratio less than 0.070 = negative, * the ratio in the range of 0.070 – 0.090 = questionably positive, * the ratio above 0.090 = strong positive. Table (2) explained the relationship between *Escherichia coli* serotypes and their toxin production showing that (3) isolates were verotoxin (VT) producers, (2) strains were heat labile toxin (LT) producers; none of strains were heat stable toxin (st) producers, the results agreed with which found by **De Rycke et al., 1987, Donta et al., 1974 and Dean et al., 1972**

As shown in table (5) a total of 34 reproducible DNA fragments which were produced by the five primers. The length of polymorphic bands ranged from 2101-165 bp. Although many fragments appeared common to several strains, the patterns were qualitatively sufficient for accurate strain differentiation. The amplification resulted in characteristic bands of approximately 113, 750 and 175 bp in *E.coli* isolated of serogroup O_2, O_{26} and one untypable *E.coli* strain. In general, the RAPD patterns from non-pathogenic *E.coli* were less complex, often producing single low MW DNA bands with RAPD primers. Serotype O1is characterized by a fragment of 300pb approximate size. Serotype O2 is characterized by a fragment of 1000 bp approximate size and the untypable serotype in lane 3 is characterized by a fragment of 450 bp approximate size. Serotype O26 showed a characteristic polymorphic band of 750 bp and the untypable serotype in lane 5 is characterized by the absence of the 450 bp amplicon. Fragments of 1500 bp and 1900 bp approximate size characterize the untypable serotype in lane 6.

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

Table (3): Detection of heat stable enterotoxin produced by *E.coli*

Serotypes of *E.coli* isolates	Mean ratio of fluid accumulation	Interpretation
O_1	0.056	ST –ve
O_2	0.061	ST –ve
O_{26}	0.064	ST –ve
Untypable	0.062	ST –ve

ST = heat stable

Table (4): Serological identification of different *E.coli* serovars obtained from collected samples.

Samples	No. of +ve	Serological identification		
		O : K1	No.	%
Diseased	15	O_1: K1 O_2: K1 O_{26}: K1 untypable	2 3 1 9	13.3 20 6.7 60
Healthy	5	untypable	5	100 %
Total	20			

Table (5): RAPD profile of *E.coli* serotypes

No	O1: K1	O2: K1	u	O26: K60	U	U
1	-	-		2010*	-	2101
2	1966	-		1966	-	1966
3	-	-		1900	-	1900
4	-	-		1500	-	1500
5	-	1000				
6	-	113		-	-	-
7	-	-		750	-	-
8	602	602		602	-	602
9	537	-		-	-	-
10	519	519		519	-	519
11	500	-		-	-	-
12			450			
13	-	-		440	440	440
14	320	320		-	-	-
15	250	320		250	-	-
16	-	-		-	-	175
17	165	-		165	-	-

**** Molecular weight of bands; u = Untypable***

Concerning to antibiotic susceptibility of *E.coli* to different antibiotics the results revealed that Ciprofloxacin and gentamycin were the most effective drugs against the isolated *E.coli*, table (1).Our results agreed with **Enas, 2008 and Roy et al., 2006.**

CONCLUSION

1. An attention should be directed toward the untypable serotypes of *E.coli* where some of them may be of toxin producer causing a public health problems.
2. Use of Antibiotics in these birds should be controlled and kept under veterinary supervision and investigation to avoid the flourish up of new serotypes resistant to antibiotics resulting in epidemiological problems.
3. Regular veterinary clinical examination supported by sensitivity test is indicated for providing the drug of choice for proper treatment.

REFERENCE:

Baker, J.R. (1996): Causes of mortality and morbidity in exhibition budgerigars in the United Kingdom. Vet. Rec. Aug. 17,139: 156-162.

Char, N.L. and Rao, M. R. (1991): Zoonotically significant serotypes of *E.coli* isolated from pathologically condition of animal and poultry. Indian Vet. J. 68(11): 1003-1006.

Darrel, K.; **Styles and Flammer K. (1991):** Congo red binding of *Escherichia coli* isolated from the cloaca of psittacine birds. Avian diseases 35:46-48.

De Rycke, J.; Guillot, J.F. and Bolvin, R. (1987): Cytotoxins in non-enterotoxogenic strains of *Escherichia*

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

coli isolated from feces of diarrheic calves. Vet. Microbiol., 15:137-150.

Dean, A.G.; Ching, Y.p; Willams, R.G.; and Harden, L.B. (1972): Test for *Escherichia coli* enterotoxin using infant mice application in a study of diarrhea children in Honolulu. J. Infec. Dis., 125:407-411.

Donta, S. T.; Moon, H.W. and Whipp, S.C. (1974): Detection of heat-labile *Escherichia coli* entero-toxins with the use of adrenal cells in tissue cultures. Science. 183:334-336.

Emery, D.A.; Nagaraja, K.V.; Shaw, D.P.; Newman, J.A. and White, D.G. (1992): Virulence factors of *Escherichia coli* associated with colisepticemia in chickens and turkeys. Avian, Dis., 36, 504-511.

Enas, M.S.A. (2008): Studies upon some bacterial isolates affecting budgerijars M.V.Sc., (Wild life and Zoo Medicine). Fac. Vet. Med., Suez Canal University.

Finegold, S.M. and Martin, W.J. (1982): Diagnostic Microbiology, 6 Th Ed., C.V. Mosby Co. St. Louis, Toronto, London.

Flammer, K. and Drewes, L. A. (1988): Species related differences in the incidence of gram-negative bacteria isolated from the cloaca of clinically normal psittacine birds. Avian Dis., 32: 79-83.

Forshaw, J. (1973) . Landsdowne Editions, Australia, 1973. William T Cooper (illustrator). 1st Edition.

Giannella, R. A. (1976): Suckling mouse model for detection of heat-stable *Escherichia coli* enterotoxin: Characteristics of the model. Infect. Immune. 14(1):95-99.

Graham, C.L. and Graham, D.L. (1978): Occurrence of *E.coli* in feces of psittacine birds. Avian Dis. 22: 717-720.

Krieg, N.R. and Holt, J.G. (1984): Bergey's Mannal of systematicology. Vol. 1, William and Baltmore, London.

Minsky L, Petrak ML, 1982. Diseases of the digestive system. In: Diseases of cage and aviary birds [ed. by Petrak, M. L.]. Philadelphia, Pennsylvania, USA: Lea & Febiger, 432-448.

Roy, P., Purushothaman, V.; Kotees-waran, A. and Dhillon, A.S. (2006): isolation Characterization and Antimicrobial Drug Resistance Pattern of *Escherichia coli* isolated from Japanese quail and their Environment. J. Appl. Poultres., 15:442-446.

Salehi, M. and Ghanbarpour, R. (2010): Phenotypic and genotypic properties of *Escherichia coli* isolated from colisepticemic cases of Japanese quail. Tropical Animal Health and Production J. Vol. 42, No. 7:1497-1504.

Sojka, W. J. 1965. Escherichia coli in domestic animals and poultry. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalaski, J.A. and Tingey, S.V.(1990): "DNA polymor-phism amiplified by arbitrary primers are useful as useful as genetic markers". Nucleic Acid Res., 18:6531-6535.

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg