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ISOLATION,IDENTIFICATIONAND MOLECULARCHARACTERIZATION OF PSEUDOMONASAERUGINOSAISOLATED FROM INTERNALORGANS OF WILD BIRDS IN EGYPT

By

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ABSTRACT

Pseudomonas infection of birds is of great importance because epidemics may spread rapidly through poultry flocks causing mortality in all ages. The most predominant *pseudomonas* species causing mortality among birds specially chickens was *pseudomonas aeruginosa.* The organisms are ubiquitous, often associated with soil, water and humid environments. A total number of 304 different organ samples (liver, kidney, heart and lung) from 76 examined resident and migratory wild birds were examined bacteriologically for isolation of *pseudomonas aeruginosa*. Our results revealed that 25% from bird samples were positive. Culture character as well as identical biochemical identified the organism. Pure isolates were confirmed by PCR using (*tox* A gene).

Key words:

Pseudomonas aeruginosa, Wild birds, poultry, PCR, tox a gene.

INTRODUCTION

Smith *et al.*, (1978); concluded that, the role of birds as vectors of disease transmission to domestic livestock has been attributed to environmental contamination of water supplies, pastureland and feed by avian feces. Various species of free living birds, because of their propensity to nest and roost near human activity, may harbor and disseminate various species of bacterial microorganisms to domestic birds and animals As *E. coli, Pseudomonas aeruginosa, Klebsiella* spp*., Salmonella* spp*., Pasteurella* spp*., S. aureus and Proteus spp*. were recovered from hoopoe, ibis, sparrow, doves and quails with variable rates **(El-Sheshtway and Moursi, 2005; Soad and Wafaa, 2003).** *Pseudomonas aeruginosa* is a common avian pathogen, causing disease principally as a secondary invader. Localized infections with *Pseudomonas aeruginosa* occur in the upper respiratory tract causing rhinitis,

sinusitis and laryngitis **(Gerlach, 1994).** Infections are also associated with septicemia and haemorrhagic enteritis in psittacines, corneal ulcers in captive cranes **(Miller *et al*., 1995)** and mass mortality in free-living flamingos **(Kock and Kock, 1995)**. This present study was aimed to investigate the presence of *P. aeruginosa* in free living and the risk obtained by transmitting this bacterium to domesticated birds.

MATERIAL AND METHODS

Birds: 76 apparently healthy resident and migratory wild birds (10 Hooded Crow, 12 Cattle Egret, 13 Rock Pigeon 11 Laughing Dove, 10 shoveler duck, 10 Cotte duck and 10 Green Winged Teal duck) were collected by hunting from different localities in Egypt.

Sampling:

The specimens (heart blood, lung, liver, kidney and intestine) were taken under aseptic conditions for bacterial isolation.

Isolation and identification of *Pseudomonas aeruginosa*:

The samples collected from organs were taken aseptically from apparently healthy free-living birds and inoculated in nutrient broth and incubated at 37 °C for 24 hours, then subcultured onto selective medium (MacConkey agar and Pseudomonas agar base medium with C-N supplement) and incubated at 37 °C for 24 hours to observe the non-lactose fermenting colonies. Also sub cultured onto nutrient agar plate to observe the pigmentation. On bacteriological examination of pure cultures, they identified as Pseudomonas aeruginosa according to **(Cowan, 1974)** and showed the same biochemical characteristics described by **Cruickshank *et al.* (1975).** *Pseudomonas aeruginosa* was Gm-ve, rods, highly motile, hemolytic, and produced a bluish green pigment on agar plates. It was unable to grow at 4 °C, but grew very well at 42 °C. It shows sugar fermentation of Glucose, mannose and Xylose, while sucrose, lactose and maltose weren't. It gives negative results for Indole, MR, VP, starch hydrolysis. Also, shown positive result for citrate utilization and gelatin hydrolysis.

Genomic DNA Extraction:

A rapid boiling procedure was used to prepare template DNA from bacterial strains according to **(Reischl, 1994).** Two to 5 loops of bacterial isolates taken from the nutrient agar plate were collected and suspended in 200 µl of RNA-DNA free water. After boiling for 10 min, the suspension was centrifuged for 2 min. to sediment bacterial debris. The supernatant was aspirated and from which 5 µl was used directly for PCR amplification.

PCR for detection of *Pseudomonas aeruginosa.*

a. Primers used for *pseudomonas aeruginosa*.

Primers of Exotoxin A gene (toxa A) of *pseudomonas aeruginosa* were selected from the published paper **(Al- Daraghi and Abdullah 2013).**

Table (1): Primer sequences for specific Exotoxin A gene (*toxA*) of *Pseudomonas aeruginosa*.

primer name (Target gene) toxA	Oligonucleotide sequence (5-3`)	Amplicon size (bp)
ETA1	F:GACAACGCCCTCAGCATCACCA	398 bp
ETA2	R:CGCTGGCCCATTCGCTCCAGCGCT	

b. Amplification of Exotoxin A (toxA) in P. aeruginosa isolates.

The PCR amplification was carried out in thermal Cycler (Ptc-100 Peltier thermal cycler MJ Research) in 25 µl reaction volume; 3 µl of DNA as template, 5 µl of 5X of PCR master mix and 1 µl of 10 picomole of each primer and complete water to 25 µl**.** gene was amplified using sets of specific primers as described in (Table1).

c. PCR cycling program was performed in the thermal cycler as follow: initial denaturation at 95 ℃ for 2 min, then 30 PCR cycles were run under the following conditions: 94 ^{0}C for 1 min, 68 ^{0}C for 1 min, and 72 ℃ for 1 min. Final extension were done at 72 ℃ for 7min. A negative control PCR mixture with no template DNA was included.

RESULT

Table (2): prevalence of *Pseudomonas aeruginosa* isolated from different wild bird species.

Bacteria	Wild Birds							
Bacteria	Resident birds	Number examined	Positive	Prevalence %	Migratory birds	Number examined	Positive	Prevalence %
Pseudomonas aeruginosa	Hooded Crow	10	1	10	Shovler duck.	10	6	60
	Cattle Egret	12	0	0	Cotte duck	10	2	20
	Rock Pigeon	13	3	23.1	Green Winged Teal duck	10	3	30
	Laughing Dove	11	4	36.4				
Total isolates		46	8	17.4		30	11	36.6

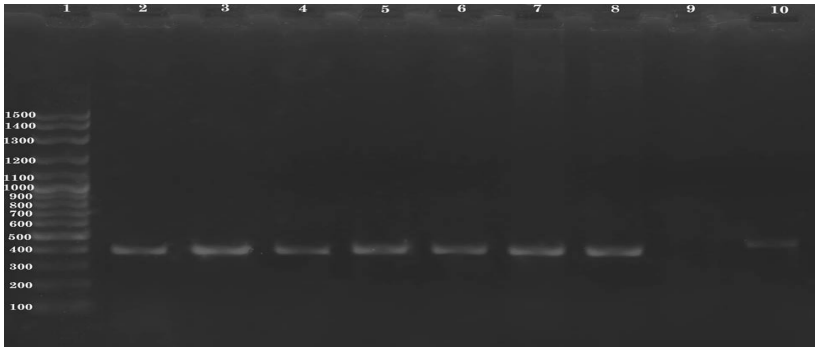

Fig. (1): PCR products of the examined pseudomonas aeruginosa isolates from different wild bird species. Lanes 1, 2, 3, 4, 5 and 6 shows specific bands for Exotoxin A gene (toxa A) gene at 398 bp

DISCUSSION

Today many different species of wild birds are worldwide in distribution. Little is known about the incidence of these enteropathogens in wild birds near poultry facilities or their transmission to poultry **(Craven *et al.,* 2000). Walker *et al.,* (2002);** reported that, *Pseudomonas Aeruginosa* is a common avian pathogen which principally affects the upper respiratory tract, causing rhinitis, sinusitis and laryngitis. Infections are also associated with septicemia and haemorrhagic enteritis in psittacines, corneal ulcers in captive cranes and mass mortality in free-living flamingos. *P. aeuroginosa* is considered to be an environmental infection and it is found in soil, water, feed and farm equipments. It is however difficult to clear the farm from the organism since it has high resistant to various antibiotics and may be resistant to conventional disinfectants. We isolated *P. aeruginosa* from different wild bird species by prevalence rate 25% similar to (**Brittingham *et al.,* 1988)** who isolated *Pseudomonas* spp. from 22% of the tested wild birds and agree with **(Teresa *et al.,* 1999)** who isolated *E.coli, Pseudomonas, Enterobacter and Staphylococcus aureus* from wild birds. Our results higher than **Maha E. Awad-Alla *et al*, (2009)** who isolated *pseudomonas* from internal organs of 25 hunted Crows by rate (12%). The highest isolation was detected from heart blood, lung, and liver and **(Bowman and Jacobson 1980)** who isolated it from 5% of the psittacines they tested. **Satish and Priti, (2015)** reported that, the organism was lethal with 100% mortality when injected intramuscularly into 7 days old chicks (10 out of 10 died). Also Outbreaks of *Pseudomonas aeruginosa* infection with a mortality rate that may reach 90% among chicks have been reported **(Narula and Kuppuswamy, 1969)**. Symptomless 'carrier' birds may contaminate shared food and water bowls.

CONCLUSION

From the present results we can concluded that, the *P. aeruginosa* are found by high rate in wild birds and can transmitting to poultry farms causing high economic loses. It is however difficult to clear the farm from the organism since it has high resistant to various antibiotics and may be resistant to conventional disinfectants. Hence, prevention of *Pseudomonas* invasion is an indispensable duty to any farm. The farm management should take stringent measures against all the possible sources of infection.

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