رقم البحث (16)

INCIDENCE OF BACTERIA AND FUNGAL AGENTS IN EMIGRATED BIRDS AT NORTH SINAI

BY

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ABSTRACT

In the present study a total of 36 isolates were isolated out of 228 samples taken from hearts blood, tracheal exudates, lungs, air sacs, livers, and joints of 38 migrating birds belonging to five species including (Calandrella cinerea, Muscicapa striata, Calidris minuta, Buteo buteo and Alaemon alaudipes) by incidence rate (14.5%). The type of isolates were 33 bacterial isolates, 12 E. Coli (5.26%) and serotyped into 3o1,3o111,4o119and 2o127, 7 (3.07%) Proteus mirabilis, 8 (3.51%) Pseudomonas aeruginosa, 3 (1.31%) Salmonella spp. and 3(1.31%) Staphylococcus aureus and 3 mycotic isolates (2 isolates Aspergillus fumigatus and one isolate Penicillium spp.

INTRODUCTION

The phenomenon of bird migration with its large scale dimension has attracted the attention of naturalistis for centuries. World wide billion of birds leave their breeding grounds every autumn to migrate to areas with seasonally more favorable conditions. Many of these migrants travel only over a few hundred kilometers but others cover distances equivalent to circumference of earth. Among these long distance migrants are several billion birds that invade Africa every autumn from their west and central palaearctic breeding areas **(E.Gwinner 1990).** The potential for transport and dissemination of certain pathogenic microorganisms by migratory birds is of concern. Migratory birds might be involved in dispersal of microorganisms as their biological carriers, mechanical carriers, or as carriers of infected hemato-phagous ecto parasites**(e.g., ixodid ticks) (Hubalek 2004).** Many species of microorganisms pathogenic to homoeothermic vertebrates including human have been associated with free-living migratory birds. Migratory birds of divers species can play significant roles in the ecology and circulation of some arboviruses (e.g., eastern and

western equi encephalomyelitis and sindbis alphaviruses, West Nile and St. louis encephalitis flaviviruses), influenza virus, Newcastle disease virus, duck plague herpes chlamydophila psittaci, Anaplasma phagocytophila, Borrelia burgdorferi sensu lato, Campylobacter jejuni, Salmonella enterica, Pasteurella multocida, Mycobacterium avium, candida spp. and avian heamatozoan. the efficiency of dispersal of pathogenic microorganisms depends on a wide variety of biotic and a biotic factors affecting the survival of the agent in, or disappearance from a habitat or ecosystem in a new geographic area **(Hubalek 2004) .**

The present study was under taken in order to evaluate the presence of bacteria and fungus in migrating birds in North Sinai Governorate and bacterial sensitivity for different antibiotic .

MATERIAL AND METHODS

Material :- The examined birds were collect from Elarish , Beer Elabed , Rafah and Elshekh Zewaied area at North Sinai governorate

Samples: - 38 birds of different species *(*Calandrella cinerea, Muscicapa striata, Calidris minuta ,Buteo buteo and Aleamon alaudipes *)* were ,examined and looked like apparently healthy. Samples from heart blood, trachea, lung, air sac, liver and joint were collected from each bird and were submitted for bacteriological and serological examination at bacteriological unit of poultry diseases and researches department at Animal Health Research Institute at Dokki.

Bacteriological examination:-

For salmonella isolation all organs samples were transferred into teterathionat broth and seleint F broth, then incubated at 35 -37̊C for 16-18 h according to *Quinn et al*. (1994). Aloopful from the enriched broth was streaked onto selective media XLD and S.S agar plat. The plates were incubated at 37̊C for 24-48h.The suspected colonies were picked onto nutrient agar and incubated at 37˚C for 24h for purification and Biochemical and serological identification according to *Edwards and Ewing* (1972) and *Quinn et al*.(2002).

For E coli isolation the collected samples were cultured on MacConkey broth and sub cultured on MacConkey agar media and E.M.B agar ;both incubated aerobically at 37℃ for 24h. From the suspected colonies film were stained with gram's stain and examined microscopically. Each suspected colony was picked up,purefied and the pure isolates were stabbed onto slant nutrient agar tube for biochemical and serological identification according to *Quinn et al*. (2002).

For proteus and pseudomonas spp. all organs samples were cultivated in nutrient broth and subcultured on nutrient and MacConkey agar plates.The seeded plates were incubated aerobically at 37℃ for 24h. The suspected colony was subjected to morphological examination by gram's stain and microscopical examination. The suspected colonies were stabbed onto nutrient agar tube for biochemical identification according to *Quinn et al*. (2002).

For isolation of Staphylococcus. spp. all organs samples were cultivated in nutrient broth and subcultured on blood agar and manitol salt agar then incubated at 37℃ for 24h. The suspected colony was subjected to morphological examination by gram's stain and microscopical examination. . The suspected colonies were inoculated onto slope nutrient agar tube for preservation and biochemical and serological identification according to *Quinn et al*. (2002).

Antimicrobial susceptibility tests : According to *Cruickshank et, al*; (1975) .Muller-Hinton agar (Oxoid, Basingstoke, UK) was prepared in a uniform thickness (4 mm). The following anti-microbial agents (Oxoid co.), which represent the commonly used antibiotics, were tested: Ampicillin (10µg), Cefotaxim (30µg), Ciprofloxacin (5µg), Enrofloxacin (15µg), Gentamycine (10µg), Oxytetracycline (30µg), Cholormphenicol (30µg), Erythromycin (15µg), Streptomycin (10µg), Trimethoprim (30µg) and Nalidixic acid (30µg).

For mould isolation samples were first cultivated on sabouraud dextrose agar (treated with penicillin and streptomycin) then incubated at 25℃ for 72h -7days and examined daily. The colonies were identified macroscopically and microscopically according to *Laron* (1987) and *Koneman et. Al*., (1992).

RESULTS

Table (1): Bacterial species and rate of isolates from migrating birds according to the site of isolation.

Isolates	Site Of Isolation							
	heart blood	Tracheal exudat	lung	air sac	liver	joint	total	%
E .coli.	2	3	2	2	3	- VE	12/228	5.26
Proteus mirabilis.	- VE	2	1	1	3	- VE	7/228	3.07
Pseudomonas aeruginosa	-VE	1	3	3	1	- VE	8/228	3.51
Salmonella spp.	- VE	- VE	1	- VE	2	- VE	3/228	1.31
Staphylococcus aureus	- VE	- VE	- VE	- VE	- VE	3	3/228	1.31
TOTAL	**2/38**	**6/38**	**7/38**	**6/38**	**9/38**	**3/38**	**33/228**	**14.47**
%	**5.3**	**15.7**	**18.4**	**15.7**	**23.6**	**7.8**		

Table (2): Bacterial and Fungal species isolated from migrating birds according to the species of birds

Species	Isolates						
	Number of birds	E. coli	Sal. Spp.	Proteus mirabilis	Ps. aeruginosa	Staph. aureus	Fungus
Calandrella cinerea	12	3	-ve	3	2	2	2
Muscicapa striata	14	4	2	- ve	3	- ve	- ve
Calidris minuta	5	2	1	- ve	- ve	1	- ve
Butea buteo	3	1	- ve	2	- ve	- ve	- ve
Alaemon alaudipes	4	2	- ve	2	3	- ve	1
Total	**38**	**12**	**3**	**7**	**8**	**3**	**3**
%		**31.57**	**7.89**	**18.4**	**21.0**	**7.8**	**7.9**

Table (3) : Serotyping of *E. coli* isolated from migrating birds

Source of isolates	Total no. of samples	Number of isolates	% No. of isolates	*E.coli* serogroups			
				O1	O111	O119	O127
Calandrella cinerea	72	3	4.2	1	1	_	1
Muscicapa striata	84	4	4.8	_	2	1	1
Calidris minuta	30	2	6.7	1	_	1	_
Buteo buteo	18	1	5. 5	_	_	1	_
Alaemon alaudipes	24	2	8.4	1	_	1	_
Total	**228**	**12**	**5.3**	**3**	**3**	**4**	**2**

Table (4): Fungal species isolated according to the site of isolation

Site of isolation	Number examined	Number positive	Fungal Isolates		%
			Aspergillus fumigates	Penicillium spp.	
Lung	38	2	1	1	5.2%
Air sac	38	1	1	_	2.6%
Total	76	3	2	1	3.9%

DISCUSSION

Domestic and wild birds and animals have been always considered very important reservoir of agents of human infections, particularly birds, because of their great mobility from a continent to another or within the limits of some ecosystem may transfer pathogenic microorganisms (*Levre et al*;(1989).

Birds have been thought to play a role in transmitting infectious agent like Influenza , Borrelia and Salmonella (*Palmgren et al*., 1997). So, the present survey was aimed to study the prevalence of bacteria in migrating bird and to investigate the role of migrating birds in the dispersal of enteropathogenic bacteria in North Sinai Governorate .

In the present study a total of 36 isolates were isolated out of 228 samples taken from heart blood, tracheal exudates, lung , air sac , liver and joint of 38 migrating birds belong to 5 species including (*Calandrella cinerea*, *Muscicapa striata*, *Calidris minuta*, *Buteo buteo*, and *Alaemon alaudipes*) by incidence rate (14. 5 %). The bacterial isolates were 12 E. coli (5. 26%) ,7 (3.07%) *proteus mirabilas*, 8 (3.47%) *pseudomonas aeruginosa*, 3 (1.31 %) Salmonella spp. and 3 (1.31%) *Staphylococcus aureus* (Table, 1), and 3 mycotic isolates (2 isolates *Aspergillus fumigatus* and one isolate *Penicillium spp*. By incidence rate (3.9%) as shown in (Table 4).

In this work *E. coli* was isolated in an incidence of (5.31%), 12 isolates from all species (4 isolates from *Muscicapa striata* species, 3 from *Calandrella cinerea*, 2 isolates from *Calidris minuta* and *Alaemon alaudipes* species and one isolate from *Buteo buteo* species). The isolates were divided into 4 serogroups and the most frequently serogroups were O119 ,O111 ,O1 and O127 (Table 2, 3). Previously (*Hussong et. al*., 1979) carried out quantitative and qualitative analyses of the intestinal flora of migrating Canada gees and whistling swans, They found that migratory bird harbor significantly more fecal coliform than streptococci and they reported that from 44 migratory water fowl Enteropathogenic *Escherichia coli* were detected in seven birds. In the same time our results is higher than that reported by *Brittingham et al*., (1988) who isolated *E. coli* from cloacal swabs of migratory birds by incidence rate (1%), While the given results is lower than that reported by (*Aruji et al*., 2004) whereas they isolated *E. coli* by the highest rate of isolation (21%) and they reported that the most frequently identified serogroups were O8 ,O114 and O144. On the other hand our results is agree with the results of some

others such as *Petermanns et al* ., 1989 ; *Aquirre et al*., 1992 ; *Tsubokura et al*., 1995 ; *Middleton* and *Ambrose* 2005, who reported that *E. coli* was the commonest bacterium isolated from organs, intestinal tract and cloaca of migratory birds and migratory water fowls.

In the present study *proteus mirabilis* was isolated by incidence rate 7(3%), 3 isolates from *Calandrella cinerea* species and 4 isolates (2 isolates from *Buteo buteo* species and 2 isolates from *Alaemon alaudipes* species). The highest site of isolation from the birds is the liver (3 isolates) followed by tracheal exudates (2 isolates) and after that the lung and air sac (1 isolates), this results is higher to some extent than that reported by *Zenoble et al* .,(1983) who isolated proteus spp.from migratory psittacin birds by incidence rate 1%. While *Bangert et al*.,(1988) isolated newly described species of Proteus (*Proteus penneri*) from the feaces of migratory wild birds. Also in Japan *Aruji et al* ., (2004) isolated *Proteus mirabilis* from migratory birds captured from July to December 2002 at Uenozoo, Tokyo .

The results presented in Table (1)declared that the incidence rate of isolation of *pseudomonas aruginosa*, Salmonella spp. and *staphylococcus aureus* are 8 (3.5%), 3 (1.31%) and 3 (1.31%) respectively from all samples . Higher isolation rate were from lung and air sac in case of *pseudomonas* , while in case of Salmonella spp., its isolated from liver (2 isolates) and lung (1 isolates) but *Staphylococcus aureus* isolated only from joint (3 isolates). This results show partial similarty and sometimes disagree with several authors such as *Szeness et al*., (1979) they isolated *salmonella spp., Staphylococcus aureus* and *Pseudomonus aeruginosa* from intestinal content and bile of migratory birds (stock ducks). *Zenoble et al* ., (1983) they isolated *Saphylococcus epidermis* (25%), *Staphylococcus aureus (*5%) and (1%) *pseudomonas spp*. from eye of import psittacin birds. *Kirkpatrick* and *Trexler* (1986) were isolated *Salmonella entertidis* and *Salmonella Newport* from migrating Falconiform birds (*Buteo jamaicensis*) by incidance rate (1.9%). *Brittingham et al*., (1988) determined that the prevalence of bacteria isolated from cloacal swabs of wild and migrating birds were as follows pseudomonas spp.(22%), salmonella spp. (0%) staphylococcus spp. (15%). *Levre et al*., (1989) isolated 8 strains belonging to the genus salmonella (3.68%) from the intestine of migrating birds, the isolates were identified as *Salmonella typhimiurium* (7strains) and *Salmonella blokley* (1 strains). *Petermann et al*., (1989) found salmonella in the organs of (5%) and in the intestinal tract of (3%) of the migrating birds, the

species of salmonella most frequently isolated was *Salmonella typhimiurium* varieties Copenhagen, also they recorded both *Pseudomonas spp.* and *Staphylococcus spp. Hubalek et al*., (1998) isolated *Pseudomonas stutzeri* from faecal sample of migrating birds by isolation rate (4.5%) they also recovered *pseudomonas spp. Refsum et al*.,(2002) isolated *Salmonella enterica* serovar Typhimurium from migratory birds and migratory water fowl by incidence rate (54%). *Aruj et al*., (2004) identified *Pseudomonas maltophila* and *staphylococcus spp.* from migratory birds captured from Tokyo, Japan. *Shawkey et al*., (2005) isolated species of the poorly defined genus pseudomonas from the feathers of the migrating birds, they suggested that birds may have acquired many of these bacteria from the environment. *Lillehaug et al*., (2005) isolated *Salmonella diarizona* 14: k:253 from feacal samples collected from gray leg gees migrating northwards, they demonstrated that the wild birds species may constitute a reservoir for important bird pathogens and zoonotic disease agent in Norway. *Kobayashi et al*., (2007) isolated 19 strains of *Salmonella typhimiurium* from 328 cloacal swabs of wild and migrating birds.

As shown in table (4) *Aspergillus fumigates* and *Penicillium spp.* were only the identified mycotic species during this study and were isolated from lung by incidence rate (5.2%) for both, but *Aspergillus fumigates was* isolated also from air sac by incidence rate (2.6%). These results are similar to those reported by *Buxton & Sommer* (1980). *Bolske & Morner* (1982) who revealed mycotic air sacculitis, bronchitis and pneumonia in a common buzzard (*Buteo buteo*). *Astorga et. al*.,(1994) who isolated *Aspergillus fumigates* (1.1%) from migrated water fowl in southern Spain. Also this results supported by *Cork et. al*., (1999); *Leota et. al*., (2002); *Atasever & Gumussoy* (2004) and *Low et. al*., (2005) .

Tsiodras et. al., (2008) summarized that wild birds and especially migratory species can become long distance vectors for a wide rang of microorganisms. Several wild and migratory birds serve as reservoirs and or mechanical vector (simply carrying a pathogen or dispersing infected arthropod vectors) for numerous infectious agent. Migratory species may play a significant role in the epidemiology of influenza A virus, araboviruses such as West Nile virus and enteric bacterial pathogens .

Our results of antibiogram in vitro showed that *E. coli* isolates were resistant to ampicillin, oxytetracycline, erythromycin, and cholormphenicol and sensitive to cefotaxim, ciprofloxacin, enrofloxacin, and gentamycin. This result supported by *Aruji et al*. (2004) and *Middleton & Ambrose (2005)*.

The Salmonella spp. was sensitive to ampicillin, ciprofloxacin, enrofloxacin, oxytetracycline, cholormphenicol, streptomycin, trimethoprim and nalidixic acid. This result agreed with *Gopee et al*. (2000*); Smith et al. (*2002*) and Kobayashi et al.* (2007*)*. The *Staphylococcus aureus* was highly sensitive to ciprofloxacin, enrofloxacin, and gentamycin and moderate to ampicillin, cefotaxim and trimethoprim and resistance to oxytetracyclin, cholormphenicol, erythromycin and nalidixic acid. This result agreed with *Mulla et al*. (2007). While, *Pseudomonas aeurginosa* was sensitive to ciprofloxacin, enrofloxacin, gentamycin, Cholormphenicol, oxytetracyclin and streptomycin but resistence to ampicillin, Cefotaxim, erythromycin, trimethoprim and nalidixic acid. This result agreed with *Ehab et. al*. (2006) and *Simon et. al*. (2006). *Proteus mirabilis* was sensitive to ciprofloxacin, enrofloxacin, gentamycin, ampicillin, Cefotaxim, erythromycin, and nalidixic acid but resistance to Cholormphenicol, oxytetracyclin, streptomycin and trimethoprim. This result agreed with *Ehab et al.* (2006) and *Simon et. al*. (2006).

The results of this study indicates that a migratory birds are considered potential reservoir or a carrier of bacterial and fungal agents and may therefore play a role in the epidemiology of economically important and zoonotic disease .

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الملخص العربي

مدى تواجد البكتيريا والفطريات في الطيور المهاجرة في محافظة شمال سيناء

منى عبداللاه احمد – احمد فرج عبد النبي- نجوى عبد العزيز شلبي

في هذه الدراسة تم عزل ٣٦ عتره ميكروبات من ٢٢٨ عينة أخذت من القلب وافرازات القصبة الهوائية , رئتين، أكياس هوائية، الكبد، والمفاصل من ٣٨ طيور مهاجرة تنتمي إلى خمسة انواع (كالاندريلا سينريا، موسيكاب اسينيريا سترتيا، كاليدريس مينيويا، بوتيو بوتيووالايمون الايوبيدس بنسبة (١٤,٥ %). وكانت انواع المعزولات هي ٣٣ عتره بكتيرية ، ١٢ (٥,٢٦ %) الميكروب القولوني، ٧ (٣,٠٧ %) بروتيوس ميرابيلس، ٨ (٣,٥١) سيودوموناس ارحينوز، و٣ (١,٣١ %)عترة سالمونيلا و٣ (١,٣١ %) ستافيلوكوكوس اريوس،٣ معزولات من الفطر (٢ أسبيرجيلوس فيوميجاتس وواحد بينيسيليوم . وكانت الانواع السيرولوجية للمكروب القولوني هي : O١١٩، أو O١١١، أو O١ و ١٢٧ O.