CHARACTERIZATION OF *E.COLI* ISOLATED FROM SEAGULL AND WILD BIRDS WITH SPECIAL REFERENCE TO PLASMID PROFILE

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Abstract

A total of 100 fresh faecal samples and faecal swabs were collected from seagulls and wild birds; 46% were positive for *Escherichia coli*.

Serological identification of isolated strains of *E.coli* from seagulls and wild birds showed that they belonged to "7" different "O" serogroups which were O_1, O_{18}, O_{55}, O_{126} from seagulls and O_{26}, O_{55}, O_{114} from wild birds.

The antibiogram of the isolated serogroups of *E.coli* revealed that all tested serogroups of *E.coli* were sensitive to cefaperazone except serogroup (O_1). On the contrary, with ceftazidine and subctam-ampicillin all tested *E.coli* serogroups were resistant. Meanwhile, the examined strains gave variable results with the remaining used chemotherapeutics.

Plasmid analysis of seven *E.coli* isolates demonstrated significant homology between them which ranged from 30-50 Kbp.

INTRODUCTION

Escherichia coli organisms are frequently isolated from humans and animals in a variety of serogroups. Although the predominant serogroups associated with human infection are $O_{157:}$ $H_{7,}$ non-O_{157} also cause human disease *(*Griffin and Tauxe, 1991)

Many studies in the world suggest that wild animal populations can serve as reservoirs for pathogenic bacteria important for domestic animals and humans. Considering the alaming trend towards growing antibiotic resistance among micro-organisms isolated from domestic animals and humans, the problem emerges from contamination of various environments with the resistant bacteria. Antibiotics can be administered for prophylactic purpose in animals and humans, or they can play a role as growth promoters in food animals. Thus, use of antibiotics can contribute to selection of resistant micro-organisms (Schwarz and Chaslus-Danda, 2001). Although wild animals do not come naturally into contact with antibiotics, these animals can be infected with resistant bacteria and serve as reservoirs, vectors and bioindicators of resistant bacterial pathogens and genetic determinants of antibiotic resistance in the environment (Cole *et al.,* 2005).

Birds are potential carriers of *E.coli* because they have a wider ranging living area and have been implicated in the transmission of various pathogenic bacteria such as *Campylobacter* spp, *Salmonella* spp. *and Escherichia coli.*

Antibiotic-resistant *Escherichia coli* can spread to the wild, infecting wild animals and thus creating new host reservoirs of resistant bacteria in the environment. Wild bird- populations can be vectors of *E.coli*, salmonellae and *campylobacter* spp., and faeces from bird flocks contribute to an increasing level of coliform bacteria contamination of drinking water reservoirs. Wild birds can transmit these pathogens to humans directly by contaminating agricultural fields or surface water used for drinking, recreation or crop irrigation (Cole *et al. ,* 2005).

Hence, the goal of this study was directed mainly to throw a light on the presence and prevalence of enteric pathogen of public health importantce as *E.coli* isolated from seagulls and wild birds. Also, complete biochemical and serological identification was done and studying the effect of different chemotherapeutic agents on various serogroups of *E.coli* which had been isolated in the present work as well as applied plasmid profile among different isolated serogroups.

MATERIALS AND METHODS

1. Sampling and cultivation

A total of 100 fresh faecal samples and faecal swabs were collected from seagulls and wild birds at El-Reian natural reserve during the period from January to September 2008. All faecal samples and faecal swabs were inoculated directly into nutrient broth and incubated for 24 hours at 37°C, then, subcultured onto MacConkey's agar. The inoculated plates were incubated at 37°C for 24-48 hours. The isolation, purification and biochemical identification of bacterial isolates were carried out according to Koneman *et al.* (1996).

2. Serological identification

Antisera of *Escherichia coli* were used for serological identification of somatic antigen "O" using slide agglutination test according to Edwards and Ewing (1972).

The *E.coli* immun-O-sera (poly valent sera, 8 vials monovalent sera, 43 vials) were obtained from DENKA SEIKEN Co. LTD Tokyo, Japan.

3. Antibiogram pattern

Antibiogram was applied on the different "O" serogroups of *E.coli* using *in vitro* disc diffusion technique according to Quinn *et al.* (1994) using Mueller Hinton agar plates. Thirteen of chemotherapeutic agents were used to determine sensitivity of different serogroups of *E.coli.*

The results were interpretated according to Oxoid Manual Company (Oxoid Manual, 2000).

Plasmid profile

Preparation and purification of plasmid DNA (miniprep) were performed according to Ausubel *et al.* (1987). The identification of the extracted plasmid DNA using agarose gel-electrophoresis was performed according to Sambrook *et al.* (1989).

RESULTS

1. Bacteriological examination

Out of 100 examined faecal samples and faecal swabs collected from seagulls and wild birds 46% were positive for *Escherichia coli*.

2. Serological identification

Serogrouping of (46) isolates of *E.coli* recovered from seagulls and wild birds revealed (7) different "O" serogroups and "11" strains which were untypable as illustrated in Table 1.

3. Antibiogram pattern

Antibiogram pattern of pathogenic *E.coli* serogroups recovered from seagulls and wild birds were recorded in Table 2 which shows that all tested serogroups of *E.coli* were sensitive to cefoperazone except serogroup (O_1). On the other hand, with ceftazidine and subctam-ampicillin all tested *E.coli* serogroups were resistant, while with the remaining used chemotherapeutics, examined strains gave variable results.

4. Plasmid analysis

Plasmid profile analysis of *E.coli* serogroups (Table 3) revealed that O_1 and O_{18} (seagulls) harboured a plasmid of 30-32 Kbp, while, O_{126} and O_{55} (seagull), O_{26} (wild birds) harboured the same molecular weight plasmid of 39.6 Kbp.

O_{55} (wild bird) had plasmid of 34 Kbp, therefore O_{114} (wild bird) harboured the largest plasmid of 49.6 Kbp.

Table 1. Serological identification of pathogenic strains of *Escherichia coli* isolated from seagulls and wild birds.

E.coli isolated from seagulls			*E.coli isolated from wild birds*		
Serogroup	No.	%	Serogroup	No.	%
O_1	4	8.69	O_{26}	8	17.39
O_{18}	3	6.52	O_{55}	5	10.86
O_{55}	7	15.21	O_{114}	4	8.69
O126	4	8.69	untyped	8	17.39
Untyped	3	6.52	-	-	-
Total	21	45.65	Total	25	54.34

Table 2. Antibiogram pattern of pathogenic serogroups recovered from seagulls and wild birds.

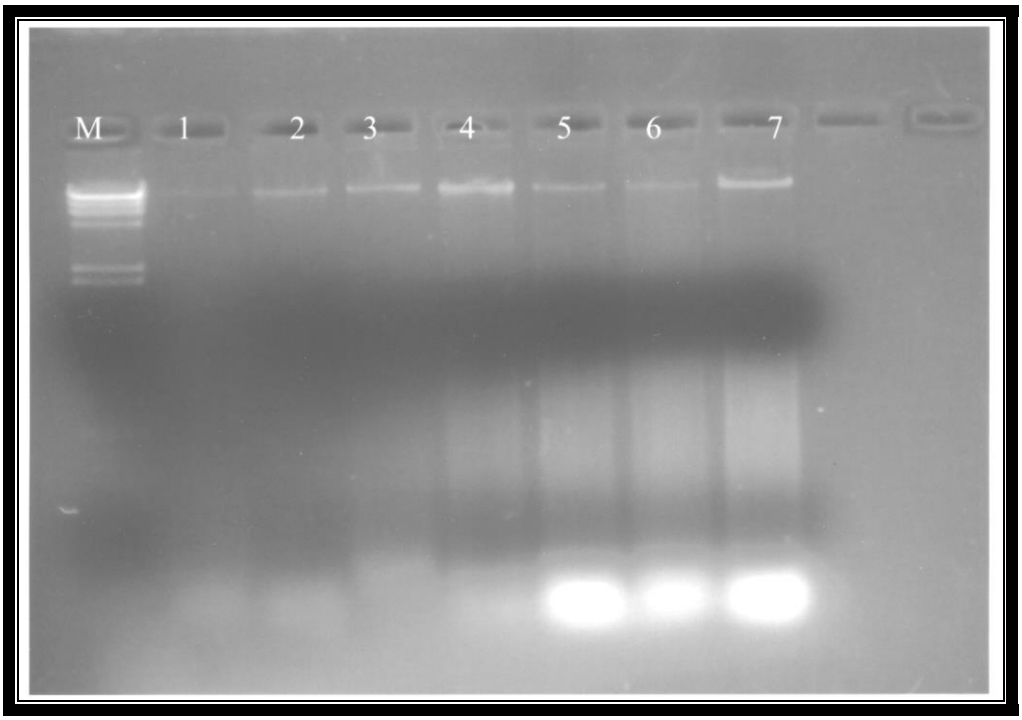
No.		*Escherichia coli* isolated from seagulls				*Escherichia coli* isolated from wild birds		
		O_1	O_{18}	O_{55}	O_{126}	O_{26}	O_{55}	O_{114}
1	Aminosidin	S	S	S	M	M	S	R
2	Amoxicillin clavulanic acid	R	R	S	S	S	S	R
3	Cefoperazone	R	S	S	S	S	S	S
4	Ceftazidime	R	R	R	R	R	R	R
5	Ciprocin (Ciprofloxacin)	R	R	R	S	S	S	S
6	Flucloxacillin	R	R	S	R	R	R	S
7	Gentamicin	R	R	S	S	S	S	M
8	Nalidixic acid	R	M	R	S	R	R	S
9	Ofloxacin	R	R	R	R	S	R	S
10	Pefloxacin	R	R	R	S	S	S	S
11	Subctam-Ampicillin	R	R	R	R	R	R	R
12	Tobramycin	R	R	M	M	R	S	M
13	Cefozon	R	M	S	S	M	R	M

N .B. *S: Sensitive* *M: Intermediate* *R: Resistant*

Table 3. Plasmid profile analysis of *E.coli* serogroups.

No.	*E.coli* serogroup	Source of serogroup	Plasmid MW/Kbp
1	O_1	seagulls	30.288
2	O_{18}	seagulls	31.680
3	O_{126}	seagulls	39.661
4	O_{26}	wild birds	39.661
5	O_{55}	seagulls	39.661
6	O_{55}	wild birds	34.390
7	O_{114}	wild birds	49.652

Photo 1. Plasmid profile analysis of *E.coli* serogroups.



- M: Molecular marker
- 1-7: plasmid lanes of *E.coli* isolated from seagulls and birds.

DISCUSSION

E.coli has been widely isolated from domestic and wild animals, pets and house flies and also from gulls in England and Japan (Gannon *et al. ,* 1990 and Makino *et al.* 2000), who reported that although it is not clear that seagulls-origin Shigha toxin-producing *Escherichia coli* (STEC) are pathogenic for human, the Stx_1 and Stx_2 toxin produced by them would probably be toxic to human, because they had the vero cell cytotoxicity similar to human-origin STEC. These results suggested that seagull-origin STEC might potentially be pathogenic for human.

The concentrations of *Escherichia coli* associated with gull and wild bird faeces suggest that they may be significant source of this indicator bacterial contamination of reservoirs beach sediments and coastal waters (Obiri-Danso and Jones, 2000).

Bacteriological examination of the faecal samples and faecal swabs revealed that 46% were positive for *E.coli*. These results agree to certain extent to those reported by Makino *et al.* (2000), who isolated *E.coli* strain from a seagull in Japan. Also, Welsh *et al.* (1997) isolated *E.coli* from intestinal, cloacal swabs and faecal samples from enteric ratites in an incidence of 35.9%. while, Ali and Ibrahim (2004), isolated *E.coli* 40.38% from enteritis of ostriches.

Serological identification of the isolated strains of *Escherichia coli* recovered from seagulls and wild birds revealed "six" different "O" serogroups, which were as follows: (O_1, O_{18}, O_{55}, O_{126}, O_{26} and O_{114} and "11" strains which were untypable.

These findings are in agreement with those recorded by previous workers as Ali and Ibrahim (2004) who isolated different sergroups O_1, O_8, O_{15}, O_{18}, O_{26}, O_{112}, O_{119}, O_{143}, O_{144}, O_{157}, O_{164}, O_{168}, and O_{169} from ostriches. On the contrary, Makino *et al.* (2000) mentioned that serogroups of *E.coli* isolated from seagulls in Japan were O_{136}: H_{16} and O_{153}: H^-.

Although-gulls and wild birds do not come naturally into contact with antibiotics, these birds can be infected with resistant *E.coli*, and potentially serve as their reservoirs, vectors and bioindicators in the environment.

Table 2 showed that the antibiogrm patterns of the isolated *E.coli* from faecal samples and faecal swabs from seagulls and wild birds revealed variable results against different antibiotic discs which had been used. All tested *E.coli* serogroups were sensitive to cefoperazone except serogroup (O_1) but with ceftazidine and subctam-ampicillin, all test *E.coli* serogroups were resistant, while, with remaining chemotherapeutics, examined strains gave variable results.

The present results coincide with observation reported by Dolejska *et al.* (2007), who mentioned that the most prevalent resistant phenotypes among *E.coli* isolates from young Black-headed Gulls in three locations in the Czech Republic were resistant to tetracycline, followed by ampicillin and streptomycin. The same situation can be found in various European livestock and human populations (Bywater *et al.* 2004).

A high occurrence of resistant *E.coli* isolates obtained from poultry has been documented by Kolar *et al.* (2002) in the Czech Republic, but, unfortunately, data concerning the antibiotic susceptibility of *E.coli* isolates from other animal species are not available in other countries. In addition, Guerra *et al.* (2003) reported that the most prevalent resistance *E.coli* isolates from cattle, swine and poultry were to tetracycline, ampicillin, streptomycin and sulphamethozaxole. Contrary to these results, Galland *et al.* (2001) recorded that *E.coli* O_{157}: H_7 was susceptible to trimathoprim.

The study suggests that the most frequent antibiotic-resistant phenotypes in seagulls and wild birds correlate with the consumption of antibiotic compounds in human and veterinary medicine. The antibiotic with highest consumption in veterinary medicine is tetracycline followed by beta-lactams, sulphonamides, macrolides and aminoglycosides. In human medicine, the most frequently used antibiotics are beta-Lactams followed by tetracycline, sulphonamides and macrolides.The source of

resistant strains among *E.coli* isolates from seagulls and wild birds can be found in poultry and livestock as well as in the human population (Dolejska *et al.* 2007).

Plasmid profiles of seven isolates from seagulls and wild birds were analysed. Possibility associated with natural plasmid ranged from 30 ≥ 50 Kbp. There were high degree of similarity between the molecular weight of plasmid bands among different isolates of seagulls and wild birds. These results considered the highest than observed by Tricia *et al.* (2006), who analysed plasmid from *E.coli* strains isolated from broiler chicken and human. They reported that the plasmid band molecular weight ranged from 2 ≥ 12 Kbp.

CONCLUSION

It can be stressed on the role of seagulls and wild birds in transmission of resistant *E.coli* strains and act as host reservoir for the several pathogenic strain of *E.coli*. Also, the present study throws light on the prevalence of multiple drug resistant *E.coli* serogroups among seagulls and wild birds, a possibility associated with the presence of natural plasmid in the tested isolates.

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تعريف ميكروب الإيشيرشيا كولى المعزول من الطيور البرية وطائر النورس وخاصة دراسة البلازميد بروفيل

عائشة رجب على ، هالة إسماعيل شعراوى ، حنان محمد محمد

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تم تجميع ١٠٠ عينة براز ومسحات شرجية من طائر النورس والطيور البرية وكانت نسبة عزل الميكروب القولونى (٤٦ %).

الفحص السيرولوچى للميكروب القولونى المعزول كان تحت ٧ مجموعات سيرولوچية كالتالى وكان تحمل الأنتيجين البدنى O_{126}, O_{55}, O_{18}, O_1 من طائر النورس والمجموعات السيرولوچية O_{114}, O_{55} من الطيور البرية.

بإجراء إختبار الحساسية للمجموعات السيرولوچية المعزولة أظهرت النتائج أن كل المعزولات حساسة للمضاد الحيوى (سيفايرازون) ماعدا المجموعة السيرولوچية (O_1)– وعلى العكس كل المجموعات كانت مقاومة للمضاد الحيوى سفتازين– والسبكتام– مع الإمبسلين– ومع بقية المضادات الحيوية المستخدمة كانت النتائج مختلفة مع المجموعات السيرولوچية المختلفة.

وسجل تحليل البلازميدات للمجموعات السيرولوچية السبعة المعزولة تماثلا واضحاً بينها يتراوح بين ٣٠–٥٠ Kbp.