CHARACTERIZATION OF \textit{E. coli} ISOLATED FROM SEAGULL AND WILD BIRDS WITH SPECIAL REFERENCE TO PLASMID PROFILE

AISHA R. ALI, HALA I. SCHARAWE AND HANAN M. IBRAHIM

Animal Health Research Institute, ARC, Ministry of Agriculture, Dokki, Giza

(Manuscript received 21 June 2009)

Abstract

A total of 100 fresh faecal samples and faecal swabs were collected from seagulls and wild birds; 46\% were positive for \textit{Escherichia coli}. Serological identification of isolated strains of \textit{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 \textit{E. coli} revealed that all tested serogroups of \textit{E. coli} were sensitive to cefaperazone except serogroup (O$_{1}$). On the contrary, with ceftazidine and subctam-ampicillin all tested \textit{E. coli} serogroups were resistant. Meanwhile, the examined strains gave variable results with the remaining used chemotherapeutics. Plasmid analysis of seven \textit{E. coli} isolates demonstrated significant homology between them which ranged from 30-50 Kbp.

INTRODUCTION

\textit{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 alarming trend towards growing antibiotic resistance among microorganisms 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 \textit{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 importance 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 (O1). 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 O1 and O18 (seagulls) harboured a plasmid of 30-32 Kbp, while, O126 and O55 (seagull), O26 (wild birds) harboured the same molecular weight plasmid of 39.6 Kbp. O55 (wild bird) had plasmid of 34 Kbp, therefore O114 (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.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>No.</th>
<th>%</th>
<th>Serogroup</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>4</td>
<td>8.69</td>
<td>O26</td>
<td>8</td>
<td>17.39</td>
</tr>
<tr>
<td>O18</td>
<td>3</td>
<td>6.52</td>
<td>O55</td>
<td>5</td>
<td>10.86</td>
</tr>
<tr>
<td>O55</td>
<td>7</td>
<td>15.21</td>
<td>O114</td>
<td>4</td>
<td>8.69</td>
</tr>
<tr>
<td>O126</td>
<td>4</td>
<td>8.69</td>
<td>untyped</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Untyped</td>
<td>3</td>
<td>6.52</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>45.65</td>
<td><strong>Total</strong></td>
<td>25</td>
<td>54.34</td>
</tr>
</tbody>
</table>
Table 2. Antibiogram pattern of pathogenic serogroups recovered from seagulls and wild birds.

<table>
<thead>
<tr>
<th>No.</th>
<th>Escherichia coli isolated from seagulls</th>
<th>Escherichia coli isolated from wild birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₁</td>
<td>O₁₈</td>
</tr>
<tr>
<td>1</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>9</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>10</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>11</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>12</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>13</td>
<td>R</td>
<td>M</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>E.coli serogroup</th>
<th>Source of serogroup</th>
<th>Plasmid MW/Kbp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O₁</td>
<td>seagulls</td>
<td>30.288</td>
</tr>
<tr>
<td>2</td>
<td>O₁₈</td>
<td>seagulls</td>
<td>31.680</td>
</tr>
<tr>
<td>3</td>
<td>O₁₂₆</td>
<td>seagulls</td>
<td>39.661</td>
</tr>
<tr>
<td>4</td>
<td>O₂₆</td>
<td>wild birds</td>
<td>39.661</td>
</tr>
<tr>
<td>5</td>
<td>O₅₅</td>
<td>seagulls</td>
<td>39.661</td>
</tr>
<tr>
<td>6</td>
<td>O₅₅</td>
<td>wild birds</td>
<td>34.390</td>
</tr>
<tr>
<td>7</td>
<td>O₁₁₄</td>
<td>wild birds</td>
<td>49.652</td>
</tr>
</tbody>
</table>
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 Shiga toxin-producing *Escherichia coli* (STEC) are pathogenic for human, the Stx₁ and Stx₂ 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: (O₁, O₁₈, O₅₅, O₁₂₆, O₂₆ and O₁₁₄) 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 serogroups O₁, O₈, O₁₅, O₁₈, O₂₆, O₁₁₂, O₁₁₉, O₁₄₃, O₁₄₄, O₁₅₇, O₁₆₄, O₁₆₈, and O₁₆₉ from ostriches. On the contrary, Makino *et al.* (2000) mentioned that serogroups of *E. coli* isolated from seagulls in Japan were O₁₃₆: H₁₆ and O₁₅₃: 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 antibiogram 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 (O₁) 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 sulphamethoxazole. Contrary to these results, Galland *et al.* (2001) recorded that *E. coli* O₁₅₇: H₇ was susceptible to trimethoprim.

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.
REFERENCES


CHARACTERIZATION OF *E. COLI* ISOLATED FROM SEAGULL AND WILDBIRDS

**تعريف ميكروبِ الإشيشيا كولى المعزل من الطيور البرية وطائر النورس وخاصة دراسة البلازميد بروفيل**

عائشة رجب عمى ، هالة إسماعيل شعراوى ، حنان محمد محمد

معهد بحوث صحة الحيوان- مركز البحوث الزراعية - وزارة الزراعة - الدقي - جيزة

تم تجميع 100 عينة برز ومسحات شرطية من طائر النورس والطيور البرية وكانت نسبة عزل الميكروب القولوني (46%).

الفحص السيرولوجي للميكروب القولوني المعزول كان تحت 7 مجموعات سيرولوجية كالتالي: $O_{26}$، $O_{18}$، $O_{55}$، $O_{126}$ من طائر النورس والمجموعات السيرولوجية من الطيور البرية.$O_{55}$، $O_{114}$

بإجراء اختبار الحساسية للمجموعات السيرولوجية المعزولة أظهرت النتائج أن كل المعزولات حساسة للمضادات الحيوية (سيفازابور) ماعدا المجموعة السيرولوجية ($O_{1}$)- وعلى العكس كل المجموعات كانت مقاومة للمضادات الحيوية سفاتازين- والسيكتام- مع الإبسيلين- ومع بقية المضادات الحيوية المستخدمة كانت النتائج مختلفة مع المجموعات السيرولوجية المختلفة.

وسجل تحليل البلازميدات للمجموعات السيرولوجية السبعة المعزولة تماثل واضحًا بينها يتراوح بين 0.50-0 Kbp.