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Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis in and Around Harare, Zimbabwe

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SUMMARY. Colibacillosis, a disease caused by avian pathogenic *Escherichia coli* (APEC), can lead to great economic losses in the poultry industry. The aim of this study was to determine the prevalence of antibiotic resistance and antibiotic resistance patterns in APEC in Zimbabwe. From 503 chickens diagnosed with colibacillosis, 103 E. coli isolates were obtained. Isolation and identification of E. coli were carried out using microscopy and biochemical tests. The disc diffusion method was used to determine antibiotic susceptibility of the isolates to 8 commercial antibiotics. Many isolates exhibited resistance to more than one antibiotic. Antibiogram profiles indicated maximum resistance to tetracycline (100%), bacitracin (100%), and clavulanic acid (100%) and a high prevalence of resistance to ampicillin (94.1%). However, there were high prevalences of sensitivity to ciprofloxacin (100%) and gentamicin (97.1%). The isolates showed moderate rates of sensitivity to chloramphenicol and neomycin. All isolates in this study showed multidrug resistance because they were all resistant to 3 or more antibiotics. Seven multidrug resistance patterns were observed. The most common pattern (resistance to ampicillin, bacitracin, clavulanic acid, and tetracycline) was exhibited by 30 isolates. Our findings show that there is emerging drug resistance in APEC associated with colibacillosis in Zimbabwe. The observed high level of multidrug resistance could hamper the treatment of colibacillosis in Zimbabwe.

RESUMEN. Reporte de Caso—Resistencia contra antimicrobianos de cepas *Escherichia coli* aisladas de pollos con colibacillosis en Harare, Zimbabue y en zonas aledañas.

La colibacillosis es una enfermedad causada por cepas de *Escherichia coli* patógenas para aves (con las siglas en inglés APEC), esta enfermedad puede producir grandes pérdidas económicas en la industria avícola. El objetivo de este estudio fue determinar la prevalencia de la resistencia a antimicrobianos y los patrones de resistencia de *E. coli* patógenos para aves en Zimbabue. A partir de 503 pollos diagnosticados con colibacillosis, se obtuvieron 103 aislamientos de *E. coli*. El aislamiento e identificación de *E. coli* se llevaron a cabo utilizando microscopía y pruebas bioquímicas. Se utilizó el método de difusión en disco para determinar la susceptibilidad de los aislamientos para ocho antibióticos comerciales. Muchas cepas mostraron resistencia a más de un antibiótico. Los perfiles de los antibiógramas indicaron la máxima resistencia para la tetraciclina (100%), bacitracina (100%), y clavulánico (100%) y una alta prevalencia de la resistencia a la ampicilina (94.1%). Sin embargo, había una alta prevalencia de la sensibilidad a ciprofloxacina (100%) y gentamicina (97.1%). Las cepas mostraron tasas moderadas de sensibilidad a cloranfenicol y neomicina. Todas las cepas aisladas en este estudio mostraron resistencia a múltiples fármacos, ya que fueron resistentes a tres o más antibióticos. Se observaron siete patrones de resistencia a múltiples fármacos. El patrón más común (resistencia a la ampicilina, bacitracina, clavulánico, y tetraciclina) fue exhibido por 30 aislamientos. Estos resultados muestran que existe una resistencia emergente de drogas en cepas de *E. coli* patógena para aves asociadas con colibacillosis en Zimbabue. El alto nivel de resistencia a múltiples fármacos observado podría dificultar el tratamiento de la colibacillosis en Zimbabue.

Key words: *Escherichia coli*, colibacillosis, antibiotic resistance, Zimbabwe, poultry

Abbreviations: APEC = avian pathogenic *E. coli*, CVL = Central Veterinary Laboratories

In chickens, colibacillosis refers to any local or systemic infection caused entirely or partially by *Escherichia coli* (16). Strains of *E. coli* found to be virulent in poultry are classified as avian pathogenic *E. coli* (APEC) (1). In poultry, colibacillosis causes a variety of disease manifestations, including yolk sac infection, respiratory tract infection, swollen head syndrome, septicaemia, and enteritis (13,20). Avian colibacillosis has been recognized to be a major infectious disease in poultry of all ages. This disease contributes significantly to economic losses, mainly due to morbidity and mortality of affected birds (6,7,13).

The great diversity among APEC strains limits the possibilities of vaccination. Several vaccines based on attenuated or killed strains have been found to give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient (13). There remains no effective commercial vaccine to control colibacillosis (11), hence antimicrobial drugs remain important in reducing both incidence and mortality associated with this disease (4,23).

However, for colibacillosis, as for other diseases in veterinary and human medicine, concern remains over the emergence, selection, and dissemination of antibiotic-resistant microorganisms. Antibiotic-resistant *E. coli* can be transferred from animals to humans through consumption of contaminated food and food products, and this is a public health concern (14). A number of reports have shown an increased prevalence of resistance to antibiotics by APEC (12,19,21,22).

In Zimbabwe, bacterial infections are treated without first establishing an antibiogram. Without surveys to determine antibiotic resistance profiles of pathogenic microbes, the use of antibiotics can be quite irrational. This study was therefore done to determine the
Table 1. Antibiotic susceptibility profiles of E. coli isolates from chickens with colibacillosis.

<table>
<thead>
<tr>
<th>Antibiotic (conc., µg)</th>
<th>Resistant no. (%)</th>
<th>Intermediate no. (%)</th>
<th>Susceptible no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>103 (100)</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>3 (1)</td>
<td>2 (1.5)</td>
<td>97 (9.7)</td>
</tr>
<tr>
<td>Neomycin (10)</td>
<td>56 (54.4)</td>
<td>39 (37.9)</td>
<td>8 (7.7)</td>
</tr>
<tr>
<td>Chloramphenicol (30)</td>
<td>38 (36.9)</td>
<td>47 (45.6)</td>
<td>18 (17.5)</td>
</tr>
<tr>
<td>Ampicillin (10)</td>
<td>97 (94.3)</td>
<td>5 (4.7)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>103 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bacitracin (10)</td>
<td>103 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol (5)</td>
<td>103 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

prevalence and patterns of antibiotic resistance in APEC isolates that cause colibacillosis in Zimbabwe. This was done in order to provide information on the current status of antibiotic resistance patterns of these isolates. This information could aid in controlling colibacillosis.

MATERIALS AND METHODS

Sample collection. Commercial poultry farmers from the Harare and Mashonaland provinces brought their dead or sick chickens to the Poultry Section of the Central Veterinary Laboratories (CVL), Harare, over a period of seven months from July 2011 to January 2012. Post-mortem examinations were carried out on 1049 chickens. Among these, 503 were diagnosed with avian colibacillosis. Organs showing characteristic lesions consistent with colibacillosis were inspected and random samples were collected for further analysis. A total of 103 organ samples from different chicken samples were used for E. coli isolation.

Isolation and identification of E. coli. Yolk sac, heart, or liver samples were swabbed with a sterile cotton swab and inoculated on 3% sheep blood agar (New England Biolabs, Midrand, South Africa) and incubated aerobically at 37°C for 24 hr. Suspected E. coli colonies were then inoculated on MacConkey agar plates and incubated as above. The identification of E. coli was performed according to methods described by Burrow and Pelham (3). Biochemical tests were carried out including the Gram stain and the catalase, oxidase, indole, and citrate tests.

Antimicrobial susceptibility testing. The disc diffusion method was used to determine antibiotic susceptibility of the isolates on Mueller Hinton agar (Oxoid, Basingstoke, Hampshire, UK). Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), cloranfloxacin (5 µg), gentamicin (10 µg), neomycin (10 µg), and bacitracin (10 µg). All antibiotic disks were from Oxoid. The plates were incubated at 37°C for 24 hr, and inhibition zones were measured. The results were interpreted according to the Clinical and Laboratory Standards Institute (5) guidelines. E. coli ATCC 25922 was used as a reference strain in all tests.

RESULTS

The prevalence of avian colibacillosis was 48% as of the 1049 birds brought to the CVL. 503 were confirmed to have avian colibacillosis. Diagnosis was confirmed by post-mortem examination, and isolation of E. coli was done from organs of diseased poultry showing clinical lesions. A total of 103 E. coli isolates were obtained from randomly selected diseased organs (hearts, livers, and yolk sacs). The antibiotic resistance profiles of the 103 E. coli isolates are presented in Table 1. A high level of antibiotic resistance was observed, as many isolates exhibited resistance to more than one antibiotic. Most E. coli isolates were, however, sensitive to ciprofloxacin, chloramphenicol, and gentamicin (Table 1). The prevalence of antibiotic resistance phenotypes of all E. coli isolates is presented in Table 2. All isolates were resistant to at least 3 antibiotics, and 7 different antibiotic resistance patterns were observed. The most common resistance pattern, exhibited by 30 isolates, was resistance to bacitracin, tetracycline, clonazepam, and ampicillin (pattern D), and the least common resistance pattern was pattern F exhibited by only 1 isolate (Table 2).

DISCUSSION

In this study 103 E. coli isolates were obtained from the livers, hearts, and yolk sacs of chicken carcasses with confirmed colibacillosis. The E. coli isolates showed high sensitivity to ciprofloxacin and gentamicin, but many were resistant to tetracycline, bacitracin, clonazepam, and ampicillin (Table 1). Moderate sensitivity was shown to neomycin and chloramphenicol (Table 1).

Our findings are similar to previous studies from Spain (4), Germany (8), Jamaica (14), Nigeria (17), and Bangladesh (10), which found that E. coli isolates were highly sensitive to ciprofloxacin and gentamicin. The high sensitivity to ciprofloxacin might be because it is a broad-spectrum antibiotic that is still relatively new and has limited use by poultry farmers (10). Our findings differed from those of a study done in Sudan by Oweis et al. (16), who found that avian E. coli isolates were highly resistant to ciprofloxacin but were sensitive to gentamicin, and one in Iran (23) that found a high incidence of resistance of E. coli to gentamicin.

In this study, all 103 E. coli isolates were resistant to tetracycline (Table 1). This was expected, as most studies on avian E. coli have consistently shown a high resistance to tetracycline (9,19,23). This is probably due to the increased use of antibiotics as food additives, for example, tetracyclines, bacitracin, and clonazepam are widely used in poultry industries for growth promotion or prevention of diseases (12,15,16,18). This could explain the high resistance of E. coli isolates to these three antibiotics (Table 1). E. coli isolates were also generally resistant to ampicillin (94.1%), a finding in agreement with several previous reports (17,23), but differs from findings in other countries (14,19). The high resistance to ampicillin could be as a result of the unrestricted use of this prescription drug for treatment of livestock in Zimbabwe. However, it is important to note that general data on the use of antibiotics in the poultry industry in Zimbabwe are not readily available.

Table 2. Antimicrobial resistance patterns of the 103 E. coli isolates.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>No. of isolates</th>
<th>Resistance patterna</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>Bac, Tet, Cox</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>Bac, Tet, Cox, Amp</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>Neo, Chlo, Bac, Tet, Cox</td>
</tr>
<tr>
<td>D</td>
<td>14</td>
<td>Chlo, Bac, Tet, Cox, Amp</td>
</tr>
<tr>
<td>E</td>
<td>29</td>
<td>Neo, Bac, Tet, Cox, Amp</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>Geo, Neo, Bac, Tet, Cox, Amp</td>
</tr>
<tr>
<td>G</td>
<td>23</td>
<td>Neo, Chlo, Bac, Tet, Cox, Amp</td>
</tr>
</tbody>
</table>

aAmp: ampicillin; Bac: bacitracin; Chlo: chloramphenicol; Cip: ciprofloxacin; Cox: clonazepam; Geo: gentamicin; Neo: neomycin; Tec: tetracycline.

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