ISOLATION OF ESCHERICHIA COLI FROM YOUNG LIONS, DOGS AND SHEEP WITH DETERMINATION OF ANTIBIOTIC RESISTANCE

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ABSTRACT

E. coli were isolated from fecal samples and the antibiotic resistance pattern of E. coli was determined by means of disc diffusion assay. The resistance pattern determined streptomycin, amoxicillin, ciprofloxacin, ceftriaxone amoxicillin/clavulanic acid, cefepimeand azteronam. E. coli were isolates reported as resistant to more than five antibiotics (multidrug-resistant). This might result from expansion of the antibiotic resistance image among animals and from the animals to human living in close contact with them. The present study designed to isolate Escherichia coli and estimate its antibiotic resistance that isolated from young lions, dogs and sheep to establish occurrence of levels antibiotic resistance.

Keywords: Escherichia coli, young lion, dogs, sheep, antibiotic.

1. INTRODUCTION

The medical hazards of antibiotics resistance have a wide history that prevails over decades and even up today due to unnecessary use of antibiotics has brought new challenges to the public health (1). The wild and pet animals contribute to the mainfactor in the extent of infections. This actuality is supported by the outcomes of many studies which reported that the majority of outbreaks in humans over a decade (1990-2000) that involved transmission through animals including the spread of multidrug-resistant (MDR) zoonotic pathogens (through zoo animals to humans) (2). The majority of bacteria which are gram negative pathogens (3). The approximation of antimicrobials usage in feed of farm animals and poultry leads to modification of intestinal flora by creating a selective pressure in favor of resistant bacterial pathogens that get transferred into humans through the environment and food chains (4). Similarly, the genetic modifications, receptor insensitivity and decreased drug uptake and R factors contribute significantly in the spread of resistance to antimicrobials (5). Escherichia coli is an inhabitant of normal flora of the gastrointestinal tract of humans and animals, and is believed to facilitate food digestion through enzyme synthesis, however, few of them are potentially pathogenic (6). This study was conducted to determine the resistance E. coli isolated from young lions.

2. MATERIAL AND METHODS:

Sample collection: The fresh fecal samples which divided into four young lions, four young dogs and four sheep were obtained from Central Veterinary Hospital in Baghdad / Ministry of Agriculture and clinical cases reported to the Department of Internal and Preventive Medicine / College of Veterinary Medicine / University of Baghdad. E. coli were isolated from all representative fecal samples.

Sample processing and identification: The microbial colonies were obtained by serial dilution method. Colonies were cultured on sterile nutrient agar plates and the isolated E. coli strains were identified by biochemical tests (7).

Antimicrobial agents: Nine different antimicrobial discs were tested using ofloxacin 5 μg, amoxicillin 25 μg, cefepime 30 μg, amoxicillin-clavulanic acid 30 μg, ciprofloxacin 5 μg, ceftriaxone 10 μg, aztreonam 30 μg, meropenem 25 μg and streptomycin 10 μg. The standard antimicrobial powders were purchased commercially for MIC viz: Meropenem (Astra Zeneca, UK), amoxicillin-clavulanic acid and amoxicillin (Astra Zeneca, UK), ceftriaxone (Macterpvt Ltd., USA) streptomycin (Astra Zeneca, UK) and ciprofloxacin (Astra Zeneca, UK). The antibiotics used during this study were selected on the basis of information regarding their use in feed and frequency of usage against infections.

Antimicrobial resistance screening: Disc diffusion assay, Susceptibility tests were performed by Bauer-Kirby (8) disc diffusion method on Muller Hinton agar. The results were expressed as susceptible/resistant according to diameter of zone of inhibition around each antibioidisc (9). The Minimum inhibitory concentration (MIC) of the antimicrobial agents was determined by agar dilution method. The sterilized Muller Hinton agar media was cooled to 50°C and about 19 ml of this was added to sterilized test tubes that contained 1 ml of different concentrations of antibiotics. This mixture was thoroughly mixed and poured into pre-labeled sterile petri plates. That having only growth media were prepared with a similar procedure to serve as comparison with the petri plates containing
antibiotics. The concentrations of the antibiotics used in this test ranged from 30 mg to 0.117 mg/ml. The suspensions of the microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated onto the series of agar plates using micropipette (0.05 μl approximately). The plates were then incubated at 37°C for 24 hs.

3. RESULTS

E. coli. bacteria isolated from fresh feces and swabs were collected of young lions, dogs and sheep. All most of these strains bacteria showed resistance towards cephalosporin antibiotics (100%). It was also observed that about 50 to 75% of E. coli strains were resistant to more than five antibiotics (Therefore, they could be considered as multi-drug resistant, MDR). The general resistance pattern of E. coli and the MIC levels of the antibiotics reported were resistant to AML, STP, FEP, ATM, AMC, CIP, CRO (Table 1).

<table>
<thead>
<tr>
<th>animal</th>
<th>Resistance pattern</th>
<th>antibiotic</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young lions</td>
<td>AML, FEP, STP, ATM, AMC, CRO</td>
<td>AML</td>
<td>0.7-0.725</td>
</tr>
<tr>
<td></td>
<td>AML, FEP, STP, AMC, CRO</td>
<td>MEM</td>
<td>0.35-0.225</td>
</tr>
<tr>
<td>dogs</td>
<td>AML, STP, AMC, CRO</td>
<td>FEP</td>
<td>0.6-0.65</td>
</tr>
<tr>
<td></td>
<td>AML, MEM, STP, FEP, ATM, AMC, CIP, CRO</td>
<td>STP</td>
<td>0.31-0.345</td>
</tr>
<tr>
<td>sheep</td>
<td>AML, ATM, AMC, CRO</td>
<td>ATM</td>
<td>0.7-0.726</td>
</tr>
<tr>
<td></td>
<td>STP, ATM, AMC, CRO</td>
<td>AMC</td>
<td>0.7-0.736</td>
</tr>
</tbody>
</table>

AML amoxicillin, MEM meropenem, STP streptomycin, FEP cefepime, OFX ofloxacin, ATM azteronam, AMC amoxicillin/clavulanic acid, CRO ceftriaxone, and CIP ciprofloxacin

4. DISCUSSION

During the present study much higher levels of resistance were determined among fecal samples representative of these farmanimals compared to that reported earlier(10,11). The resistance pattern of E. coli revealed nearly similar results. Furthermore, significantly elevated resistance levels were reported towards all antibiotic classes, especially towards the cephalosporins. This fact may be as result of ESBLs production by E. coli strains which is regarded as one of the most important resistance factors in gram negative bacteria(12). It was noted that all representative strains exhibited resistance to more than five antibiotics, which confined them multidrug resistant as reported earlier (11,13). Additionally, the E. coli strains isolated from hog dea were also resistant to meropenem, which is an indicator of higher levels of antibiotic resistance. Likewise, the isolated E. coli strains showed significantly elevated levels of corresponding MIC values that were in close agreement with results of disc diffusion assay. These findings may confirm the presence of resistance plasmids in these isolates as reported previously (14). This is perhaps due to frequent use of antimicrobials in the feed and for prophylaxis that resulted in the development of selective pressure towards the ultimate emergence of antibiotic resistance (15). During the present investigation, about 50 to 75% of E. coli isolates were observed to be resistant to more than three antimicrobial agents (MDR). Mainly the high resistance levels could be indicative of this finding (15). There is strong evidence that the excessive use of antimicrobial agents and the circulation, and amplification of antimicrobial resistance genes in the environment may also result in the emergence of multidrug resistant strains E. coli (12). It is concluded that E. coli strains isolated from animals in a farm house were highly resistant and the antibiotics significant majority of isolates were observed as multidrug resistant.
REFERENCES


