Detection of virulence and antimicrobial susceptibility testing of *Escherichia coli* O157:H7 isolated from pets and stray dogs

Araf Abdulrahman Yousif, Mustafa Salah Hasan, Thaar Mohammed Najim and Mohammed Ali Hussein

Abstract

The main objective of this study was to detect the virulence of *E. coli* O157:H7 by using Congo red dye and to determine the pattern of antimicrobial susceptibility for twenty six isolates of *E. coli* O157:H7 obtained from Department of internal and preventive medicine/College of Veterinary Medicine/University of Baghdad isolated from diarrheic and non-diarrheic dogs found in Baghdad Province, the study extended from March 2015 to August 2015. Seven antimicrobial discs were used in this study Chloramphenicol (30µg), Ampicillin (10µg), Trimethoprim plus sulfa (1.25 µg), Ciprofloxacin (5µg), Erythromycin (15µg), Gentamycin (120 µg), Cephalexin (30 µg), Trimethoprim (5 µg). The result showed that 24 isolates gave a positive result for Congo red, whereas only two isolates give a negative result. Results of antimicrobial susceptibility showed that the isolates susceptible to Ciprofloxacin at a percentage (53.84 % ; 46.15 %) respectively. In conclusion, isolates of *E. coli* O157:H7 isolated from fecal samples from diarrheic and non-diarrheic dogs were having the multidrug resistant phenomena and most of isolates were positive to Congo red dye which indicated this organism as an invasive pathogen.

Keywords: *E. coli* O157:H7, dogs, antimicrobial susceptibility. Congo red dye, pets and stray dogs.

1. Introduction

*Escherichia coli* O157: H7 is regarded as an important global zoonotic food borne pathogen, which produces life threatening diseases in humans, such as hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) [1]. *E. coli* O157:H7 has been detected in the feces of apparently healthy cattle, sheep, goats, pigs, dogs, bats and avian species [2-4]. Also, this pathogen has been documented in dogs by [5, 6]. Dogs and puppies regarded as important reservoirs for *E. coli* O157:H7 which is one of the main causes of diarrhea and other diseases in human [7]. Also, Yousif *et al.* [8] reported that *E. coli* O157:H7 was important pathogen in dogs.

Transmission pathways of STEC were the direct contact between humans and pet animals as well as fecal and urine contamination [9, 10]. Diarrheic and non-diarrheic dogs of all ages may serve as potential sources of multi-drug resistant STEC O157:H7 transmissible to humans [11]. *Escherichia coli* O157:H7 were isolated from dogs fecal sample which has class 1 integrons and they conclude that the presence of class 1 integrons serving as reservoirs of antibiotic resistance genes [12]. Many microorganisms carrying integrons can change and adapt quickly to different environmental niches and remain stable in several microbial lineages highlights not only its adaptive value, but also the potential for selection and dissemination of multiresistant bacteria and it is known that antibiotic resistant bacteria will increase a lot [13]. Paula and Marin [14] concluded that the carrying of MDREC organisms by dogs represents a potential hazard for people having contact with such animals, running the risk of spreading resistance genes; they also found a high percentage of STEC strains isolated from diarrheic dogs, presenting a multidrug-resistance phenotype.

Most studies showed that *E. coli* O157:H7 isolates from different sources were resistant to most antibiotic. The study of Disassa *et al.* [15] indicated that the *E. coli* O157:H7 isolates from milk samples were resistant to most of the antimicrobials; these were resistant to tetracycline, streptomycin and kanamycin. Beyi *et al.* [16] found that *E. coli* O157 which detected in carcass swabs and cutting board swab samples from butcher shops. All isolates were susceptible to...
five drugs 100%, but five isolates were resistant to amoxicillin, two isolates to streptomycin and three isolates to chloramphenicol. One isolate was resistant to two drugs and another to three drugs. Abdissa et al. [17] were isolated E. coli O157:H7 from abattoir samples, fecal sample and the carcass swabs and found that the isolates 100% susceptible to cefotaxime, ceftriaxone, gentamycin, kanamycin and nalidixic acid.

Congo red (CR), a simple acid dye, has been used to differentiate invasive pathogens such as E. coli [18]. Some authors have purposed the utilization of this characteristic as virulence marker to E. coli strains [19, 20]. This present study was conducted to determine the in vitro virulence factors and correlation with the resistance pattern of E. coli O157:H7 isolated from pets and stray dogs.

2. Materials and methods
2.1 Isolates of E. coli O157:H7
A twenty six (n = 26) E. coli O157:H7 isolates obtained from Department of internal and preventive medicine/ College of Veterinary Medicine/ University of Baghdad isolated from diarrheic and non-diarrheic dogs found in Baghdad Province; the study extended from March 2015 to August 2015; these isolates confirmed in a previous study by Hasan et al. [7], which possess five virulence gene (rfbO157, flicH7, Stx1, Stx2 and eaeA), these genes confirmed by conventional and real-time PCR by Yousif et al. [8].

2.2 In Vitro pathogenicity testing by using Congo Red dye
The Congo red agar was made by using Trypticase Soy Agar with an additional 0.15% bile salts and 0.03% dye, 0.2% sorbitol according to Zahid et al. [21]. Congo Red binding activity test was performed and the colonies appeared must examine at 18, 24, 48 and 72 hours post incubation.

2.3 Antimicrobial sensitivity test (Disk diffusion test)
Antimicrobial discs were purchased from Mast diagnostics company (U.K.). Susceptibility to 8 antimicrobials was determined (Chloramphenicol (30µg), Ampicillin (10µg), Trimethoprim plus sulfa (1.25 µg), Ciprofloxacin (5 µg), Erythromycin (15µg), Gentamycin (120 µg), Cephalexin (30 µg), Trimethoprim 5 µg). The disk diffusion susceptibility method is simple and practical and has been well-standardized [22]. At least 4-5 well isolated colonies of the same morphological type were selected from chrome agar; they were transferred to a tube containing 4-5ml of nutrient broth. The inoculated broth was incubated at 35-37 °C for 18 hrs. until the appearance of visible turbidity.

A sterile swab was dipped into the standardized suspension of bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid level.

Then, the swab was streaked in three directions over the entire surface of the Muller Hinton agar. The inoculated plates were allowed to dry for 5 minutes. Then the discs of antibiotic were placed onto agar surface using a sterile forceps. After that the plates were inverted, placed at 37 °C incubator aerobically for 18-24hrs [23]. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter by using a caliper. The diameter of the zone was related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the criteria published by [24, 25].

2.4 Statistical analysis
All data were analyzed statistically by using the Microsoft program (SPSS).

3. Results and Discussion
The present study results showed that the differentiation between virulent and non-virulent E. coli O157:H7 isolates were the presence of red colonies which recorded as Congo Red positive and white to grey colonies indicate that did not bind the dye and considered as Congo Red negative (Fig. 1).

![Fig 1: Congo red positive (above), Congo red negative (right).](image)

Out of twenty six E. coli O157:H7 isolates, 15 isolates were given a positive result for Congo red, whereas 11 isolates give a negative result (Table 1).

Table 1: No. of isolates +ve and−ve to Congo red.

<table>
<thead>
<tr>
<th>No of isolates</th>
<th>Congo red +</th>
<th>No Of Isolates</th>
<th>Congo red -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total -</td>
<td>15</td>
<td>Total</td>
<td>11</td>
</tr>
</tbody>
</table>

Table (2) showed the sensitivity and resistance of the 26 isolates to antimicrobial drugs, most of isolates +ve to Congo red resistant to 4-7 antimicrobial while the isolates –ve to Congo red dye were showing resistance to 3-5 antimicrobial.
The present study results revealed that most isolates were susceptible to ciprofloxacin at a percentage (80.76%) followed by gentamycin and trimethoprim plus sulfa at a percentage (53.84%; 46.15%) respectively, whereas most isolates showed resistance to other antimicrobials at a different percentage which were mostly Congo red positive (Table 3, Fig. 2 and 3).

Table 3: Antimicrobial susceptibility testing of *E. coli* O157:H7 isolates according to its virulence

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Sensitive isolates (%)</th>
<th>Congo red + (%)</th>
<th>Resistance (%)</th>
<th>Congo red + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>3(11.54)D</td>
<td>Zero (0.0)E</td>
<td>23(88.46)C</td>
<td>15(65.2)A</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1(3.84)E</td>
<td>1 (100)D</td>
<td>25(96.14%)B</td>
<td>14(56)B</td>
</tr>
<tr>
<td>Trimethoprim+ sulfa</td>
<td>12(46.15)C</td>
<td>2 (16.66)C</td>
<td>14(53.85%)D</td>
<td>13(92.85)C</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>21(80.76)A</td>
<td>10(47.6)A</td>
<td>5(19.24%)F</td>
<td>5(100)E</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1(3.84)E</td>
<td>Zero (0.0)E</td>
<td>25(96.14%)B</td>
<td>15(60)A</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>14(53.84)B</td>
<td>3(21.43)B</td>
<td>12(46.14%)E</td>
<td>12(100)D</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>Zero(0.0)F</td>
<td>Zero (0.0)E</td>
<td>26(100%)A</td>
<td>15(57.7)A</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between sensitive and resistant isolates.

Fig 2: Resistance to antimicrobials at a different percentage which were mostly Congo red positive
The antimicrobial resistance of bacteria has become one of the most important therapeutic problems in human and veterinary medicine. The present study analysed the occurrence of antimicrobial resistance in E. coli O157:H7 isolated from dogs due to its properties as a multidrug resistant bacteria. From the results of this study, most E. coli O157:H7 isolates which were showing a high degree of resistance and most of these isolates were Congo red positive, this result was compatible with the result of Sharma et al. which concluded that Congo red agar was had 100% specificity for pathogenic invasive E. coli. Also, Corbett et al. found that the Congo red medium was used to identify the virulent form of Escherichia coli. A direct correlation was found between the ability of clinical isolates of pathogenic E. coli to bind Congo red dye and their ability to cause infection in pigeon.

Zahid et al. reported that Congo red agar can differentiate pathogenic strains from commensals. Binding of Congo Red is associated with the presence of virulence genes and genes for multiple resistance to antibiotics. The results of antimicrobial sensitivity test were in agreement with Oseki, who found that the most isolates of E. coli O157:H7 were sensitive to Gentamicin. And with the results of Galland et al., that recorded most isolates of E. coli O157:H7 were susceptible to antibiotics used in feedlots, such as trimethoprim-sulfamethoxazole. Antimicrobial sensitivity experiments by Shao et al. showed that 16 of the E. coli O157:H7 strains non-O157 STEC of different serotypes were multidrug resistant, Nalidixic acid showed the highest rate of resistance among the strains, followed by trimethoprim and sulfamethoxazole, and sulfisoxazole, resistance to cefepime or imipenem was not detected, the 16 strains of STEC isolated from animal stools in this area were resistant to a number of antibiotics, with many strains displaying multidrug resistance. Also, the current results were similar to study of Osaili et al. who found that Escherichia coli O157:H7 isolates revealed extensive resistance to Erythromycin and high susceptibility to Gentamicin and Ciprofloxacin. A study of Reuben and Owuna was done to determine the antibiotic resistance patterns of E. coli O157:H7 from Nigerian fermented milk consumed in Nigeria, the antibiotic susceptibility profile showed that all the isolates were resistant to multiple antibiotics such as Chloramphenicol, Erythromycin, Sulphamethoxazole/Trimethoprim, while most isolates were sensitive to ciprofloxacin and gentamicin. Mora et al. showed that a higher percentage of resistant strains of STEC O157:H7 were recovered from bovine and beef meat

human and ovine sources, the most common antimicrobial resistance was Ampicillin (10%) and Chloramphenicol (7%).

Our results compatible with a study reported in Egypt to evaluate the antibiotic susceptibilities of E. coli O157:H7 isolated from different water sources and establish a correlation between the presence of virulence genes of E. coli O157:H7 with the resistance to six antibiotic groups (amoxicillin, cefixime, ciprofloxacin, tetracycline, clarithromycin and streptomycin), the result showed that all bacterial isolates were sensitive to ciprofloxacin and showed variable degrees of resistance to other antibiotics.

Also, Edge and Hill found low levels of resistance to ciprofloxacin. High levels of resistance to ampicillin were expected due to fact that ampicillin is older antibiotics that have been extensively used over the years.

The current study revealed a high rate of antimicrobial resistance among E. coli O157:H7 isolated from dogs, this was in agreement with a result of Ojo et al. The different results of resistance could be attributed to the use of non-specific antibiotic and random administration of antibiotic.

4. Conclusions

Finally, it can be concluded that, due to misuse of antibiotics, E. coli O157:H7 strain develop strategies for resistance to most of antimicrobial agent, only all isolates were significantly susceptible to ciprofloxacin only, so that this antibiotic can be considered a drug of choice for this bacteria. And most isolates were positive to Congo red dye which indicate that these strains isolated from dogs were virulent so Congo red agar can be used as guide for the presence of virulence factors of E. coli O157:H7. There is compatable results between postivitivy to Congo red dye and the resistance of bacteria to most antibiotic.

5. Acknowledgment

Grateful thanks by all authors to support of this work by College of Veterinary Medicine, Department of Internal and Preventive Veterinary Medicine, University of Baghdad, Iraq.

6. References

2. Ojo OE, Ajwape A, Otesile E, Owodari A. Detection of shiga toxin-producing Escherichia coli in poultry birds in...


