Characterization of Escherichia coli from diarrheic calves with special reference to plasmid profile

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Abstract: Out of 120 fecal samples collected from diarrheic calves at different localities in AL-Qalyoubia Governorate, 43 were positive for Escherichia coli with an incidence of 35.83%. Serogrouping of E. coli isolates recovered 8 belong to different "O" serogroups which were O₂₅, O₁₁₁, O₂₆, O₁₅₃, O₈, O₁₈, O₁₆₆, O₁₅₇; and "5" isolates were untypable. Antibigram pattern of isolated E. coli serogroups showed that all tested serogroups of E.coli were sensitive to enrofloxacin, and neomycin, all groups were resistant to cefadroxil, colistin sulphate, Tobramycin and ampicillin. Meanwhile variable results were recorded with the remaining used chemotherapeutic agents. Plasmid profile analysis of various E. coli serogroups revealed that E. coli serogroups O₁₁ revealed 4 plasmids with their super coiled forms with a molecular weight ranging from 1-7 Kbp, while E. coli serogroup O₁₅₃ revealed 3 plasmid with molecular weight 2.5-7 Kbp and O₁₈ harbored 2 plasmid with molecular weight 1.8-2.5 Kbp, while E. coli serogroups O₂₆, O₅₅, O₈ revealed 1 plasmid with the same molecular weight 7 Kbp. On the other hand E. coli serogroups O₁₆₆ have no plasmid.

Keywords: diarrheic calves, plasmid profile, Escherichia coli

1. Introduction

E. coli is the most frequently encountered Gram negative organism causing diarrhea. Diarrhea represents an increasing and recurrent problem in young calves, from one week up to 12 weeks age, especially in suckling beef calves, China et al., (1998) and William and Zimmer (2013). Certain pathogenic strains cause enteric diseases ranging in symptoms from Cholera –like diarrhea to severe dysentery, other E. coli may colonize the urinary tract, resulting in cystitis or pyelonephritis Donneberg and Whittam (2001).

Saridakis et al., (1997) isolated 12 serotype of E. coli from diarrheic calves while O₉₆: H₁₃, O₁₁₁: H₂₅ and O₁₁: H, being the most prevalent. Also, Fectrau et al., (2001) isolated 25 E. coli strains from bactericidal calves for the presence of virulence factors. Isolates of pathotypes O₇₈, O₂₁₉ were positive but O non-typable were associated with a high mortality rate.

Ratchtrachenchai et al., (2004) determined 20629 E. coli strains isolated from children with acute diarrhea sing 43 monovalent O antisera. The following serogroups were determined O₆, O₃, O₅₆, O₁₈, O₂₅, O₂₆, O₁₁₉, O₁₂₆, O₁₂₇ₒ₄, O₁₂₆, O₁₄₆, O₁₅₉ and O₁₆₆. They recorded that were only four serogroups that were exclusively associated with a single pathotype: O₂₆, O₁₂₄, O₂₃ and O₁₆₄.

The properties which allow pathogenic E. coli to invade infect and damage host cells are conferred by adhesions, toxins and hemolysins (Beutin 1991).

Diarrhoeagenic E. coli are recognized as five major pathotypes: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC) Shiga-like toxin-producing (STEC) or enterohaemorrhagic (EHEC) and enteraggregative (EAEC) E. coli Nataro and Kaper (1998). Griffin and Tauxe (1991) mentioned that E. coli serotype O₁₅₅: H₁ associated with human infection and death in United States, while Blanco et al., (2003) reported that outbreak of disease have been traced to non-O₁₅₅: STEC serotypes. They include O₁:NM, O₂₆: H₁₁, O₂₆: H₁₁, O₂₆: NM, O₂₆: H₁₁, O₂₆: NM, O₁₁₁: NM, O₁₁₁: H₁₁, O₁₂₈: H₂ and O₁₂₈: NM. Two of these serotypes (O₁₁₁: NM and O₂₆: H₁₁) can cause post weaning diarrhea in calves.

The emergence and dissemination of antimicrobial resistance E. coli has been well documented as a serious problem worldwide Schroeder et al., (2002). Antimicrobial therapy is an important tool in reducing both the incidence and symptoms associated with diarrhea in cattle. However resistance to existing antimicrobials is widespread and of concern to veterinarians Blanco et al., (1998). Also, the genes encoding these factors have been found on large conjugative plasmids and the occurrence of antimicrobial resistance genes on these same plasmids. It impossible that use of antimicrobial agent may select for persistence of E. coli containing such plasmids Johnson et al., (2002) and O’Brien et al., (1992) mentioned that
plasmids were the major vector in the dissemination of resistance genes through bacterial population.

The aim of this study was directed mainly to determine the presence and prevalence rate of enteric pathogen of public health importance as *E. coli* isolated from diarrheic calves. 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 and determination of plasmid profile.

2. Material and Methods
2.1. Collection of Samples:
A total of 120 fresh faecal samples collected from diarrheic calves suffering from profuse watery diarrhea tinged with blood, fever and anorexia aged from 1 week to 2 months at different localities in AL-Qalyoubia Governorate.

2.2. Isolation and identification:
All fecal samples were inoculated directly into nutrient broth and incubated for 24 hours at 37°C, then sub cultured onto MacConkey agar and EMB agar the inoculated plates were incubated at 37°C for 24-48 hours. The suspected colonies were identified morphologically, culturally and biochemically according to Koneman et al., (1996).

2.3. Serological identification:
Antisera of *E. coli* were used for serological identification of somatic " O" using slide agglutination test according to Edwards and Ewing1972).
Antisera -O-sera were obtained from DENKA SEIKEN CO LTD Tokyo, Japan.

2.4. Antibiotic pattern:
Antibiogram was applied on the different isolated serogroup of *E. coli* using in vitro disc diffusion technique according to Quinn et al., (2002) and performed on Mueller Hinton agar plates and 11 Discs of chemotherapeutic agents were used (enrofloxacin, neomycin, amoxyccillin, oxytetracyclin, chloramphenicol, streptomycin, cefadroxil, colistin sulphate, gentamicin, tobramycin, and ampicillin).
The results were interpreted according to National Committee for Clinical Laboratory Standards (2000).

2.5. Plasmid analysis:
Plasmid DNA was extracted from cultured cells following alkaline lysis method of plasmid preparation according to Vincent et al., (2010) using QIAGEN plasmid MINI KITS.

The samples were processed using Gel electrophoresis to identify the number of plasmid copies present in different isolates. For this purpose, an Agarose Gel of 0.9% was used. Staining of DNA fragments was carried out using ethidium bromide and they were visualized U.V. Trans illumination.
Standard DNA molecular weight marker were used to estimate of the plasmid size .The standard DNA molecular weight marker used in the present study were 1Kb ladder, 1 Kb plus DNA ladder and Lambda DNA 1 MIU 1 digest. The molecular weight of each plasmid was detected using Hind 111 Lambda phage digest. Plasmid analysis was performed in Biotechnology Center, Faculty of Veterinary Medicine, Cairo University.

3. Results
3.1. Bacteriological identification:
Out of 120 faecal samples were collected from diarrheic calves at different localities in AL-Qalyoubia Governorate. 43 samples were positive for *Escherichia coli* with an incidence of 35.83%.

3.2. Serological identification:
Serogrouping of (43) isolates of *E. coli* recovered from diarrheic calves revealed (8) different "O" serogroups and "5" strains were untypable as illustrated in Table(1).

<table>
<thead>
<tr>
<th>Table 1. Certain characteristic of study sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serogroups</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>O55</td>
</tr>
<tr>
<td>O111</td>
</tr>
<tr>
<td>O26</td>
</tr>
<tr>
<td>O153</td>
</tr>
<tr>
<td>O8</td>
</tr>
<tr>
<td>O18</td>
</tr>
<tr>
<td>O86</td>
</tr>
<tr>
<td>O157</td>
</tr>
<tr>
<td>Untyped</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* the percent was calculated according to the No. of total isolates (43)

3.3 Antibiotic pattern:
Antibiogram pattern of pathogenic *E. coli* serogroups recovered from diarrheic calves were recorded in Table (2) which shows that all tested serogroups of *E. coli* were sensitive to enrofloxacin, and neomycin. On the other, *E. coli* were resistant to cefadroxil, colistinsulphate, tobramycin and ampicillin, while variable results were recorded with the remaining used chemotherapeutic agents.
Table 2. Antibiogram pattern of \textit{E. coli} serogroups recovered from diarrheic calves

<table>
<thead>
<tr>
<th>Chemotherapeutic agent</th>
<th>Conc. µg</th>
<th>O55</th>
<th>O111</th>
<th>O26</th>
<th>O153</th>
<th>O8</th>
<th>O18</th>
<th>O86</th>
<th>O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>10</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>25</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>M</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Oxytetracyclin</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>M</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Colistin sulphate</td>
<td>50</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>M</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tobramicin</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

3.4. Plasmid analysis

Table 3. Plasmid profile analysis of different \textit{E- coli} serogroups recovered from diarrheic calves

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid and number</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>O11</td>
<td>4 plasmids with their super coiled form</td>
<td>7 Kbp, 3.5 Kbp, 2 Kbp, 1 Kbp</td>
</tr>
<tr>
<td>O153</td>
<td>3 plasmids</td>
<td>7 Kbp, 3 Kbp, 1 Kbp</td>
</tr>
<tr>
<td>O157</td>
<td>3 plasmids</td>
<td>7 Kbp, 2.5 Kbp, 2.3 Kbp</td>
</tr>
<tr>
<td>O18</td>
<td>2 plasmids</td>
<td>2.5 Kbp, 1.8 Kbp</td>
</tr>
<tr>
<td>O26</td>
<td>1 plasmid</td>
<td>7 Kbp</td>
</tr>
<tr>
<td>O55</td>
<td>1 plasmid</td>
<td>7 Kbp</td>
</tr>
<tr>
<td>O8</td>
<td>1 plasmid with their super coiling</td>
<td>7 Kbp</td>
</tr>
<tr>
<td>O86</td>
<td>No plasmid</td>
<td>-</td>
</tr>
</tbody>
</table>

4. Discussions

Pathogenic form of \textit{E. coli} associated with human and animal diseases are remarkably diverse. Diseases that affect young domestic animals during the first few months of life causes great losses for animal industry. The immune system of animals at young age is not well developed and the maternal
immunity would not with stand variable infections
Mohamed(1993). The most important bacterial enteropathogens are enterotoxigenic E.coli causing
diarrhea in calves the disease is usually referred to as
colibacillosis and include either enteric or systemic
colibacillosis Wary et al., (1993). Bacteriological
examination of 120 faecal samples collected from
diarrheic calves revealed that 43 isolates of E.coli
percentage of 35.83%. These findings nearly coincides
with that of Mahmoud (1993) and khan and khan
(1997) who isolate E.coli from diarrheic calves at
different percentages. Also, Vagh and Jani (2010)
isolated different serotypes of E.coli from the young
cattle and buffal calf calves affected with calf scour.

Serogrouping of Bacterial isolates of
enteropathogenic E.coli collected from diarrheic
calves revealed 8 different “O” serogroups and
"A" strains were untyped. The most prevalent
serogroups were O55, O111, O26, O153, O8, O16, O157.
These results agree to certain extended results
recorded with Holland (1990) who also mentioned
that the ETEC isolated from calves was limited to a
few serogroups especially O8, O9 and O101, while
Donkersgoed (1999) isolated E.coli O157 from faecal
samples collected from cattle in Canada, also
Champman et al., (1993) isolated E.coli O157 from
bovine rectal swabs in an incidence of 15% in North
America. They suspected that food of bovine origin
considered as a vehicle of infection and is food born
Zoonoses Holland et al., (1999) isolated E.coli
serogroups O5, O26, O111 from both apparently
healthy and diarrheic calves, and recorded that E.coli
strains O26 are more prevalent in calves with diarrhea.

Thin et al., (2011) isolated different serogroups of
E.coli from diarrheic calves in Vietnam and the
most prevalent serogroups were O15, O26, O101 and O157.

Antimicrobial therapy is an important tool in
reducing both the incidence and symptoms associated
with diarrhea in cattle, resistance has become an
important problem worldwide. Bacterial resistance to
antimicrobial agents has been emerging are rapidly
dispersing among many nosocomial and
community-acquired pathogens Tenover (2001). The
development of antibiotic resistance in E.coli has
important clinical implications. The development of
resistance to older agents such as Ampicillin and
Trimethoprim-sulfamethoxaxol, as well as the
emerging problem of fluoroquinolone resistance may
substantially limit our antibiotic choices Karlowsky
et al., (2002). In the present study, the results of
testing the E.coli serogroups against different
antimicrobial agents are illustrated in Table (2). All
tested serogroups were found to be sensitive to
enrofloxacin and Neomycin.

These results coincide with that obtained by
Basoglu et al., (1999) who recorded that E.coli
isolated from diarrheic calves were sensitive to
enrofloxacin and cholestyamine. On the contrary, all
tested serogroups were resistant to cefadroxil,
colistinsulphate, tobramycin and Ampicillin, also
variable results were recorded with the remaining
tested antibiotic. These finding in agreement with
those reported by Blanco et al., (1996) who recorded
that the highest percentage of antibiotics resistance
recorded with Tetracycline, streptomycin,
sulphadiazin, ampicillin, kanamycin, chloramphenicol, trimethoprim and cortrimoxaole in
treatment of E.coli in diarrheic lambs in Spanish, also
reported that emergence and dissemination
of antibacterial resistance in E.coli O8 may complicate
treatment of certain urinary tract and enteric infection
in human and animals. Meanwhile Mora et al.,
(2005) recorded that most strains of E.coli showed
resistant to five or more antibacterial agent(MDR) multiple drug resistant. The wide spread
uses of antibacterial agents promoted the increasing
frequency of E.coli multdrug resistance isolates in
bovine which facilitate the spread of resistant plasmid
to other bacteria. In addition Nyman et al., (2010)
suggest multiple drug resistant in E.coli from
Swedish dairy calves.

Antimicrobial resistance plasmids have been
increasingly associated with both Gram positive and
Gram negative bacterial infections. This trend is
accelerated by the fact that E.coli is a common
enteric commensal of mammals and a common case
of human infections. As such E.coli strain are
routinely exposed to a wide range of antimicrobial
agents. E.coli also has a very wide natural
distribution and a propensity to plasmid carriage
Scherley et al., (2003) Resistance to various antibiotic
is relatively common in clinical pathogens in Turkey
and also common E.coli strains Ozden et al., (2003)
and it is frequently plasmid-mediated Neu (1992). In
this study, plasmids were screened to determine their
antibiotic resistance profiles and focusing on the
changes on molecular level, could provide valuable
insights for its management, these results showed a
more than one plasmids in one strains.as shown in
table (3).

It was observed that there was no correlation
between plasmid occurrence and multiple antibiotics
resistance for all isolates because with serogroup O16
which have no plasmid and produce multiple drug
resistance with many antibiotics, also some
serogroups contain 4 or 3 or 2 plasmids. These results
are congruent with a molecular weight ranging 1-7
Kbp to the results reported by Celebi et al., (2007)
who evaluate the plasmid and antibiotic resistance
patterns among 118 uropathothogen E.coli strains
from infected urinary system plasmids were detected

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113 strains (97%). Some isolates harbored up to 10 plasmids ranging from 1-19 Kb in size.in the contrary (Vincent, et al. 2010) 

Also Nasreen et al. (2009) mentioned that some isolates of E.coli possess single sized plasmid while other had multiple plasmids with different size ranged from 2.3 Kbp to 26 Kbp, very high antibiotic resistance was detected from isolates possessing high molecular weight plasmids. 

Meanwhile Vincent et al., (2010) reported that (62.9%) of clinical isolates of E.coli O157 which have Multidrug resistance harbored plasmids all of which were no less than 2.1 Kbp in size but only 35% contained multiple plasmids. Moreover Nasreen et al., (2009) reported that the clinical isolates of E.coli gain resistance to numerous of antibiotics by various mechanisms .This drug resistance increases as function of time and their microorganisms exposure to many factors (antibiotics, chemicals) besides, the bacteria acquire resistance through different route, such as natural or intrinsic resistance (inaccessibility of the target, multidrug inactivation), mutational resistance(drug target site modification reduce permeability or uptake, metabolic by pass and derepression of multidrug efflux) All these mechanisms of antibiotic resistance warrant a detailed investigation of multiple factors, with prioritization of the studies of molecular characterization.

Such multidrug resistance has serious implications for the empiric therapy of infections caused by E.coli and for the possible co-selection of antimicrobial resistance mediated by multidrug plasmid .Thus, the study confirm the important role of plasmid numbers that controls the resistance characteristics in E.coli.

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