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 O_{55}, O_{111}, O_{26}, O_{153}, O_8, O_{18}, O_{86}, $O_{157,}$ and "5" isolates were untypable. Antibiogram 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, Tobramicin 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 O_{111} revealed 4 plasmids with their super coiled forms with a molecular weightranging1-7 Kbp, while *E.coli* serogroup O_{153} revealed 3 plasmid with molecular weight 2.5-7 Kbp and O_{18} harbored 2 plasmid with molecular weight 1.8-2.5 Kbp, while *E.coli* serogroups O_{26}, O_{55}, O_8 revealed 1 plasmid with the same molecular weight 7 Kbp. On the other hand *E.coli* serogroups O_{86} have no plasmid.

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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 sever 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 O_{26}: H_{11}, O_{119}: H_{25} and O_{11}: H, being the most prevalent. Also, **Fectrau** *et al.,* **(2001)** isolated 25 *E.coli* strains from bacteremic calves for the presence of virulence factors. Isolates of pathotypes O_{78}, O_{119}, were positive but O nontypable 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 O_6, O_8, O_{15}, O_{18}, O_{25}, O_{86}, O_{119}, O_{126}, O_{127}a, O_{128}, O_{146}, O_{159} and O_{166}. They recorded that were only four serogroups that were exclusively associated with a single pathotype: O_{20}, O_{124}, O_{52} and O_{164}.

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 enteroaggregative (EAEC) *E.coli* **Nataro and Kaper (1998) . Griffin** and **Tauxe(1991)** mentioned that *E.coli* serotype O_{157}: H_7 associated with human infection and death in United States, while**(Blanco** *et al.,* **2003)** reported that outbreak of disease have been traced to non-O_{157}STECserotypes. They include O_5: NM, O_6: H_{31}, O_{26}: H_{11}, O_{26}: NM, O_{48}: H_{21}, O_{91} : NM, O_{111}: NM, O_{113}: H_{21}, O_{128}: H_2 and O_{128}: NM.Two of these serotypes (O_{111}: NM and O_{26}: H_{11}) 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.,* **(200 2)** 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 from1week 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^{o}C, then sub cultured onto MacConkey agar and EMB agar the inoculated plates were incubated at 37^{o}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. Antibiogram 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, amoxycillin, oxytetracyclin, chloramphenicol, streptomycin, cefadroxil, colistin sulphate, gentamicin, tobramicin, and ampicillin).
The results were interpretated 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 1. Certain characteristic of study sites

Serogroups		
	No	%*
O55	7	16.27
O111	6	13.95
O26	5	11.62
O153	5	11.62
O8	4	9.32
O18	4	9.32
O86	4	9.32
O157	3	6.97
Untyped	5	11.62
Total	43	100

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

3.3 Antibiogram 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, tobramicin and ampicillin, while variable results were recorded with the remaining used chemotherapeutic agents.

Table 2. Antibiogram pattern of *E.coli* serogroups recovered from diarrheic calves

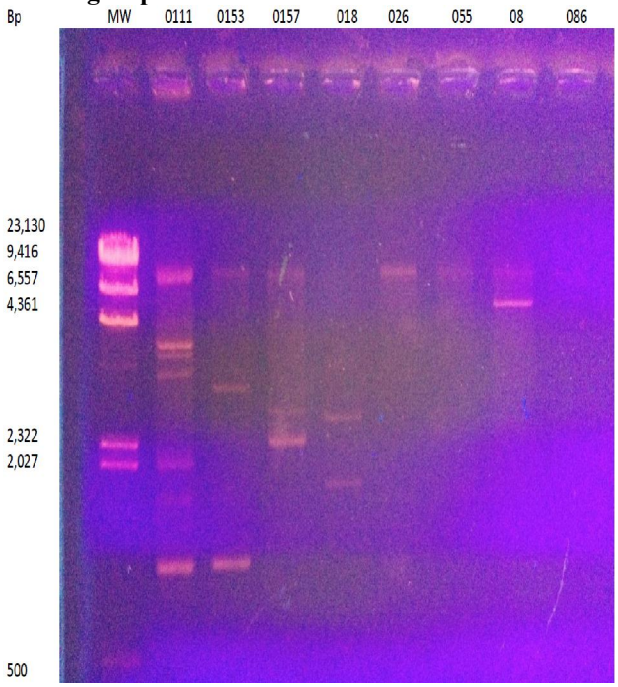
Chemotherapeutic agent	Conc. µg	O55	O111	O26	O153	O8	O18	O86	O157
Enrofloxacin	10	S	S	S	S	S	S	S	S
Neomycin	30	S	S	S	S	S	S	S	S
Amoxycillin	25	R	R	R	R	R	R	M	R
Oxytetracyclin	30	R	R	S	R	R	R	S	S
Chloramphenicol	30	R	R	R	R	R	R	M	R
Streptomycin	10	R	R	S	R	R	R	R	R
Cefadroxil	30	R	R	R	R	R	R	R	R
Colistinsulphate	50	R	R	R	R	R	R	R	R
Gentamicin	10	R	R	R	R	M	R	S	R
Tobramicin	10	R	R	R	R	R	R	R	R
Ampicillin	10	R	R	R	R	R	R	R	R

3.4. Plasmid analysis

Table 3. Plasmid profile analysis of different *E- coli* serogroups recovered from diarrheic calves

Strain	Plasmid and number	Molecular weight
O11	4 plasmids with their super coiled form	7 Kbp 3.5 Kbp 2 Kbp 1 Kbp
O153	3 plasmids	7 Kbp 3 Kbp 1 Kbp
O157	3 plasmids	7 Kbp 2.5Kbp 2.3Kbp
O18	2 plasmids	2.5 Kbp 1.8 Kbp
O26	1 plasmid	7 Kbp
O55	1 plasmid	7 Kbp
O8	1 plasmid with their super coiling	7 Kbp
O86	No plasmid	-

Plasmid Profiling Analysis of different *E.coli* serogroups recovered from diarrheic calves:



4. Discussions

Pathogenic form of *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 **Mahmoud(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*in percentage of 35.83% .These finding 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 buffalo calves affected with calf scours.

Serogrouping of Bacterial isolates of enteropathogenic *E.coli* collected from diarrheic calves revealed 8 different "O" serogroups and"5"strains were untyped. The most prevalent serogroups were O_{55}, O_{111}, O_{26}, O_{153}, O_8, O_{18}, O_{157} .These results agree to certain extended those recorded with **Holland (1990)**who also mentioned that the ETEC isolated from calves was limited to a few serogroups especially O_8, O_9 and O_{101}, while **Donkersgoed (1999)** isolated *E.coli* O_{157} from faecal samples collected from cattle in Canada, also **Champman** *et al.,* **(1993)** isolated *E.coli* O_{157} 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 Zoonozes **Holland** *et al.,* **(1999)** isolated *E.coli* serogroups O_5, O_{26}, O_{111} from both apparently healthy and diarrheic calves, and recorded that *E.coli* strains O_{26} 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 O_{15}, O_{20}, O_{103} and O_{157}

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 disseminating 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-sulfamethoxazol, as well as the emerging problem of fluroqinolone 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 inTable (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, tobramicin 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, kanamysin, chloramphenicol, trimethoprim and cortrimoxaole in treatment of *E.coli* in diarrheic lambs in Spanish, also **Schroeder** *et al.,* **(2002)** and **Guerra** *et al.*, **(2003)** reported that emergence and dissemination of antibacterial resistance in *E.coli* O_{26}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* multidrug 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 **Sherley** *et al.,* **(2003)** Resistance to various antibiotic is relatively common in clinical pathogens in Turkey and also common *E.coli* strains **Özden** *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 O_{86} which have no plasmid and produce multiple drug resistance with many antibiotics, also some serogroups contain4 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

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* O_{157} which have Multidrug resistance harbored plasmids all of which were no less than 2.1 Kbpin 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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