Isolation of *Escherichia Coli* from Diarrhea and Test Their Pathogensity and Susceptibility Pattern for Antibiotic

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Abstract:

This study was designed to isolate *Escherichia coli* from diarrhea in children, and to test susceptibility pattern for antibiotic and pathogenesis of *Escherichia coli*. 150 samples of stool gathered from patients children in different region of Dhi-Qar city in south of Iraq for the period January 2015 to June 2015. the isolate was microscopically and biochemically examined and diagnosed by Api 20E kit Results of the bacterial growth showed that 111 samples were positive bacterial growth with percentage (74%), 84 isolate belong to *Escherichia coli* with percentage 75.6%. The sensitivity of isolates was examined for antibiotics, the isolates showed a height resistance for Ampicillin, while the others showed highest sensitivity for Erythromycin, and Co – trimoxazol. The study showed the ability of *Escherichia coli* to elicit an inflammatory response in mouse intestine after experimental infection that induced by orally dosing with *Escherichia coli*.

Key word: Enteropathogenic *E.coli* , pathogensis ,antibiotic resistance.

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Introduction:

the *E.coli* belong to *Enterobacteriaceae* which is live as harmless commensals in animal intestines (Qadri et al. ,2000) *Escherichia coli* is one of the most common causes of morbidity and mortality in children with diarrhea all over world particularly in developing countries (Enayat *et al*.,2011). Diarrheal diseases continue to be a health problem worldwide. (Passariello *et al*. ,2010; Kosek . ,2003).

For most patients, the illness is a self-limited one. But, disease can cause severe fluids and electrolytes loss, which require prompt treatment. The management of acute diarrhea is based on replacement of fluids. However, antibiotic might be required for the management of the same cases and may reduce the duration of disease, but use is restricted due to emergence of resistance or due to lack of availability in some countries (Phavichitr and Catto-Smith. ,2003).

Antibiotic therapy in hospitals is possibly the most important factor that increases antibiotic-resistant microorganisms (Tacconelli *et al* . ,2009) . The emergence, propagation, accumulation, and maintenance of antimicrobial resistant pathogenic bacteria have become significant health concerns, and lead to increased morbidity, mortality, and health-care costs as a result of treatment failures and longer hospital stays (Levy and Marshall. ,2004; Salma. ,2008).

Despite progress made during the last decade regarding the study of *E.coli* pathogenesis, relatively little is known about *E.coli* -induced physiological changes. In order to adequately define these changes, an animal model is needed. Animal models have been used to study host responses to EPEC homologues; these models include rabbits infected with rabbit *E.coli* (Tauschek *et al* .,2002; Vallance and Finlay. , 2000) there are limitations to the use of this model, such as a paucity of genetic and immunological resource(Abe *et al* . ,1998). Mouse models have the advantage of allowing the use of genetically modified animals for further studies.

The aim of current study is to isolate *Escherichia coli* from diarrhea in children, and to test susceptibility pattern for antibiotic and pathogenesis in mice as model.

Material and Method:
Sample collection:

150 samples of stool collect from the patients children affected with diarrhea   from different region Dhi-Qar, Iraq, during January 2015 – June 2015.

Isolation of *E.coli*:

A swab of faecal sample was cultured directly on MacConkey agar, Eosin methylene blue(EMB). Petri dishes were kept in the incubator for 24 hours at 37ºC (Collee *et al* .,1996). After 24 hours, the plates were examined and studied carefully for the presence of characteristic colonies of *E.coli* . Microorganisms grown on MacConkey agar are capable of metabolizing lactose which produces acid by-products that lower the pH of the media which causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony (Koneman, 2005). Different biochemical tests (Silva *et al*., 1980) were performed for the identification of *E. coli* (Table 1). Api 20E kit (biomeriux, france) also used for identification of the bacteria.

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Table (1): show the Biochemical test of *E. coli*

Indole Test	Methyl Red Test	Voges Proskeur Test	Simmon's Test	
			Ammonium acetate test	Ammonium CitrateTest
+	+	-	+	-

Antibiotic susceptibility test:

All *E.coli* isolates were tested for their susceptibility toward Gentamycin, Rifampicin, Ampicillin, Tetracycline, Co-trimoxazol, and Cefotaxime following the procedure of Bauer et al .,(1966)

Experimental Design (Pathogenesis study):

There were two groups of mice, each group contained three mice, first group Mice were infected with *E.coli* in dose about 0.25ml/mice which contained 1.5×10^8 for 7 days the second group injected with(0.25 ml) phosphate puffer saline ,the animals were observed daily for activity level and water intake, and weight was measured. At various times following infection, animals were sacrificed, and intestinal tissues were processed for further analysis.

Results and Discussion:

Isolation of *E.coli*

According to the cultural, microscopical, biochemical in addition to Api 20E kit Figure (1), One hundred and eleven specimens were positive for bacterial culture. *E. coli* formed 75.6% of total positive specimens.

The dominance with these percentage is because of E.*coli* have many virulence factors such as Attachment and Effacing Factor (AEF) and Fimbrial Adherence Factor (FAF) which make these bacteria able to attached to epithelial layer of intestine ,the *E.coli* also able to produce enterotoxins (Qadri *et al.* ,2000).

Figure (1) Result of API 20 system of EPEC

Antibiotic susceptibility

Results demonstrate that all E. coli isolates were resistant to ampicillin with percentage 100%; while Erthromycin recorded the lowest resistance percentage (14.1%) Table (2).

Table (2) Comparison of nine antibiotic resistance patterns of the E. coli strains isolated from patients children with diarrhea

Antimicrobial agent	No.of isolate		Rate of resistant %
	Resistant	Sensitive	
Gentamycin	36	48	42.8
Ampicillin	84	0	100
Tetracycline	66	18	78.57
Co-trimoxazol	20	64	23.8
Erthromycin	12	72	14.2
Cefotaxime	72	12	85.7

Accurate use of antimicrobials may be beneficial in preserving antimicrobial efficacy and substantially reducing diarrheal illness. However, antibiotic therapy can further increase drug resistance in microorganisms (Tacconelli *et al.*,2009). In this study, we examined antimicrobial resistance of *E. coli* isolates from diarrhea. The highest levels of resistance were observed against Ampicillin and Tetracycline for pathogenic *E. coli*, which may be caused by the frequent use of these antibiotics and the transfer of plasmids between bacteria (Roberts ,2003; Uma,2009). In the *Enterobacteriaceae*, resistance to Ampicillin is mainly because of ß-lactamases (Kliebe *et al.* ,1985).

Pathogenesis Study
Macroscopical Examination
        To define host responses to *E.coli* infection, the mouse intestines was examined . The colon of uninfected mice contained formed pellets of stool beginning just distal to the cecum. However, the proximal colon of animals infected with *E.coli* for 10 days contained semisolid stool, and formed stool pellets were not seen until the distal colon also, the cecum appeared to be slightly engorged in *E.coli* infection.

Histopathological Examination
        The colon of control animals revealed sparse intraepithelial lymphocytes (IELs) and lamina propria polymorphonuclear leukocytes (PMNs), consistent with the normal mucosal histology of mice. In contrast, the numbers of both IELs and lamina propria PMNs were significantly increased in the colon of EPEC-infected mice figure (2). Because intestinal inflammation has been linked to increased goblet cell differentiation (Surawicz *et al.*,1994; Ciacci *et al.* ,2002; Conour *et al.* ,2002; Seto *et al.* , 2003). *E.coli* infection also caused a significant increase in the number of goblet cells. In contrast, acute inflammation, as evident from intraepithelial PMNs and occasional crypt abscesses, occurred in a patchy distribution in the intestine of *E.coli* infected mice and was not present in the intestine of control mice . Together, these data show that *E.coli* elicits an inflammatory response in the mouse intestine.
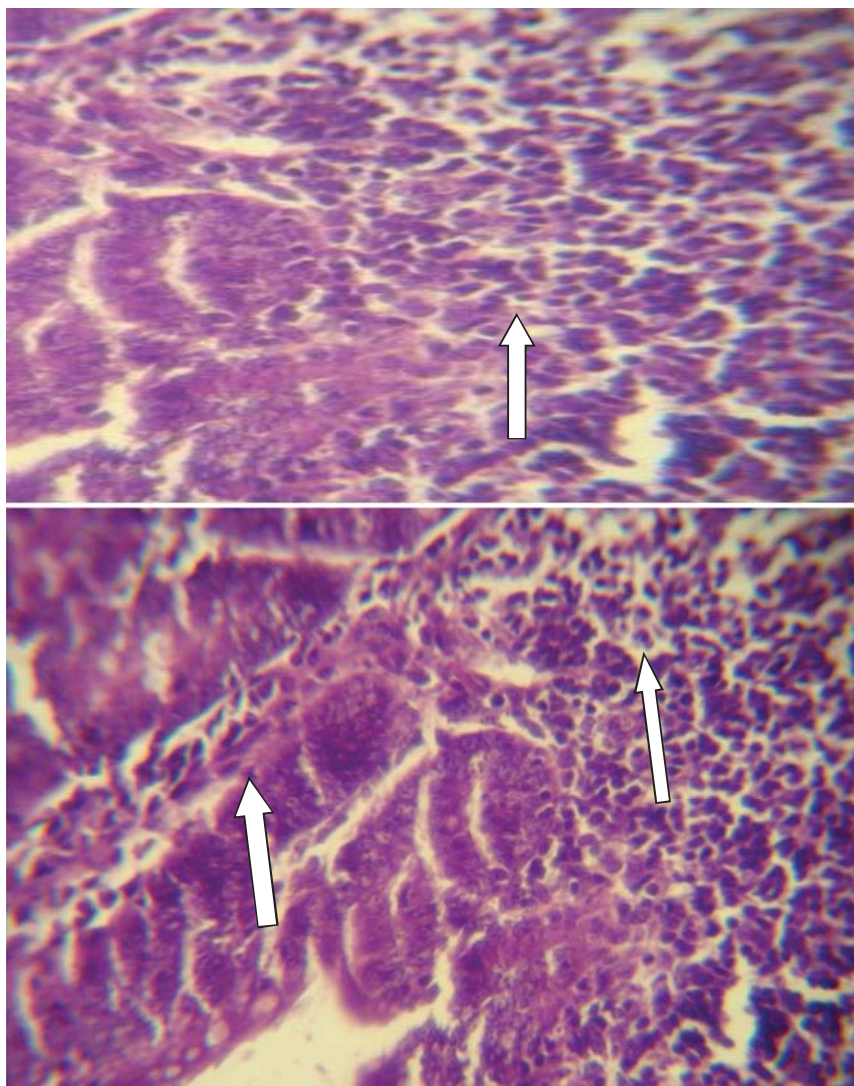
Figure (2): Histological section of intestine of mice infected with *E.coli* showed both IELs and lamina propria PMNs were increased in epithelial lining of intestine ← (H&E 40 X).

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