Estimating LD50 and ID50 Doses of Typical Enteropathogenic Escherichia coli Isolated from human Infantile Diarrhea in Mice

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Abstract:
A systematic study bases on the tow aspects which were bacteriology and clinical singe aspects were done experimentally to Enteropathogenic Escherichia coli (EPEC) isolate from cases of human infantile diarrhea on (BALB/e) mice. The present investigation was carried out on twenty mice of males sex with age range (6 - 8) week old ,which used to estimating lethal dose (LD50) and infective dose of this organism.

In the bacteriological aspect, the results showed the LD50 were (1x106 cells) and infective dose (LD50) were (1x108 cells) of typical enteropathogenic E. coli (EPEC) in mice when inject intraperitoneally. but
The results of the pathological aspect revealed that enteropathogenic E. coli cause dullness , fever ,anorexia , mild thirst , decrease activity .

Keywords: *EPEC; LD50; ID50; Mice and Diarrhea.*

I. INTRODUCTION

Escherichia coli bacteria that are more able to produce disease of the intestinal tract than that of any other organ systems, have all been named earlier as enteropathogenic E. coli. Today the literature describes six main pathotypes of E.coli bacteria differing from each other in their pathogenetic potential (i.e. adhesiveness, invasiveness and toxin production): enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), enteroaggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC) (1).

EPEC is a major cause of infantile diarrhea among children in developing countries (2). The central mechanism of EPEC pathogenesis is a lesion called 'attaching and effacing (A/E), which is characterized by intimate adherence of bacteria to the intestinal epithelium (3). The eaeA gene, which is located in the 'locus of enterocyte effacement' pathogenicity island (LEE), and the bfpA gene, located on a plasmid called the EPEC adherence factor (EAF) have been used for the identification of EPEC and for subdivision of this group of bacteria into typical and atypical strains (4).

Typical and atypical enteropathogenic E.coli (EPEC) strains differ in several characteristics of EPEC. A leading cause of infantile diarrhea in developing countries , is rare in industrialized countries .Where EPEC seems to be a more important cause of diarrhea for EPEC. Also the only reservoirs is humans; for atypical EPEC , both animals and human can be reservoirs. Typical and atypical EPEC also differ in genetic characteristics, serotype, and virulence. Atypical EPEC is more closely related to shiga toxin-producing E.coli (STEC), and strains appear to be emerging pathogens (5) .

To our knowledge, worldwide, typical EPEC's in vivo virulence at the level of infective dose (ID50) and lethal dose (LD50). So that this study was designed to show typical EPEC isolated from cases of human infantile diarrhea. The study included inspection of clinical signs and calculation of ID50 and LD50 by using male BALB/c mice as an animal experimental model depend on (6).

II. MATERIALS AND METHOD

Bacterial isolates:

EPEC isolate was provided at Bacteriology laboratory/ Al-Kut Obstetrics Hospital/Ministry of Health/ Al-Kut city/ Wassit Province/ Iraq. From a 2 years old infant suffered from watery diarrhea for more than 10 days.

Laboratory animals:

A total number of 20 mice (BALB/c) of both sexes with age range (6-8) weeks old were adapted for two weeks before started experiment by reared in separated clean and disinfected cages, they were fed on ad libitum commercial assorted pellets and clean water.

Gram stain:

A single colony from nutrient agar was taken by a loop and spread on a clean slide with fixing by heat, and then staining with gram stains according to (7) and then examined the bacterial cell under oil immersion by using light microscope. Biochemical tests:

Biochemical tests were conducted according to (8)

Preservation of bacterial isolates:

The isolates after definitive diagnosis were cultured on slant of brain heart infusion agar, incubated 37 ˚C for 24 hours; they may be re-cultured monthly to maintain their viability and activity (8) . But The definitive isolates were cultured on brain heart infusion broth and glycerol 20% and incubated at 37 ˚C for 24 hours, then after turbidity occurred, stored in a deep freezing.

Estimating the Infective dose (ID) as follows:-

The bacteria:

Enteropathogenic E. coli (EPEC) was grown on TSA plates at 37°C for 16-18 hr. harvested in PBS (PH=7.2). centrifuged at 200 g for 15 min , and resuspended by using (1) ml of PBS (Ph=7.2) and tenfold dilution (10-1,10-2.10-3,10-4,10-5,10-6,10-7,10-8,10-9 and 10-10) were done.The viable count of the bacteria in each diluent were made according to method (9). Selected the diluents which had these concentrations: (1 ×108 cells) , (1×109 cells) , (1×1010 cells) and (1 ×1011 cells) for drenching the mice groups.

Mice groups:

Twenty healthy mice were divided into 5 groups, each group contained four mice.

EPEC isolate

Was identified serologically as EPEC belonged to serotype O111: K58 by using polyvalent and monovalent antisera and biochemical test (10).

Experimental animals:

In experiment: Twenty mice were used in this experiment (four group each group contains four mice) to estimate the infective dose (ID) and LD50 of the E. coli.

Four groups of mice injected I/P with one of the calculated CFU/ml diluents 0.5 ml and the five groups injected I/P PBS (Ph=7.2) and considered as a control group. All groups were observed for 5 days to calculate the live and dead mice and estimate the LD50 according to (11) but the infective dose (ID) was estimated by choosing the group of mice which showed clinical signs with no mortality. All groups of mice were examined daily for clinical signs which showed after induced infection. The infective dose was considered as the lowest bacterial concentration which when injected the mice which showed clinical signs with no mortality (12).

Data Analysis

Statistical analysis was conducted to determine the statistical differences among the tested groups by using ready–made statistical design: statistical package for social science (SPSS).

III. RESULTS AND DISCUSSION

Identification of the bacteria :

The identification of the bacteria was ensured according to the 8 by following these steps :

Cultural characteristics :

The colonies appeared small, smooth , rounded and pale colonies in agar , trypticase soya agar and brain heart infusion agar , while in MacConkey agar plates which was also used to differentiate lactose from non lactose fomenters. The isolates were diagnosed biochemically according to (13).

Microscopic examination:

The bacteria appeared in light microscope as gram negative, coccobacilli and single cells bacteria after 18 – 24 hours post incubation at 37°C .

Biochemical identification: The biochemical identification of the bacterium showed that this bacterium were catalase positive , oxidase , lactose formative and indole positive . TSI (triple sugar iron) a positively indicator for E. coli in a result : yellow (in slant) ⁄ yellow (in bottom) with gas and no H2S production , motile , have ability to gas production , unable to liquefied the gelatin and urease negative show in table(1)

Table 1: Results of some biochemical of E. coli .

Biochemical test	Oxidase test	Catalase test	Urease test	Motility	Indole	TSI test H2S Co2
Result	-Ve	Ve+	Ve-	Ve+	Ve+	A⁄A - +

Results of experiment:

Estimating the LD50 infective dose:

The results of LD50 and infective dose (ID) of typical EPEC isolate in mice were injected I⁄P with bacteria have revealed that (1x106 CFU⁄ml) and (1x108 CFU⁄ml) respectively which estimated by calculating the dead and alive mice in each group during 5 days as showed in table (2).

Table 2: Calculation of LD50 and ID50 of typical EPEC isolate form human infantile diarrhea using male BALB/c mic)(

Mice Groups	Dose (CFU/mouse)	Alive	Dead	Total alive	Total dead	Percent mortality %
1	1×1011	0/4	4/4	0	9	100
2	1×1010	0/4	4/4	0	5	100
3	1×109	3/4	1/4	3	1	25
4	1×108	4/4	0/4	7	0	0

The clinical signs were inspected every 24 h for five days by monitoring mice injected with 1×108 CFU/mouse (ID50).so the mice injected with typical EPEC showed dullness after 24 hours post infection, tending to aggregate in one place which may refer to suffering from fever, loss of appetite, thirst when compared the quantities of consumed water with that consumed in the control group , decreased activity, very mild diarrhea , the color of feces was changed from dark black as in before injecting and when compared with feces of the mice in the control group to light yellow and paste in consistency .While the mice in the group I control group did not show any clinical signs or disorders during the same period. As it is obvious from the results mentioned above, typical EPEC did not differ significantly from each other for all this study included criteria, which means that typical EPEC have the same virulence for mice injected intraperitoneally. Although mice are usually quite resistant to E. coli infection, the mouse virulence of an E. coli 018:Kl strain, isolated from a case of neonatal meningitis, was high (LD50 4 x l05 bacteria/mouse) . An intraperitoneally injection of this strain resulted in peritonitis, followed by increasing numbers of bacteria in the blood .While the duration of the infection was short. If the mice survived, the bacterial counts in the mice started to decrease by 24 h, and the animals were free from the infection within 48 hours (14).

Furthermore, if the convalescent mice were challenged after 48 h with another lethal dose of E.coli, they were highly resistant to the infection. The LD50 of the E.coli O18: K1 strain was at least 50-fold higher in the convalescent mice than in normal mice (> 2 x l07 and 4 x l05 CFU/mouse), respectively (14).

Used mice models to determine the minimum lethal dosage (MLD) of E. coli The MLD was found to be an intra-peritoneal (I/p) injection of 0.5 ml of 10 7CFU/ ml as it induced fatality in all replicates within 24 hours (15). Estimated infective dose (ID) and LD50 dose of Salmonella hader isolated from goat in mice were (1.5×107 CFU/ml) and (1.5 ×109 CFU/ml) respectively.(16). This study agree with (17) they showed mice were weight loss and symptoms EPEC colonized the intestine after challenge EPEC causes profuse watery diarrhea. Primarily in children under the age of 2 years, and mostly affects individuals residing in developing countries. In contrast , adults and children infected by EHEC bacteria can suffer from either bloody or non bloody diarrhea , and in a small percentage of cases a life-threatening complication know as hemolytic uremic syndrome occurs (18) .

Also the study difference with (19) who reported not detect any clinical or pathological signs caused the infection of pooled EPEC bacteria or rather Canadian reference EPEC strain (O45, eae-) of pooled EPEC of the oral infection (1- 5 x 10 CUF/animal) via intragastric tube of experiments in weaned pigs .

IV. CONCLUSIONS

(1) Enteropathogenic E. coli (EPEC) are invasive strain.

(2) The invasion of this strain is rapid in blood stream and reach to the internal organs of mice.

(3) The study showed that Enteropathogenic E. coli (EPEC) can cause similar clinical signs of other ssp. Of E.coli.

(4) This strain is able to cause systemic infection with very mild diarrhea, dullness, fever, anorexia, mild theist and decrease activity.

References

1-Piérard D, De Greve H, Haesebrouck F, Mainil J. O157: H7 and O104: H4 Vero/Shiga toxin-producing Escherichia coli outbreaks: respective role of cattle and humans. Veterinary research. 2012 Dec;43(1):13.

2- Abdelhamid M. Dhiarroginic Enteroaggregative, Shiga Toxin-producing Escherichia coli and Enterobacteriaceae in Hospitalized Diarrheal Children. Curr Sci Int. 2015;4:127-35.

3- Dupont A, Sommer F, Zhang K, Repnik U, Basic M, Bleich A, Kühnel M, Bäckhed F, Litvak Y, Fulde M, Rosenshine I. Age-dependent susceptibility to enteropathogenic Escherichia coli (EPEC) infection in mice. PloS pathogens. 2016 May 9;12(5):e1005616.

4- Albalawi, S. (2016). Developing a Multiplex PCR Platform to Determine the Virulence Genes of Escherichia Coli in Boulevard Lake, Thunder Bay, Ontario (Doctoral dissertation).

5- Hu, J., & Torres, A. G. (2015). Enteropathogenic Escherichia coli: foe or innocent bystander. Clinical Microbiology and Infection, 21(8), 729-734.

6- O'Ryan M, Vidal R, del Canto F, Carlos Salazar J, Montero D. Vaccines for viral and bacterial pathogens causing acute gastroenteritis: Part II: Vaccines for Shigella, Salmonella, enterotoxigenic E. coli (ETEC) enterohemorragic E. coli (EHEC) and Campylobacter jejuni. Human vaccines & immunotherapeutics. 2015 Mar 4;11(3):601-19.

7- Jawetz, J. ; Melnick, E. and Adelbergs, E. (2007). "Enterobacteriaccae" In: Review of medical microbiology ". 24th ed. by Geo. F. ; Janet S. and Stephen, MeGraw- Hill companies, U.S.A., pp.256-261.

8- Quinn, P.J.; Carter, M.E.; Markey, B. and Carter, G.R. (2004): " Clinical Veterinary microbiology" 6th ed. Mosby an imp. Wolf , London.

9- Delbeke S, Ceuppens S, Jacxsens L, Uyttendaele M. Microbiological analysis of pre-packed sweet basil (Ocimum basilicum) and coriander (Coriandrum sativum) leaves for the presence of Salmonella spp. and Shiga toxin-producing E. coli. International journal of food microbiology. 2015 Sep 2;208:11-8.

10- Gharieb, R. M., Fawzi, E. M., Attia, N. E., & Bayoumi, Y. H. (2015). CALF DIARRHOEA IN SHARKIA PROVINCE, EGYPT: DIAGNOSIS; PREVALENCE, VIRULENCE PROFILES AND ZOONOTIC POTENTIAL OF THE CAUSAL BACTERIAL AGENTS.

11- Reed, L .J. and Muench, H. (1938). "A simple method of estimating fifty percent end point". Am. J. Hyg., 27(16): 8739-8744.

12-Ramakrishnan MA, Muthuchelvan D. Influence of Reed-Muench Median Dose Calculation Method in Virology in the Millennium. Nature. 2018;8:9633.

13- Wanger A, Chavez V, Huang R, Wahed A, Dasgupta A, Actor JK. Microbiology and Molecular Diagnosis in Pathology: A Comprehensive Review for Board Preparation, Certification and Clinical Practice. Elsevier; 2017 Jun 13.

14- Vuopio-Varkila J. Experimental Escherichia coli peritonitis in immunosuppressed mice: the role of specific and nonspecific immunity. Journal of medical microbiology. 1988 Jan 1;25(1):33-9.

15-Lemuel BR, Remedios S, Flamiano MS. Use of bacteriophages as therapy for Escherichia coli-induced bacteremia in mouse models. Phil. J. Microbiol. Infect. Dis. 2004;33(2):47-51.

16-Al-Naqeeb, M.M.N. (2009)." Study the pathogenesis of Salmonella hadar which isolated from goats in mice". M.SC. thesis, Vet. Med. College/Baghdad University – Iraq.

17-Adamu MS, Ugochukwu IC, Idoko SI, Kwabugge YA, Abubakar NS, Ameh JA. Virulent gene profile and antimicrobial susceptibility pattern of Shiga toxin-producing Escherichia coli (STEC) from humans in Maiduguri, Borno State, North-Eastern Nigeria. Comparative Clinical Pathology. 2018 Mar 1;27(2):341-51.

18- Mellies JL, Lorenzen E. Enterohemorrhagic Escherichia coli virulence gene regulation. InEnterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli 2015 Jan 1 (pp. 199-209). American Society of Microbiology.

19- Abubakar RH, Madoroba E, Adenubi O, Morar-Leather D, Fasina FO. Bacterial pathogens of pigs with particular reference to Escherichia coli: A systematic review and meta-analysis. Journal of Veterinary Medicine and Animal Health. 2017 Jul 31;9(7):159-85.