------ Raf. Jour. Sci., Vol.18, No.12 Biology, Special Issue, pp.13-21, 2007 ------

Histological Effect of *Escherichia coli* Strains Isolated From Children Diarrhoea Cases on Intestinal Mucosa of Infant Mouse

Sarab D. Sulyman
Department of Biology
Collage of Science
Mosul University

(Received 22/9/2003;Accepted 13/4/2004)

ABSTRACT

In this study strains of *Escherichia coli* that isolated from children diarrhoea cases had been improved to produce different enterotoxines, heat stable enterotoxin (ST) and heat labil enterotoxin (LT) inducing positive responses by biological tests such as suckling infant mouse and rapid skin permeability test (for ST enterotoxin) and delayed permeability test of rabbit. (for LT enterotoxin) Histological effect of crud culture of *Escherichia coli* on the mucosal intestine of infant mouse had been studied and showed significant tissue damage .lncluding detachment Exfoliation and sloughing of the surtace epithial cell of mucosa of infat mouse.

These data Supported the idea that the enterotoxin is an important virulance factor in *Escherichia coli* associated diarrhoea that attak the mucosal intestine of infant mouse and microvilli of it .

التأثيرات النسيجية لبكتريا *Escherichia coli* المعزولة من حالات اسهال الاطفال على الطبقة المخاطية لامعاء الفئران الرضيعة

الملخص

تم في هذه الدراسة اثبات انتاج السموم المعوية من قبـل سـلالات بكتريـا *Escherichia coli* المعزولة من حالات اسهال الاطفال دون سن السادسة من العمر في مدينة الموصل، كالـسموم المعويـة الثابتة حرارياً (Heat stable enterotoxine ST) والسموم المعوية التالفة بـالحرارة (Heat labile enterotoxine LT) وذلك بأستخدام بعض الاختبارات البايولوجية مثل اختبار تجريع الفئران الرضيعة (Suckling infant mice) واختبار النفاذية السريع لجلد الارنب (rapid skin permeability test) بالنسبة للسموم المعوية الثابتة حرارياً واختبار النفاذية المتأخر لجلد الأرنـب (Delayed permeability skin test of rabbit) بالنسبة للسموم المعوية التالفة بالحرارة.

كما درست التاثيرات النسيجية والمرضية لمعلق هذه البكتريا على الطبقة المخاطية لامعاء الفئـران الرضيعة وتبين بانها تلتصق بالطبقة المخاطية للامعاء وتسبب ازالة الزغابات المعوية وتلفاً وهدماً لمعظم طبقات الامعاء.

INTRODUCTION

Enterotoxines are recognized as an Important pathological attribute and an important virulance factor of some diarrhoea inducing bacteria, Diarrhoeal diseases are important causes of mortality and morbidity in various age groups especially infant in developing countries (WHO,1989). *Echerrichia coli* is the head of the larg bacterial family, Enterobacteriaceae , that live in the intestinal tracts of health and diseased animals. *E. coli* is responsible for three types of infection in humans, urinary tract infection (UTI) neonatal meningitis, and intestinal disease gastroenteritis (Todar K., 2002) .

Five classes (vitro types of E.coli that cause diarrhoeal diseass are recognized, Enterotoxigenic *E. coli* (ETEC), Entero invasive *E. coli* (EIEC), Entero hemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enteroaggregative *E. coli* (EaggEC), each class fall within a serological subgroup and manifest distinct feature pathogenesis (Koneman et al., 1997).

Enterotoxigenic *Escherrichia coli* (ETEC) strains are frequent cause of diarrhoeal disease through out the world and are a major cause of diarrhoea in children (Neill et al., 1994).

(ETEC) infection usually follows ingestion of contaminated water or foods producing watery diarrhoea, nausea, abdominal cramps and low grad fever in infants (Sommerfelt, 1996) and can cause severe diarrhoea in humans and animals by production of two distinct types of toxins, a heat labil enterotoxin (LT) with two sub types (LT_1 and LT_{11}) and a family of heat – stable enterotoxin (ST_a , ST_b) (Soderlind et al., 1988 ; Handl et al., 1993). These enterotoxins reversibly ulter normal intestinal homeostasis , causing intestinal secretion and diarrhoea (Betly et al., 1986; Weikel et al., 1986) LT enterotoxin is a high molecular mass toxin (185 KDa) functionally and structurally related to *Vibrio cholera* toxin (Sprangler, 1992) ST_a are low molecular mass toxin that retain toxic activity after incubation at 100°c for 30 min, where as LT loses activity under these conditions (Weikel et al., 1986).

Heat stable toxins are classified by their methanol solubility and biological activity , (ST_a or ST_1) is methanol soluble and induces intestinal secretion in infant mice and neonatal pigs. (ST_b or ST_{11}) is methanol insoluble and induces intestinal secretion in neonatal pigs but dose not affect infant mice (lortie et al., 1991).

(LT) produced by enterotoxingenic *E. coli* (ETEC) is a causative agent of diarrhoea in humans and other animals such as pigs, chickens, and cows (Tsujii et al., 1988) LT is composed of A and B subunits, The B subunit bind to Gm_1 ganglioside on the cell membran and allows the toxin to attach to target cells (Tsuji et al., 1995).

MATERIAL AND METHODS

Escherichia coli strains were taken from Department of biology / College of science / University of Mosul. (which were isolated from diarrhael children under six years of age in Mosul .

The isolates were identified according to standard method using Biomerieux Diagnostic antisera polyvalent and monovalent antisera Biomerieux Laboratories (France).

Enterotoxigenicity of the strains were determined using different biological tests that proved the production of enterotoxins.

Preparation of Enterotoxin

Cell free culture supernatant of E. coli which disgnated (CFCS) were prepared according to the procedure of (Rahman et al., 1992). 0.1 ml of 24 h culture of test organism was added to 100 ml of BH1 broth in a 250 ml Erlenmeyer flask and further incubated at 37°C for 18 h on a rotary shaker 200 rpm/min (this bacterial suspension was ingected intragastrically in suckling infant mouse and the intestine of mice which gave positive result, was taken for histological studies).

Then culture suspension was centrifuged (600 xg , 45 min at 4°C) the supernatant was collected, membran filtered (Millipore, 0.22 Mm) and stored at 4°C as crud enterotoxines for further use.

Entrotxin Assay

Heat Stable Enterotoxin Producation(Assay Of Sta Activity) Infant Mouse Assay :

this was carried out essentially according to (Dean et al., 1982) suckling mice (3-4) days old were ingected intra gastrically by the percutanous rout with 0.1 ml of culture supernatant fluid (cfcs) the ratio of the intesinal weight to the remaining body weight were determined 4 h. post inoculation, samples yielding ratio $\geq$ 0.083 were considered positive, between 0.075–0.082 were considered doubtful and ratio $\leq$ 0.074 were considered as a negative.

Rabbit skin permeability tests

Tests were performed for both the rapid permeability factor (RPF) and delayed permeability factor (DPF) according to the procedure described by sandefur and peterson 1976.

Adult Newzeland albino rabbits were shaved and the remaining hair was removed with a depilatory cream prior to skin testing. these rabbits were injected intradermaly with 0.1 ml of (CFCS) of E. coli for both (RPF) and (DPF). these rabbits were injected also intravenously with 2 to 3 ml of 5 % (w/v) .Evan blue dye 1 hour post inoculation.

The diameter of the blue zones which. corresponds to the areas of erythema were measured in millimeter. after 30 min a reaction of at least 4 mm in diameter was regared as positive for rapid permeability factor.

The zones of delayed bluing and induration after 24 – 48 h indicated the positive results for (DPF).

Histological studies :

Intestin of infant mice that suckled with 0.1 ml of bacterial suspension of *Escherrichia coli* and showed positive responses was taken and excised and washed with normal saline and then immersed in 10 % neutral buffered formalin solution . Formalin

fixed pieces of intestin were embeded in paraffin sectioned 4 um thick and stained with hematoxylin and eosin by standard – techniques of (McGinnis et al.,1986).

RESULTS AND DISSCUSION

This study improved the ability of *Escherichia coli* strain isolated from children diarrhoeal case to produce heat labil (LT) and heat stable (ST_a) enterotoxin which are causative agent of diarrhoea in humans and other animals.

Enterotoxicity of crud preparation

Suckling infant mouse :

(CFCS) of E.coli strains was positive for the assay of ST_a activity with a ratio of gut weight to remaining body weight 0.93 as it shown in Fig (1). these results improved as other reportes that (ST_a) is not associated with the cellular fraction but found preferentially in the culture supernatant (Kupersztoch et al., 1990 ; Suarez et al., 1987) and many studies have reported poor yield of toxin from wild – type strains (Urban et al., 1990 ; Dubreuil et al., 1991).

Fig. (2) this specific rapid bluing reaction remained visible for several hours, but appeared to fade in 18 to 24 h.

Rapid and delayed skin permeability factors :

It was observed that crud filterate (CFCS) of E. coli strain exhibit a rapid vascular permeability response of skin appeared within 5 min as a solid blue spot as illustrated in In contrast the delayed permeability factor (DPF) is characterized by delayed on set of induration accompained by bluing as shown in Fig. (3).

This (DPF) is characterized as a heat labil enterotoxin and destroyed in 30 min at 75 – 100 ℃ (Sandefer and Peterson , 1976).

These results of biological tests improved the ability of E. coli to produce several types of toxins ST and LT enterotoxin which agreed with many reports .
(Busque et al., 1995 ; Tsnji et al., 1995 ; Ram et al., 1993 ; Lortie et al., 1991 ; Dubreuil, 1997).

Histological studys :

Compared with Fig.(4), (control), the result of this study indicates as shown in Fig. (5) severe tissue damage including detachment Exfoliation and sloughing of the surface epithial cells of mucosa of the infant mice. caused by enterotoxigenic *Escherrihia coli*. (Embaye et al., 1989) concluded that the initial binding of Enteropathogenic strains of E.coli to enterocytes appeared to be to the mucus layer overlying the brush border and to globet cells. this was followed by brush border effacement in areas close to the bacteria and they demonstrated that enteropathogenic E. coli can attach to gastric, duodenal, jejunal, ileal and colonic mucosa from rabbit to produce effacement of microvilli in vitro .

(Rousset et al., 1998) also indicated that the interaction of most extracellular toxin like *E. coli* enterotoxine to host cells is thought to be an important first step in pathoganic event is most likely to take place through the binding of the toxin to specific receptors on the target cells .

Finally Enterotoxigenic *E. coli* strains which produce enterotoxin are a world wide econmically important agent of diarrhoea in animals and human (Spangler, 1992).

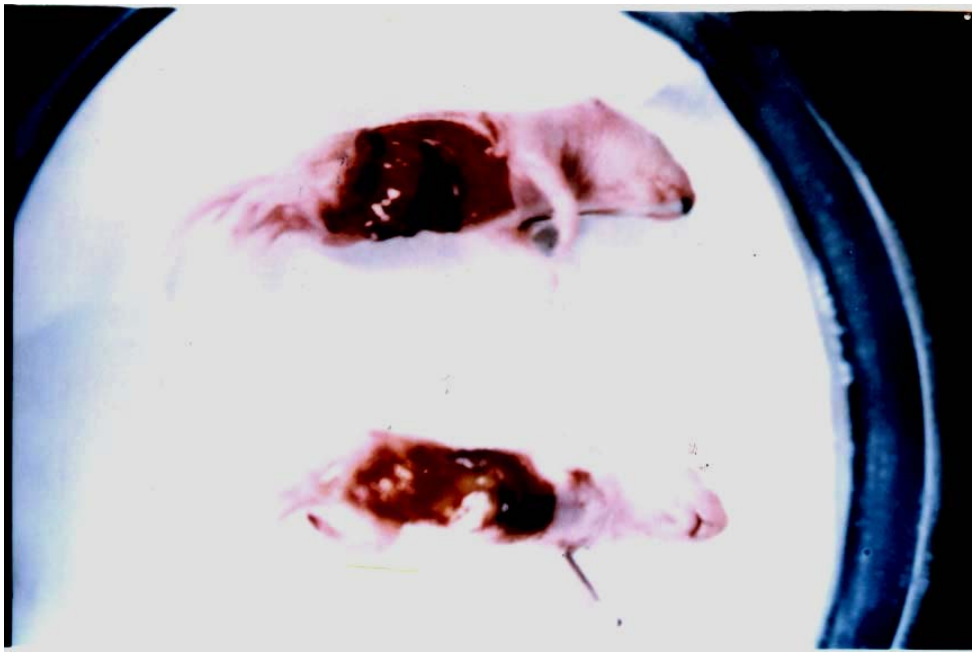

Fig. 1 : Positive result in suckling infant mouse test (mice in the upper) according to the (mice in the lower) that injected with 0.1 ml of sterilised brean heart infusion broth .

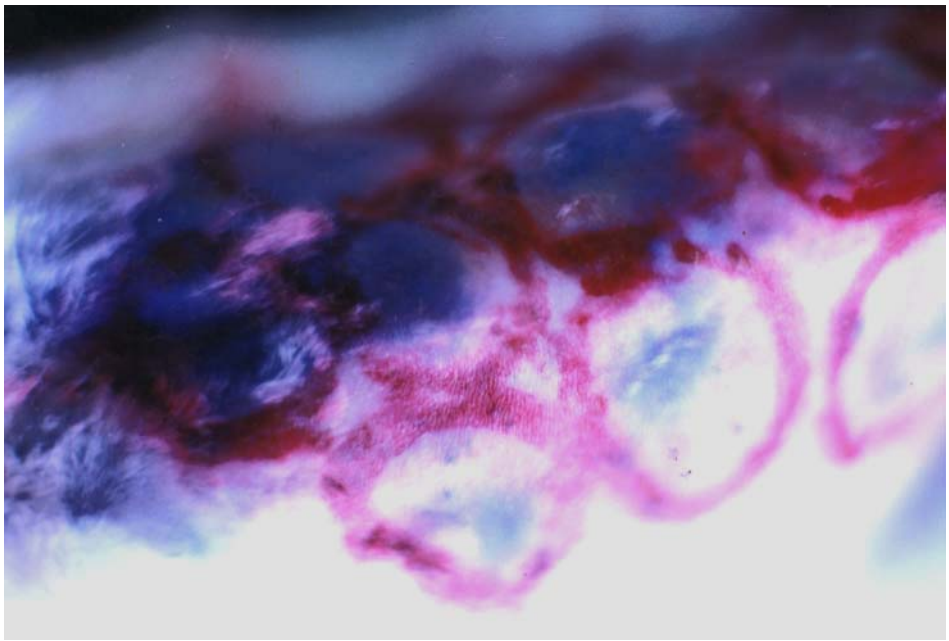

Fig. 2 : Appearance of Rapid PF when dye is administered 1 h after skin testing .

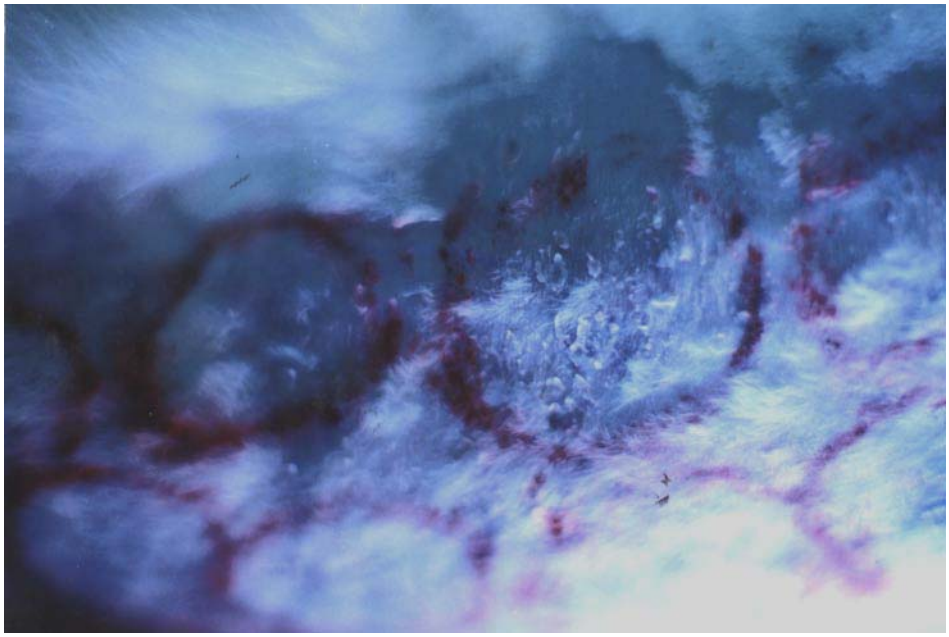

Fig. 3 : Delayed induration and bluing demonstrated within 24 h .

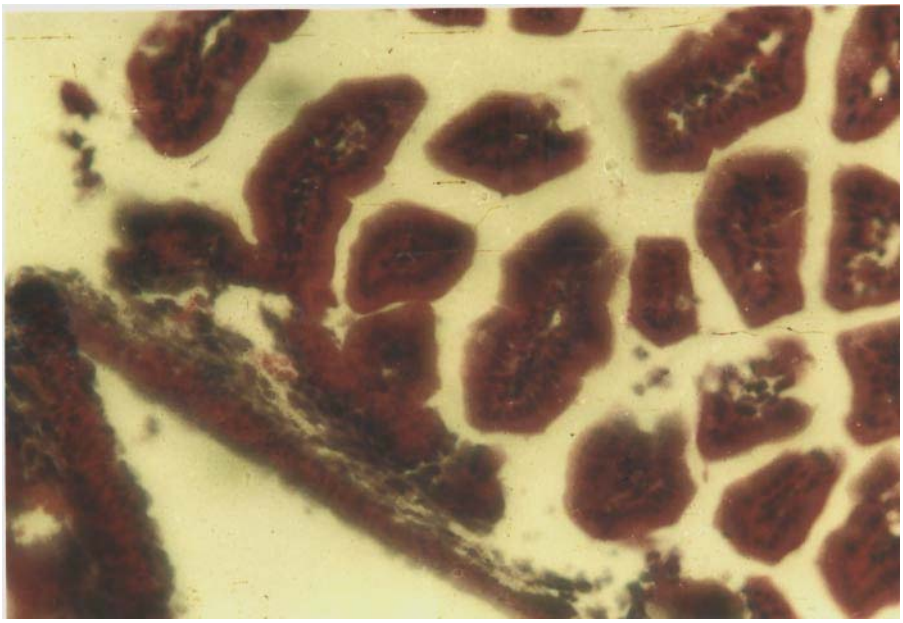

Fig. 4 : Intestinal mucosa of infant mouse suckled with 0.1 ml sterilised brain heart infusion.magnification 160 x (hematoxylin and Eosine staning).

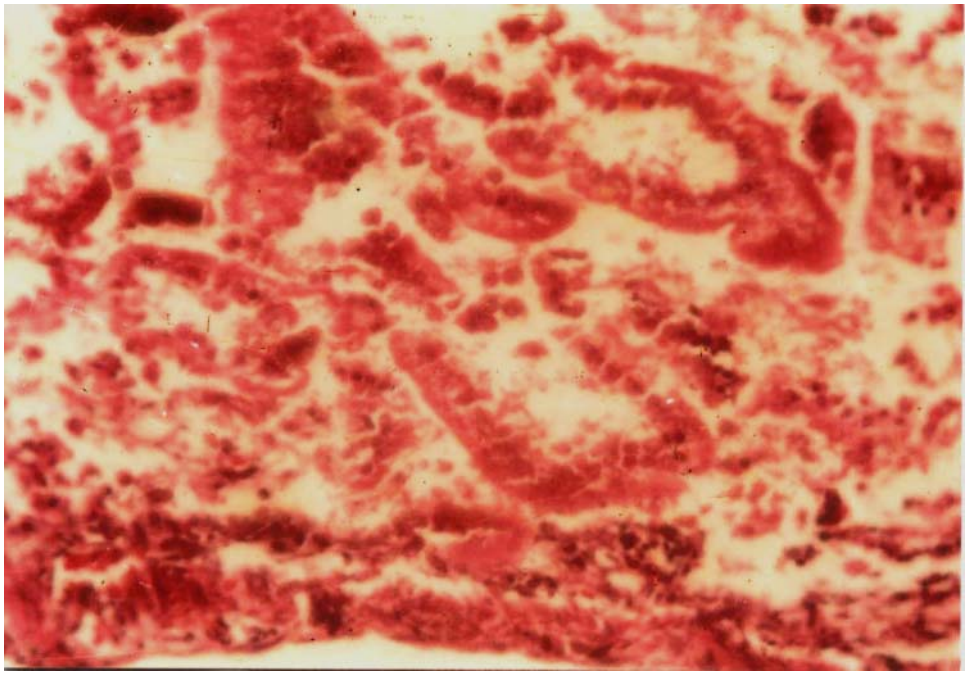

Fig. 5 : Intestinal mucosa of infant mouse suckled with 0.1ml of bacterial suspension of Escherichia coli showing the effaeement and detachment of mueosal layerss 160 x (hematoxylin and Eosin staining).

REFERENCES

Busque, P. Letellier , A. Harel, J. and Dubreuil, D., 1995. Production of *Escherichia coli* ST_b enterotoxin is subject to catabolite repression microbiology 1 : 1621p. .

Batly, M.J., Miller, V.L. And Mekalanos, J.I., 1986. Genetic of bacterial enterotoxins Annu. Rev. microbiology 40 : 577p. .

Caloca, M.J. , Suarez, S., Soler, J., 1997. Metabolic changes in red cells in response to adhession of porcin k 88 fimbriated *Escherrichia coli* veterinary microbiology . 758: 45p. .

Dean, A.G. , Ching, C.Y. , Williams, R.G. and Harden, L.B., 1982. test for *Escherrichia coli* enterotoxin using infant mice application in a study of diarrhea in Honolulu . J. infect. Dis 125 : 407p. .

Dubreuil, J.D., Fairbrother, J.M., Lallier, R. and Larivieres, 1991. production and purification of heat stable enterotoxin b from a porcin *Escherrichia coli* heat – stable enterotoxin B . proc. Natl Aced . sci. U.S.A. 90 : 3202p. .

Dubreuil, J.D., 1997. *Escherrichia coli* ST_b enterotoxin . Microbiology 143 : 1783p. .

Embaye, H., Batt, R.M. , Saunders, T.R., Getty, B. , and Hart, C.A., 1989. interaction of entero pathogenic *Escherrichia coli* with rabbit intestinal mucosa in vitro . Gastroenterology 96 : 1079p. .

Guerrant, R.L., Hugues, J.M., Lima, N.l. and Grane, J., 1990. Diarrhoea in developed and developing countries magnitude special setting and etiologies Rev . infect Dis. Suppl 12 : 841p. .

Handl, E.C. and Flock, J. I., 1992. ST_b producing *Escherrichia coli* are rarely associated with human infantil diarrhoea . J. Diarrhoeal Dis. Res 10 : 37p. .

Handl, C.E., Harel, J. Flock, J.I. and Dubreuil, J.D., 1993. High yield of active ST_b enterotoxin from a fusion protein ($MB_b - ST_b$) Expressed in *Escherrichia coli* protein Expression and purification pp.4-275 .

Koneman, E. W., Allen, S.D. , Janda, W.M. , Schrecken, Berger, P.C. , Winn W. C. , 1997. Color atlas and text book of diagnostic microbiology 5 th. ed. Lippincott – Raven publishers , philadelphia , U.S.A. : 951p.

Kupersztoch, Y.M., Tochias, k. , Moomaw, C.R. , Dreyful, A. , Urban, R. , Slaughter, C. and Whipp, SC., 1990. Secretion of a methanol – insoluble heat stable enterotoxin energy and Sec. A dependent conversion of pre – ST_b to an inter mediat indistinguishable from the extra cellular toxin J. Bacteriol 172 : 2427p. .

Lorlie, L.A., Harel, J. , Fairbrother, J.M. and Dubreuil, J.D., 1991. Evalution of three new techniques for the detection of ST_b – positive *Escherrichia coli* straine molecular and cellular probes 5 : 271p. .

Lortiel, A., Dubreuil, J.D., Harel, J., 1991. Characterization of Escherichia coli strains producing heat – stable enterotoxin b (ST_b) isolated from humans with diarrhea . J. clin microbiol 29 : 656p. .

Mc, Ginnis, M.R. , Rinaldi, M.G. , Winn, R.E., 1986. Emerging agents of phaeohyphomy Cosis pathogenic species of Bipolaris and Exserohilum J. clin microbiol pp.24-250 .

Neill, M.A., Tarr, P.I., Taylor, D.N. and Trofa, A.F., 1994. Escherichia coli in : Hui Y.H., Gorham J.R., Murrel K.D. and Cliver D.O (Eds) Fool born Disease. Hand book . Vol. 1 , Marcel Dekker New York , 169p.

Rahman, H., Singh, V.B., Sharma, V.D. and Harne, S.D., 1992. Salmonella Cytotoxic and Cytolytic factors their detection in chinease hamester ovary cells and antigenic related ness . Vet. Microbiol 31 : 379p. .

Ram, S., Khurana, S., Khurana, S.B., 1993. Coagglutination test for detection of heat labil enterotoxin producing *Escherrichia coli* strains indian J. Med . Res . [A] 97 , March 1993 : pp. 64. Rousset, E., Harel, J. and Dubreuil, D., 1998. Sulfatid3e from the pig jejunum brush border Epithial cell surface is involved in binding of E. coli Enterotoxin b infection and immunity 66 : 5650p. .

Sandefur, P.D., Peterson, J.W., 1976. Isolation of skin permeability factors from culture filterate of *salmonella typhimurium* , infect . immun 14 : 671p. .

Spangler, B.D., 1992. Structure and Function of cholera toxin and the related *Escherichia coli* heat – labil entrotoxin . Microbiol Rev. 56 : 622p. .

Soderlind, O., Thafvelin, B. and Mollby, R., 1988. Virulence factors in *Escherrichia coli* strains isolated from swedish piglets with diarrhoea. J. clin . Microbiology 26 : 879p. .

Sommer, felt H., steinsland, H., Grewal, H.M.S., Viboud, G.I., Bhandari, N. Gaastraw, Svennerholm, A.M. and Bhan, M.K., 1996. Colonization factors of Enterotoxigenic *Escherrichia coli* isolated from children in north india. the Journal of infectus Diseases 174 : 768p. .

Todar, Kenneth, 2002. Bacteriology at UW – Madison c 2002 Kenneth Todar University of Wisconsin – Madison department of bacteriology (internate).

Tsuji, T., Joya, T.E., Yao, S., Honda, T. and Miwatani, T., 1988. Purification and charactarization of heat – labil enterotoxin isolated from chicken enterotoxigenic *Escherrichia coli* FEMS Microbiol . Lett 52 : 79p. .

Tsuji, T., Watanabe, K. and Miyama, A., 1995. Monomer of the B subunit of heat labile Enterotoxin from Enterotoxigenic *Escherrichia coli* Has little ability to bind to GM1 Ganglioside Compared to its Coligenoid . Microbiol . Immunol 39 : 817p. .

Urban, R.G., Dreyfus, L.A. and Whipp, Sc., 1990. Construction of a bifunctional *Escherrichia coli* – heat stable enterotoxin (STb) – alkalin phosphatase fusion protein . infect immum 58 : 3645p. .

Weikel, C.S. , Long – Krug S. and Guerrant, R.L.,1986. E. coli ST_b . anew mechanism for Secretory diarrhoea in advances in research on cholera and related Diarrheas 333p.

World Health organization . Development of Vaccines against cholera and diarrhoea due to E. coli . Report of meeting held at the University of Maryland Baltimor . U.S.A. 6 – 9 December 1989.