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انتاج السموم بواسطة العترات المختلفة من ميكروب القولون النموذجي

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نبيلة رشوان

قد تم عزل ٤٤ عترة من ميكروب القولون النموذجي من الأطفال المصابين بالاسهال  
وكان منهم ١٩ عترة من النوع المعدي منهم ١٧ ( ٦٤ - ٣٨ % ) تتبع  $O_{55} K_{59} B_5$   
وعترة واحدة لكل من  $O_{128} K_{62} B_{12}$  ،  $O_{26} K_{60} B_6$   
واختبرت الـ ٤٤ عترة في الفئران المولودة حديثا على مقدرة افراز السموم وتوصلت  
النتائج الى مقدرة افراز السموم بواسطة ٧ ( ٣٦.٦٤ % ) من العترات المعديـة  
و ٣ ( ١٢ % ) من العترات الغير المعديـة .  
وأظهرت هذه النتائج الأهمية العلمية في استخدام هذا الاختبار للوصول الى  
العترات التي لها القدرة على انتاج السموم لميكروب القولون النموذجي .

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**TOXIN PRODUCTION BY DIFFERENT STRAINS OF E. COLI**  
(With 2 Tables)

By  
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**SUMMARY**

Forty four strains of E.coli were isolated from children suffering from diarrhoea. Among which 19 strains were typed as enteropathogenic E.coli, 17 (38.64%) strains belonged to  $O_{55}/K_{59}$  ( $B_5$ ), one (2.27%) strain to each of  $O_{26}/K_{60}$  ( $B_6$ ), and  $O_{128}/K_{67}$  ( $B_{12}$ ).

All the 44 strains were tested for toxigenicity by the infant mouse test. The results showed that enterotoxin was produced by 7 (36.84%) of the enteropathogenic strains and by 3 (12%) of non-enteropathogenic strains. These findings showed the importance of this test for the detection of toxigenic strains of E.coli.

**INTRODUCTION**

E.coli are frequently isolated from diarrhoeal stools with no association of other pathogens. This raised the possibility of the pathogenic role of these strains. Early, in 1945, Bray noticed that these pathogenic E.coli usually belonged to certain serotypes.

Certain strains of E.coli were able to produce an enterotoxin which caused fluid accumulation in ligated ileal segments of adult rabbit and other animals (DE, et al. 1956; SMITH and HELLS, 1967. There is a similar evidence from experiments in volunteers (DUPONT, et al. 1971).

Two E.coli enterotoxins have been well studied, a heat labile toxin immunologically related and similar in mechanism of action to cholera toxin, and a heat-stable toxin, a small non-immunogenic entity with an unknown mechanism of action (GORBACTS, 1974 BRINTEN, 1978; DENEKE, et al. 1980; WADSTROM, 1980 a,b).

The discovery of enterotoxigenic and entero-invasive E.coli led to investigation of the role of these two pathogenic mechanisms in E.E.C. serotypes. Such studies showed that enteroinvasive and enterotoxigenic E.coli are not restricted to recognised E.E.C. serotypes, and in fact, most E.E.C. serotypes are devoid of these recognised diarrhoeal factors (GOLD SCHMIDT and DUPONT, 1976). As a result, the significance of E.E.C. as pathogens had been challenged and E.coli of the same serotypes, isolated from urine or water, were non-pathogenic (SACK, et al. 1971). All these necessitate re-evaluation of the importance of serotyping from detection of EPEC as a sign of pathogenicity.

The aim of this work was carried out to give an idea about toxin production, by different strains of E.coli and the relation of this activity to the serotyping of the strains isolated.

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## MATERIAL and METHODS

### Biochemical identification of bacterial isolates:

Rectal swabs were collected from 60 children with diarrhoea coming to the pediatric out patients at the central hospital of Sedfa. Specimens were sent to the laboratory, where routine culture was made on Blood agar, MacConey's and nutrient agar.

A separate swab was also taken and placed in buffered glycerol-saline solution and within 24 hours it was streaked on eosin methylene blue. Colonies resembling coliforms were picked and identified in a chronological order. Identification was based on the methods of EWING and MORTIN (1974). The following tests were used on all strains: Gram stain, Triple sugar iron agar, indol, methyl red, voges-proskauer, simmon's citrate and phenyl alanine deaminase.

Strains shown not to be *E.coli* by the above reactions were subjected to further tests: motility, urease production, lactose fermentation, lysine and ornithine decarboxylase and arginine dehydrolase.

### Serological typing of isolated *E. coli* strains:

The organisms were originally stored on trypticase soya agar slants at 4°C after reconfirmation with enteropathogenic antisera. Antisera were obtained from Behring werk, marbury, W. Germany. These antisera include the polyvalent anti-OK(B) and their monospecific antisera, and also two anti OK(L) monospecific antisera for detection of K-antigen. The bacterial growth on agar slope was suspended in saline and then examined in a slide agglutination test against polyvalent anti-OK(B) antisera and then against the corresponding monospecific antisera. In case of no agglutination with the two polyvalent OK(B) antisera, the bacterial suspension was tested against the two OK(L) antisera. For O-serotyping, a heavy suspension of the organism was at first heated at 100°C for 30 minutes to destroy K antigen and then tested in a tube agglutination, against the previous mentioned OK antisera diluted 1/16. One drop of the heated bacterial suspension was added to 0.5 ml of the diluted antisera in a wassermann tube. After 2 hrs. incubation at 37°C and 18 hrs. at room temperature, agglutination was looked for. Strains giving agglutination with more than one monovalent antiserum were retested.

### The infant mouse test:

Material for the assay of enterotoxin production was prepared by a modification of the method described by SACK, *et al.* (1971). 2 ml. broth culture of each of the 44 strains grown over night was inoculated into flasks, each containing 50 ml. of trypticase soy broth. The flasks were incubated for 48 hrs. at 37°C without shaking. The culture was centrifuged at 1500- Rpm for 20 min. The supernatant was filtered through 0.45 um millipore filter and used for the test. Two mice of 1-4 days old were fed orally with 0.1 ml. of the filtrate of each strain. The mice were kept at 28°C for 4 hrs. and then killed with chloroform. The abdomen was opened, and the small intestine was examined for distention and then removed with forceps. The intestine from the two mice were weighted together and the ratio of gut weight to remaining body weight was calculated. 4 control groups, each of 2 mice, were included; the first group was fed with filtrate of the standard toxigenic strains of *E.coli*: K<sub>12</sub> 711 and K<sub>12</sub> 711 B<sub>41</sub> (the strain were obtained from the salmonella and shigella reference lab. London). The second was fed with the filtrate of a standard non-toxicogenic strain K<sub>12</sub> 1200, while the third and the fourth were fed on plain media and normal diet respectively.

Ratios of less than 0.056 were considered negative, those in the range of 0.056-0.065 were considered questionably positive, and those over 0.067 were strongly positive. Therefore the ratios of 0.065 or more were indicative of toxigenicity.

E.COLI TOXIN**RESULTS**

The result of infant mouse test are given in tables 1 and 2 from table 1 it was observed that the ratio of intestine to the body weight with the two toxigenic standard strain was 0.067 and 0.065, while in the control animals which were fed on plain media was 0.056 and in case of control animals which received normal diet was 0.055. The difference between the ratio of the two toxigenic strains on one hand and that of the three control groups on the other hand is a good indicator of toxigenicity. From table 2 it was noted that 5 out of 17 strains belonging to serotype,  $O_{55} K_{59} (B_5)$  were highly toxigenic and only one was considered questionable toxigenic. The strain belonging to serotype  $O_{26} K_{60} (B_6)$  was considered toxigenic.

From the same table, 11 strains belonging to  $O_{55} K_{59} (B_5)$  and one strain belonging to  $O_{128} K_{67} (B_{12})$  were non toxigenic. The percentage of toxigenic strains of both typable and untypable E.coli are given in the same table; it was noted that the toxigenic strains were 7 belonging to enteropathogenic E.coli, while only 3 belonged to non-enteropathogenic E.coli (untypable). The non-pathogenic E.coli were 12 belonging to untypable ones.

**DISCUSSION**

In the present study two groups of E.coli serotypes were compared for their ability to produce a soluble toxin. The first group was composed of some of the typable enteropathogenic strains while the second group was composed of untypable faecal E.coli. A special attention was taken so that the first group would contain a good number of the serotype  $O_{55} K_{59} B_5$  since it is known to be the most predominant serotypes involved in infantile summer diarrhoea in Egypt (EL-FALAKY, 1968; SOLIMAN, 1978). Production of the soluble toxin by our strains was detected by the infant mouse test as reported by SHOEB, et al. 1982. The ratio of weight of intestine of infected mice to its remaining body weight which could be considered as indicative of toxigenicity was 0.065 or more although DEAN, et al. 1972 reported that ratios less than 0.067 would be considered as negative. The slight difference observed may be due to variation in bacterial strains or in the environmental temperature.

Accordingly 36.84% of our typable strains were toxigenic while 12% of the non-typable strains were non toxigenic. These percentage are very close to those recorded by SOLIMAN (1978), since he reported that the toxigenic strains were 40% of typable and 10% of non typable strains although he used G. pigs in his study. On the other hand SACK (1975) found that all the strains of E.coli isolated from diarrhoeal cases were non-typable but at the same time toxigenic.

SHAEB, et al. (1982) reported that the toxigenic strains were 76.6% from typable E.coli and 56.6% from the untypable ones. Thier findings were higher than those of our study and such differences could be due to periodic fluctuation of the exesting bacterial strains or variation in the environmental temperature e.g. high temperature in Upper Egypt especially in summer months.

It should be noted that some of the untypable strains of E.coli are able to produce the soluble toxin as reported here and by several workers (DEAN, et al. 1972; GURWITH, et al. 1977; SOLIMAN, 1978 and SHOEB, et al. 1982). A finding which deserves more work since such strains may escape detection on routine examination of cases of diarrhaea for the enteropathogenic serotypes.

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Table (1)  
The infant mouse test of control groups

Material fed to animal	Weight of small intestine	Remaining weight	Ratio of intestine to body weight
Toxigenic strain K <sub>12</sub> 711 B <sub>41</sub>	530 mg	7.400 gm	0.067
Toxigenic strain K <sub>12</sub> 711	510 mg	7.300 gm	0.065
Non Toxigenic strain K <sub>12</sub> 1200	430 mg	7.200 gm	0.056
Plain media	420 mg	7.100 gm	0.056
Normal diet	380 mg	6.500 gm	0.055

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Table (2)  
The infant mouse test of animals tested by enteropathogenic and non entero-pathogenic E.coli

Ratio of intestine to body weight	Enteropathogenic <u>E.coli</u> serotypes												Non enteropathogenic	
	O <sub>55</sub> K <sub>59</sub> (B <sub>5</sub> )		O <sub>26</sub> K <sub>60</sub> (B <sub>6</sub> )		O <sub>128</sub> K <sub>67</sub> (B <sub>12</sub> )		Total		Non enteropathogenic		Total			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
0.090 or more	2	4.55	-	0.00	-	-	0.00	2	4.55	2	4.55	2	4.55	
0.080 - 0.089	-	0.00	-	0.00	-	-	0.00	-	0.00	1	0.00	1	2.27	
0.070 - 0.079	3	6.82	-	0.00	-	-	0.00	3	6.82	-	-	-	0.00	
0.065 - 0.069	-	0.00	1	2.27	-	-	0.00	1	2.27	-	-	-	0.00	
0.056 - 0.064	1	2.27	-	0.00	-	-	0.00	1	2.27	-	-	-	0.00	
* 0.055 - or less	11	25.00	-	-	1	2.27	2.27	12	27.27	22	50.00	22	50.00	
Total	17	38.64	1	2.27	1	2.27	2.27	19	43.18	25	56.82	25	56.82	

\* : non toxigenic.