Egypt. J. Agric. Res., 80 (4), 2002.

ESCHERICHIA COLI AS A CAUSE OF MORTALITIES AMONG SWISS MICE WITH SPECIAL REFERNCE TO THEIR ENTEROTOXIGENICITY

ROFAIIL S.K. ELHAM A.Y. AND A.M. DAOUD

Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza - Egypt

(Manuscript received 29 May, 2002)

Abstract

Bacteriological examination carried out on rectal swabs of mice, revealed that *E. coli* was the predominant organism compared with other enteric bacteria from apparently healthy mice. The percentage of isolation was 25.45%, whereas, in case of diseased mice, the percentage of isolation was 57.69%. Serological typing of *E. coli* strains isolated from diseased mice revealed that they belonged to different serogroups which included O_{119}, O_{128}, O_{127}, O_{111}, O_{85} and O_{78}. The pathogenicity test of *E. coli* isolates in mice proved that all of them were highly pathogenic. Most *E. coli* isolates were highly sensitive to gentamycin, kanamycin, chloramphenicol, neomycin and tobramycin. On studying the effect of sterile extract of *E. coli* on tissue culture, we found that 62.06% had cytopathic effect, and for detection of enterotoxins production, the infant mice used were positive for enterotoxigenic effect.

INTRODUCTION

Mice are susceptible to disease caused by *E. coli* specially enterotoxigenic *E. coli* strains, and significant economic loss is incurred due to high mortality rates. *E. coli* infection is considered allover the world as one of the most dangerous disease which affects the mice (Rao and Char, 1986). One of the major problems in mice breeding is the control of enteritis which affects the colony causing great losses (Hansen, 2000). The causative strains are called enterotoxigenic *E. coli* (Schiff *et al.*, 1972). These strains produce either one or both of two enterotoxins a heat labile or a heat stable enterotoxins (Smith and Halls, 1967).

The aim of the present study was to study enteritis in mice. This report describes the isolation, identification, serological characterization, pathogenicity of the different isolated strains from mice. In vitro, antibiotic sensitivity test of different isolated bacteria was studied, in addition to the enterotoxigenicity of isolated *E. coli* strains in infant mice.

MATERIALS AND METHODS

Samples: A total of 162 rectal swabs were collected from mice (110 from apparently healthy and 52 from mice suffering from diarrhoea) and were examined bacteriologically.

Suckling mice: A total of 232 mice, 1-3 days old were used to study the enterotoxigenic activity of the isolated *E. coli* strains.

Adult mice: A total of 60 mice of a weight ranging from 12-18 grams were used to test the pathogenicity of the isolated *E. coli* strains.

Bacteriological examination and identification of samples

Collected samples were inoculated into nutrient broth and incubated at 18-24 hours at 37°C, then, a loopful from growth was cultured onto MacConkey agar (Oxoid CM-115) and Hektoen enteric agar (Bio Merieux) plates and incubated at 37°C for 24 hours. Separate colonies were cultured onto Simmon's citrate agar medium supplemented with adonitol and incubated at 37°C for 24 hours. The presence of pink colonies (lactose fermenting) on MacConky agar plates and yellow colonies on Simmonis citrate adonitol agar medium suggested the presence of *E. coli*. Morphological, cultural and biochemical identification were carried out according to Koneman *et al.* (1997).

Serotyping of isolated strains

Isolates which were initially identified as *E. coli* were subjected to serological typing using *E. coli* polyvalent and monovalent O antisera obtained from Welcome reagents limited, England as described by Cruickshank *et al.* (1975).

Experimental infection of mice with *E. coli* strains

This was done in mice as mentioned by Holt *et al.* (1994).

Antibiogram of isolated *E. coli* strains

For antibiogram of the bacterial isolates, disc diffusion method was used as described by Russel and Ques Nel (1983).

Detection of toxigenicity on tissue culture

Behaviour of different strains of enterotoxigenic *E. coli* to produce verotoxin and other cytotoxin was tested on vero cell in tissue culture according to Rycke *et al.* (1989).

Determination of enterotoxigenicity

Enterotoxigenicity of *E. coli* strains isolated from diseased mice were tested as mentioned by Dean *et al.* (1972).

RESULTS

The results of bacteriological examination of rectal swabs isolated from apparently healthy as well as, swabs from diarrhoeic mice are shown in Tables 1 and 2 .

The results of serogrouping of *E. coli* isolated from diarrhoeic mice are represented in Table 3. Table 4 shows the pathogenicity of the isolated *E. coli* serovars to mice. The results of antibiotic sensitivity test of isolated *E. coli* strains to different antibiotics are shown in Table 5. Table 6 shows the results of detection of toxigenic *E. coli* strains in Vero cell culture. Table 7 reveals a clear evidence of enterotoxigenic nature of these *E. coli* strains. The mice givining enterotoxigenic strains had a distended and congested intestine, while, those mice givining the non enterotoxigenic strains had a normal intestine.

Table 1. Incidence of isolated bacteria from rectal swabs of apparently healthy mice.

Bacterial isolate	Number of isolates	Incidence in percent
E. coli	28	25.45
Salmonella species	19	17.27
Proteus vulgaris	15	13.64
Klebsiella pneumoniae	13	11.82
Enterobacter cloacae	9	8.18
Citrobacter freundii	8	7.27
Staphylococcus aureus	8	7.27
Pseudomonas aeruginosa	5	4.55
Shigella flexenri	5	4.55

N.B.: the percentage is calculated on the basis of the total number of examined animals (110).

Table 2. Incidence of isolated bacteria from rectal swabs of diarrhoeic mice.

Bacterial isolate	Number of isolates	Incidence in percent
E. coli	30	57.69
Salmonella species	7	13.46
Enterobacter cloacae	5	9.62
Pseudomonas aeruginosa	5	9.62
Shigella flexenri	3	5.76
Staphylococcus aureus	2	3.85

N.B.: the percentage is calculated on the basis of the total number of examined animals (52)

Table 3. Serogrouping of *E. coli* isolated from diarrhoeic mice.

Serogroup	Number of isolates	Percentage (%)
O_{119}	8	26.67
O_{128}	7	23.33
O_{127}	4	13.33
O_{111}	4	13.33
O_{85}	3	10.00
O_{78}	3	10.00
Untyped	1	3.33

N.B.: the percentage is calculated according to the total number of isolates (30)

Table 4. Pathogenicity of *E. coli* serovars to mice.

E. coli serovar	Number of died mice within the following days				
	1st	2nd	3rd	4th	5th
O_{119}	0	1	3	1	0
O_{128}	0	1	3	1	0
O_{127}	0	1	2	2	0
O_{111}	0	1	2	2	0
O_{85}	0	1	1	3	0
O_{78}	0	1	1	3	0
Control	0	0	0	0	0

N.B.: 5 mice were used for each serovar and 5 mice used as control non inoculated.

Table 5. Results of antibiotic sensitivity test applied on toxigenic *E. coli* isolated from diarrhoeic mice.

Antibiotic	Number of sensitive strains to different antibiotics											
	O_{119}		O_{128}		O_{127}		O_{111}		O_{85}		O_{78}	
	No	%	No	%	No	%	No	%	No	%	No	%
Ampicillin	0	0	1	14.29	0	0	1	25	0	0	0	0
Amoxicillin	1	12.5	0	0	1	25.00	0	0	0	0	0	0
chloramphenicol	6	75	5	71.43	3	75.00	3	75.00	2	66.67	2	66.67
Gentamycin	7	87.5	6	85.71	3	75.00	3	75.00	2	66.67	2	66.67
kamamycin	7	87.5	6	85.71	3	75.00	3	75.00	2	66.67	2	66.67
neomycin	6	75	5	71.43	3	75.00	3	75.00	2	66.67	2	66.67
Tobramycin	5	62.5	5	71.43	2	50.00	2	50.00	1	33.33	1	33.33

N.B.: the percentage is calculated according to the total number of each serovar.

Table 6. Toxigenic *E. coli* strains detected in vero cell culture.

Total *E. coli* strains examined	Toxin Dilution											
	Undiluted				1:2				1:4			
	-ve	%	+ve	%	-ve	%	+ve	%	-ve	%	+ve	%
29	11	37.93	18	62.07	13	44.83	16	55.17	27	93.10	2	6.90

Table 7. Detection of heat stable enterotoxin using suckling mice assay.

E. coli serotype	Number of +ve	%	Intestinal weight/ remaining body weight	Number of -ve	%	Intestinal weight/ remaining body weight
O_{119}	8	100.00	0.1210	0	0	0
O_{128}	7	100.00	0.1007	0	0	0
O_{127}	4	100.00	0.0967	0	0	0
O_{111}	4	100.00	0.0934	0	0	0
O_{85}	2	66.67	0.0878	1	33.33	0.0577
O_{78}	2	66.67	0.0860	1	33.33	0.0425
Control	0	0	0	116	100.00	0.0633

N.B.: Ratio of the intestinal weight/remaining body weight more than a range of 0.070-0.090 was considered as positive for toxin production Ratio of the intestinal weight/ remaining body weight less than 0.070 was considered as negative for toxin production.

DISCUSSION

The present work was conducted to investigate the recovery of *E. coli* in faeces from apparently healthy and diarrhoeic mice, the serotyping of isolates recovered from diarrhoeic mice, pathogenicity in mice, the antibiogram to commonly used chemotherapeutic agent and the enterotoxigenicity of *E. coli* strains in infant mice.

The results of bacteriological examination of rectal swabs collected from apparently healthy mice (Table 1) showed that *E. coli* was the predominant species among the other enteric bacteria. This result agreed with that obtained by Rofaiil (1997) who mentioned that *E. coli* was widely distributed as a non - pathogenic saprophytes in mice colony. The results of bacteriological examination of rectal swabs collected from diseased mice (Table 2) revealed that *E. coli* was once again the most predominant bacteria among the isolated enteric bacteria. These results were the same as those mentioned by Ramisavlijevic *et al.* (1980), who found that *E. coli* was the cause of high mortality rates among mice. Moreove, Rofaiil (2001), proved that *E. coli* was recorded among diseased mice causing high mortalities.

Strains of *E. coli* isolated from diseased mice were serotyped into 6 groups; namely, O_{119}, O_{128}, O_{127}, O_{111}, O_{85} and O_{78} (Table 3). These serotypes were recorded by Bettelheim *et al.* (1974), to be very common cause of diarrhoea in mice. On study-

ing the pathogenicity of *E. coli* isolates by experimentally infecting mice, it was found (as shown in table 4) that *E. coli* strains were highly pathogenic, since all inoculated mice died within 96 hours ,and the inoculated strains were re-isolated from the internal organs of freshly dead mice. This finding is similar to that mentioned by Holt *et al.* (1994).

The results in table (5) illustrated that *E. coli* strains were highly sensitive to gentamycin, kanamycin and chloramphenicol and less sensitive to neomycin and tobramycin and resistant to ampicillin and amoxicillin which agree with those of Russel and QueSnel (1983), Dinh and Nguyen (1995).

Table 6 revealed that, 18 isolates of 29 strains were toxigenic for Vero cells, while 11 isolates were non - toxigenic. On the other hand, by dilution of bacterial toxins into 1:2, 16 isolates gave positive cytopathic effect. However, dilution 1:4, only 2 isolates were still toxigenic. Tthese indicated the cytopathic effect of *E. coli* strains. These results agreed with Rycke *et al.* (1989) who mentioned that, *E. coli* had the ability to proliferate in small intestine and to produce enterotoxins. On studying the enterotoxigenicity in infant mice carried out on the *E. coli* strains recovered from diarrhoeic mice, it was evident that, most of *E. coli* strains were positive for the enterotoxigenic effect in suckling mice. These results are similar to those mentioned by Moon and Whipp (1971).

REFERENCES

1. Bettelheim, K.A., F.M. Bushrod , M.E. Chandler and R.A. Shooter. 1974. E*scherichia coli* serotype distribution in man and animals. J. Hyg., 73 (3): 467-471.

2. Cruickshank, R. S.P. Dugrid, B.P.Marmion and R.H.A.Swain.1975.Medical microbiology 12th Ed. Vol 2 Churchill Livingston, Edinburgh, London, Great Britain.

3. Dean, A.G., Y.C. Chin, R.G. Willions and L.B. Harden. 1972. Test for *Escherichia coli* enterotoxin using infant mice application in a study of diarrhoea in children in Honolulu. J. inf. Din., 125 (4):407-411.

4. Dinh, B.T. and T.T. Nguyen. 1995. Enterobacterial sensitivity to antibiotics. Khoa Hoc Ky thuat thu Y, 2 (3):18-24.

5. Hansen, A.K. 2000. Hand book of laboratory animals bacteriology Boca Raton, London, New York wash in Gton, DC.

6. Holt, J.G., U.R. Kreig, P.A. Sneath, J.T. Staley and S.T. Willians. 1994. Bergeys mammal of determinative bacteriology 9th Ed. Willians and will, Baltimore, London.

7. Koneman, E.W., S.D.Allen, J, and a, W. Schrechechger, MR. P. and J. R. Winner. 1997. Diagnostic microbiology, 5th Ed. J.B lippincottco. New York.

8. Moon, H.W. and S.C. Whipp. 1971. Systems for testing the enterotoxigenicity of *Escherichia coli*. Ann. N.Y.Acad. Sci., 176:197-211.

9. Ramisavlijevic, M., .B S. Markovic and C. Rusov. 1980. Enteropathogenicity of *Escherichia coli* for laboratory animals. III young mice. Veterinarski. Glasnik, 34(5):499-505.

10. Rao, M.R.K. and N.L. Char. 1986. Enterobacteria isolated from pathological condition of laboratory animals. Indian vet. J., 63 (3):179-182.

11. Rofaiil, S.K. 1997. Bacteria causing diarrhoea in Swiss mice.Thesis M.V.Sc, Fac. Vet. Med. Alexandria Univ.

12. Rofaiil, S.K. 2001. The role of some pathogenic and commensal bacteria in Swiss mice with special reference to stress factors.Thesis Ph.V.SC. Fac. Vet. Med. Alexandria Univ.

13. Russel, A.D. and L.B. QuesNel. 1983. Antibiotics: assessment of antimicrobial activity and resistance. Academic Press Inc. Society of applied bacteriology, London.

14. Rycke, J.D., E. Oswold and R. Boivin. 1989. An in-vivo assay for the detestation of cytotoxic strain of *Escherichia coli.* Annals de Recherches veterinaires, 20 (1) :39-46.

15. Schiff, L.J., P.W. Barbara, C.D. Por, and S.M. Poiley. 1972. Enteropathogenic *Escherichia coli* infectious increasing awareness of problem in laboratory animals. Lab. Ann. Sci., 22 (5):705-708.

16. Smith, H. W. and S. Halls. 1967. Studies on *Escherichia coli* enterotoxin. J. path. Bact., 93: 531-543.

الميكروب القولوني كمسبب للنفوق الجموعى فى الفئران السويسرية خاصة بالرجوع إلى السمية المعوية

صفوت كمال روفائيل ، إلهام علام يوسف، أحمد محمود داود

معهد بحوث الأمصال واللقاحات البيطرية -مركز البحوث الزراعية- وزارة الزراعة- الجيزة -مصر

فى هذه الدراسة أوضحت نتائج الفحص البكتريولوجي للمسحات الشرجية للفئران السويسرية أن الميكروب القولوني هو أكثر الأنواع التى تم عزلها بالمقارنة بالميكروبات المعوية الأخري بالنسبة للفئران السليمة ظاهريا وكذلك المصابة بالنزلات المعوية حيث كانت نسب العزل ٢٥,٤٥٪ ، ٥٧,٦٩٪ وأظهرت نتائج التصنيف السير ولوجي لعترات الميكروب القولوني والمعزولة من الفئران المصابة وجود ٦ أنواع سيرولوجية هى O_{119}، O_{128}، O_{127}، O_{111}، O_{85}، O_{78}. وبدراسة ضراوة عترات الميكروب القولوني فى الفئران وجد انها كانت شديدة الضراوة وبإجراء اختبار حساسية المضادات الحيوية وجد أن الميكروب القولوني كان شديد الحساسية للجنتاميسين والكاناميسين والكلورامفينسكول، النيوميسين والتوبراميسين وتم استخلاص للتوكسين الموجود بداخل الميكروب القولوني لمعرفة تأثيره على النسيج الخلوي فوجد أن له تأثيرا على الخلايا بنسبة ٦٢,٠٦٪ وأوضحت نتائج اختبار السمية المعوية فى الفئران الرضيعة أن معظم العترات الممرضة كانت شديدة السمية المعوية.