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With 1 table in the text.

Enzyme Immunosorbent Assay (EIA) for Detection of STa Toxin extracted from Enteropathogenic *E. coli* isolated from Neonatal dairy catte Calves

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ملخص البحث

استخدمت هذه المقابسة المناعية الأنزيمية (Enzyme immunoassay Oxoid, TD700) للكشف عن الزيفان المقاوم للحرارة (STa) فى 51 عترة لبكتريا الاشريكية القولونية والتي عزلت من مائة عجل يعانى من الاسهال في مزارع البان بالخرطوم، شمال ولاية الجزيرة وولاية النيل الابيض بالسودان. وجد منها عشر فقط موجبة لوجود الزيفينات المقاومة للحرارة

Summary

Fifty-one strains of *E. coli* isolated from one hundred diarrhoeic calves from Dairy farms in Khartoum State, Northen Gezira and white Nile State in the Sudan, were tested with Enzyme immunoassay (EIA) using commercial Oxoid, (TD,700), to determine heat-stable toxin (STa); only ten strains were positive.

Introduction

Escherishia coli (*E.coli*) has a worldwide distribution; its usual habitat is the intestinal tract of animal and man though it may contaminate vegetation, soil and water (Quinn *et. al*., 2001).

Virulence attributes of enterotoxigenic *E. coli* include the adhesion of their pili to the intestinal villous epithelial cells and the production of entrotoxins (Levine *et al.,* 1983; Evans *et al.,* 1984; Levine, 1987). Two types of enterotoxins are produced by enterotoxigenic strains of *E. coli* of various origins. They are of high molecular weight heat-labile (LT) and a low molecular weight heat-stable (STa) (Smith and Halls, 1967; Smith and Gyles, 1970; Gyles, 1971; Sack, 1975; Quinn *et a*l., 2001).

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The STa enterotoxin is unaffected by a temperature of 60^{o}C for 30 min., it occurs extracellularly in nature and is inactivated by heating to 121^{o}C.

This study was carried out to confirm the pathogenicity of *E.coli* strains isolated from diarrhoeic dairy cattle calves by detecting STa toxin production.

Materials and Methods

Bacteria:

Fifty-one *E. coli* strains, isolated from diarrhoeic calves (Ellaithi, 2004**)** were used and tested with EIA detection kits (Oxoid, TD,700) for the presence of heat-stable toxin (STa).

Enzyme immunoassay:

This enzyme immunoassay was essentially carried out as described by Carroll *et al.* (1990).

Preparation of toxins from isolated *E. coli*:

As there might be more than one strain present in the sample and to increase the probability of detecting an enterotoxin-producing strain, several cultures were prepared. The isolated *E. coli* was cultured in tryptone soya broth (Oxoid, CM 129) and incubated with continuous shaking water bath (KARL KOLB, scientific technical supplies, Buchschlag-Frankfurt, West Germany) at 37^{o}C for 18 hours. The cultures were centrifuged at 3000 rpm for 30 minutes at 4^{o}C and then the supernatants were filtered through the Millipore (SLGV) and used as test samples.

Results

Fifty-one *E. coli* strains, were tested with EIA detection kits (Oxoid, TD700) for the presence of heat-stable toxin (STa). Ten out of the fifty one strains tested (19.6%) gave positive results (Table 1).

The sensitivity of this test kit in detecting the heat-stable toxin is 10 ng/ml. Enterotoxin present at a concentration lower than this gives a negative result.

Table 1: Detection of STa enterotoxin produced by *E. coli* isolated from diarrhoeic dairy cattle calves using Oxoid test kits.

Strain no.	Serogroup	STa using EIA
3	Ont	+
4	-	+
5	Ont	+
6	08	+
8	Ont	+
11	021	+
14	-	+
16	O9	+
61	09	---
62	-	---

- = not tested for serogroup, + = positive for STa, Ont = O not typable

Discussion

Determination of biochemical reactions of bacterial isolates is still the only common routine laboratory diagnostic method for recognition and differentiation of the genera and species of the family *Enterobacteriaceae* (Barrow and Feltham, 1993; Ewing, 1986). However, Pathogenic *E. coli* can not be separated from non-pathogenic ones on the basis of their biochemical characteristics. Therefore, to determine the pathogenic potential of *E. coli* strain, One or more pathogenicity tests should be carried out (Sack, 1975; Giannella, 1976).

The yield of toxin was shown to vary from culture medium to the other (Starvic *et al.*, 1978; Bertschinger *et al.*, 1990). Production of STa toxin was tested by Oxoid toxin detection kits (EIA). EIA is a simple, satisfactory, reliable immunological test, commonly used (Wray and Woodward, 1990) and depends on a competitive enzyme immunoassay (Carroll *et al*., 1990). This test detects *E. coli* STa at or above a concentration of 10 ng/ml (Woodward and Wray 1990). Enterotoxins present at a concentration lower than this will, therefore, give negative results. Moreover, strains which gave negative result might have also possessed the toxin gene in an inactive form and thus, had produced the

toxin at levels that were suboptimal for the EIA to detect. One strain of *E. coli* produced a level of STa detectable by EIA, however, it was negative for STa when tested by suckling mouse test. This finding disagreed with Scotland and others (1989) who reported 100% harmonious association between the two methods for detection of STa production. Accordingly, this contradictory finding stresses the importance of using more than one method to confirm results; however, six isolates were STa positive when tested by the two methods.

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