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Isolation and Cytotoxic Effect of Escherichia coli on Vero cells And Kidney Cells of Bovine

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ABSTRACT

The present study was aimed to isolate the Escherichia coli from different human infection and then study its cytotoxic effect on vero cells and kidney cells of bovine. 40 samples were collected as follows: 20 isolate samples from urine, 4 isolate samples from eye swabs, 5 isolate samples from wound swabs and 11 isolate samples from stool specimen. All isolate samples were fermented glucose, maltose, sorbitol and raffinose. 84% of isolate sample were fermented sucrose, 60% of isolate samples were positive to Indole test, 80% of isolate samples were negative to urease test and 70% of isolate samples were motile. All isolate samples were negative to oxidase test, Voges-Proskaur test and H_2S, but they were positive to catalase test and MR test. 10 strain of E coli were selected according to their biochemical test and then investigate its cytotoxic activity on Vero cells and kidney cells of bovine. The results were indicated 1o strain of E coli had produced cytotoxic activity on the kidney cells of bovine, but they did not produce cytotoxic activity on the Vero cells.

Keywords: *Ecoli, Cytotoxic, Vero cells and cells of kidney.*

1. INTRODUCTION

Escherichia coli is possibly the most studied bacteria as well as it is one of the most important diseases causing agent and it contains numerous serotypes, some of them are associated with certain infections in man and animals which result in diseases throughout the world. Members of

Escherichia coli are normal habitants of the intestinal tract of vertebrates such as man. Escherichia coli is aerobic and facultative anaerobic. It is readily grown on ordinary laboratory media without addition of blood, serum, ascetic fluid or glucose. The optimum temperature for cultivation of Escherichia coli is 37ºC [1]. The present investigation was thrown some light on the incidence of Escherichia cole infection in human and related a high incidence of infections by a large variety of strains.

2. MATERIAL AND METHODS

2.1 Primary Isolation of Escherichia Coli

The sample was streaked on to blood agar and Mac- Conkey's agar. A portion of a typical well isolated colony was transferred with a wire loop to the liquid media. Inoculated solid and liquid media were incubated aerobically at 37 ºC up to 7 days and peptone water for indole was incubated for 48 hours. All cultures on solid media was examined with naked eye for growth, colonial morphology and changes in the medium, the liquid media were examined with naked eye for turbidity, colour changes and formation of sediment or pellicles. Purification of culture by sub - culturing part of a typical and well isolated colony on the corresponding medium.

2.2 Preparation of Smears

Smear was prepared by emulsifying part of a colony in saline and spread it on solid. Smears from liquid media use were prepared by a loop full on solid. The smears were allowed to dry in the air fixed by gentle flaming.

2.3 Biochemical Tests

Catalase test, oxidase test, motility test, oxidation fermentation of glucose, indole production test, sugar fermentation test, Voges-Proskeuer (VP) test, urease activity test and citrate utilization test (Biochemical tests) were performed as described [2].

2.4 Preparation of Toxins

10 strains of Escherichia coli were selected. The test organism was inoculated into nutrient broth medium and incubated at 37 ºC for 24 hours, the culture was then centrifuged at 1500 revolution/minutes for 30 minutes, the supernatant was then collected and toured into sterile medical bottles, sterilized by filtration with 0.22µ Seitz filter and stored at 20 ºC until used.

2.5 Preparation of Cell Culture

2.5.1 Vero Cell Culture

A bottles containing confluent monolayer culture of Vero cells was carried to the laboratory. The growth medium was removed and the cells were washed with PD. Two ml of warm typsin-versene solution was added and the bottle incubated at 37 ºC until cells flew freely when the bottle was titled. Few drops of calf serum were added to stop the action of typsin-versene and the suspension was centrifuged at 600 revolutions /minute for 5 minutes. The supernatant fluid was then discarded and the target cells were resuspended in 10ml of PD and thoroughly mixed with a pipette. The cells in suspension were then diluted in growth medium and sub cultured in plastic tissue culture flasks and incubated 37 ºC [3]

2.6 Bovine Kidney Cell Culture

Young animal 2-6 weeks were used. The kidney was aseptically reward after slaughter and placed in sterile Petridis and all perisental fat was reward. Kidney was then transferred to another petridish and decapsulated. The cortical tissue was sliced into small pieces with a pair of scissors and transferred to another petridish. The tissue fragments were chapped into fine pieces. The tissue fragments were then transferred to trypsinization flask

containing a magnetic bar and washed several time with PD till the supernatant fluid became clear. Warm 0.25% trypsin was added to the washed tissue fragment in amount sufficient to cover them. The contents were stirred for 30 minutes at room temperature and the supernatant fluid was discarded. Fresh pre-cooled 0.25% trypsin solution was added at a volume that was 3 times the volume of the tissue fragments. At the end of the trypsinization period the large fragments tissue were removed by filtration through sterile gauze. The dispersed cell in the filtrate was deposited by centrifugation at 600 revolutions/minutes for 5 minutes. The cells were washed once with PD containing 2% of calf serum and centrifuged. The packed cells were suspended in 10ml PD and vigorously pipette. The cell suspension was finally diluted in growth medium and distributed in the tissue culture flasks. Confluent monolayer was established within 3-5 days [3]. Tissue culture mono-layers were inoculated by 1ml of test toxin after decanting the medium. The inoculums were allowed to adsorb one hour at 37 ^{0}C before new maintenance medium was added. The medium was changed every two days and culture was observed daily for cytopathy effect (CPE).

3. RESULTS

A total of 40 samples were collected from different human infections, 30 of them were isolated and identified as Escherichia coli. Microscopic examination of the isolates revealed gram-negative short rods, occurring singly or in pairs. Growth on blood agar produced circular smooth convex colonies varying in sizes, some colonies were hemolytic and others were not. Growth on Mac Conkey's agar produced variable colony sizes with entire or irregular edges. Pink colonies on this medium were produced by isolates in variable degree indicating fermentation of lactose. Growth in liquid media, all isolated were produced heavy turbidity in peptone water. All the isolates was negative to oxidase test, positive to catalase test, Voges - Proskauer negative, MR test positive, H_2S (Pb Ac paper) negative. All the isolates were fermented glucose, maltose, sorbitol and raffinose. 84% of the isolates were fermented sucrose, 60% of isolates were positive to indole test, 80% of isolates were negative to urease test and 70% of the isolates were motile. All the isolates were fermentative. The mono-layers of Vero cells were inoculated with toxins and observed for 5 days, the control flasks showed normal tissue culture growth and there was no cytopathy effect (CPE) produced. The mono-layers of bovine kidney cells were inoculated with toxins and observed for 5 days, the control flasks showed normal tissue culture growth, but the inoculated cells showed cytopathy effect (CPE). CPE were produce essentially similar and indistinguishable from one isolate to another. The cytopathy changes consisted of groups of rounded or grape-like clusters of cells, the development of cytopathy effect was rapid appearing at 2 days post –inoculation and increased with time.

4. DISCUSSION

The results of 40 isolation samples revealed the presence of Escherichia coli in 30 samples. MacConkey's lactose bile salt agar proved to be satisfactory medium for primary isolation and these findings are agreed with [1]. Pink Colonies were produced on MacConkey's, which are agreed with [4]. The isolate samples were fermented glucose and lactose with production of acid and gas, also all the isolates were fermented maltose, sorbitose and raffinose, while some isolates did not ferment sucrose, these results are supported by finding [1] and [5].

All the isolates were failed to produce H_2S and 60% of the isolates form indole. 80% of the isolates did not develop urease. These results are agreed with [4]. Results of biochemical tests are indicating that there is large variety of sero - group involved in field. Toxins that were prepared from Escherichia coli isolates, caused morphologic change in bovine kidney cells, but have no effected on Vero cells. These results are supported the findings [6], which indicating the effect of Escherichia coli toxins in certain cells.

5. CONCLUSION

The present study was attempted to investigate the prevalence of Escherichia coli infections which are being increasing, indicating the importance growth of this pathogen. The results of isolation of 40 samples revealed the presence of Escherichia coli in 30 samples. All the isolates produced cytotoxic activity in bovine kidney cells, they did not produced cytotoxic effect in Vero cells.

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