INVESTIGATION OF THE ACTIVITY AND PATHOGENECITY OF *STAPHYLOCOCCUS AUREUS* ENTEROTOXIN C BY LIGATED ILEAL LOOP ASSAY IN RABBITS

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ABSTRACT

Three enterotoxigenic isolates of *Staphylococcus aureus* previously isolated from contaminated milk and evaluated for their enterotoxin producing ability and histopathological changes by the ligated rabbit ileal loop assay. The results of this assay revealed that crud toxin obtained by these isolates caused fluid accumulation in rabbit ileal loops. Fluid aspirated from the loops was bloody and the histopathological changes in sections were characterized by moderate to sever haemorrhage, erosion and inflammatory cells, in addition there was distortion and shift of villi. This finding established that staphylococcal enterotoxin that associated with vomiting and diarrhea , which often abate within 24 hrs., there was potential risk for more serious disturbances such as inflammation, tissue damage and toxic shock.

Key words: S. aureus, enterotoxin, ileal loop, rabbits

INTRODUCTION

Staphylococcus aureus produced large numbers of extracellular proteins and toxins. The most important toxins are called staphylococcal enterotoxins (1). Staphylococcal enterotoxins (SEs) are a family of structurally related proteins that are produced by *S.aureus* (2). The enterotoxin family now contains 17 toxins. The SE family is divided into the classical enterotoxins SEA to SEE and a group of recently discovered toxins SEG to SER , in addition the SEC has three antigenically distinct subtypes : SEC1 , SEC2 , SEC3 , and SEG have a variant form called SEGv . (3,1).

Many SEs are responsible for food poisoning , acute illness , fever , erythematous lesions , and hypotension (1) .It is estimated that about 5% of food poisoning cases in which none of the classical enterotoxins were detected can , however , be attributed to new enterotoxins (3, 4) . Since *S. aureus* may produce a large variety of

enterotoxins but 95% of poisoning outbreaks are caused by classical enterotoxins : A , B , C , D, and E (5) .

The SEs are generally heat resistant and a heat denatured enterotoxin can be renatured by prolonged storage or in the presence of urea. Toxins remain active even after boiling for 30 min. and they are stabile at 121 ◦C for 28 min. The SEs also are resist most proteolytic enzymes such as pepsin or trypsin thus keep their activity in the digestive tract after ingestion and all are capable of causing food poisoning (2, 3) .

MATERIALS AND METHODS

Bacterial strains and Ligated rabbit ileal loop assay

Three *S. aureus* strains harboring *Sec* gene detected by PCR previously isolated from milk (6) were used in this study for evaluation of their enterotoxin-production ability and histopathological changes made by the ligated rabbit ileal loop assay (7).

Enterotoxin production

Cultures for enterotoxin production were initially prepared using nutrient broth . Ten milliliter aliquots of sterile nutrient broth , in sterile tube , were inoculated each with approximately 10^5 cells/ ml according to Mc Farland standared (8), and incubated at 37 ◦ C for 48 hrs. Subsequently , the *S. aureus* strains were cultured in 10 ml. of milk at pH 8 and pH 4 that pasteurized by heating to 80 ◦C for 30 min. Cultures were incubated as above nutrient broth cultures . Following 48 hrs. incubation of the nutrient broth and milk cultures , cell free cultures supernatants were collected by centrifugation at 5000 rpm followed by filtration through 0.20 µm Millex syringe filters. The cell free filtrates were then used as crud toxin preparation (6,7).

Assay for enterotoxin activity

One to 1.5 kg body weight female rabbits were starved for 24 hrs. with water supplied and libitum. Each rabbit was anaesthetized with two ml. of ketamin injection and secured in dorsal recumbency. Following a midline incision, starting from the rectal end, the ileum was divided into segments of 5 cm. in length with string ligatures. The crud toxin preparation (0.5 ml.) was injected into different segments. Uninoculated broth and sterile saline were injected into some segments to serve as negative controls . The incisions were then sutured and the animals allowed recovering from anesthesia (6, 7).

Post – mortem examination

After 7 hrs., test animals were killed and opened immediately for examination . The gross appearance of the loops was noted , and if either the control loop contained

fluid , all tests in that rabbit were considered invalid . The length (centimeters) and volume (milliliters) of each test loop was measured. For positive loops, the volume of fluid recovered by aspiration was used to determine the dilatation index (DI) estimated as the ratio of volume of fluid to length of ileal segment. A DI > 0.2 was taken as positive . The test was done in triplicate animals (7) .

RESULTS

Diarrheagenic microorganisms including *S .aureus* are tested by ligated ileal loop assay .Three strains of PCR positive *S .aureus* isolated from contaminated milk were evaluated for their enterotoxin-producing ability and histopathological changes by the ligated rabbit ileal.

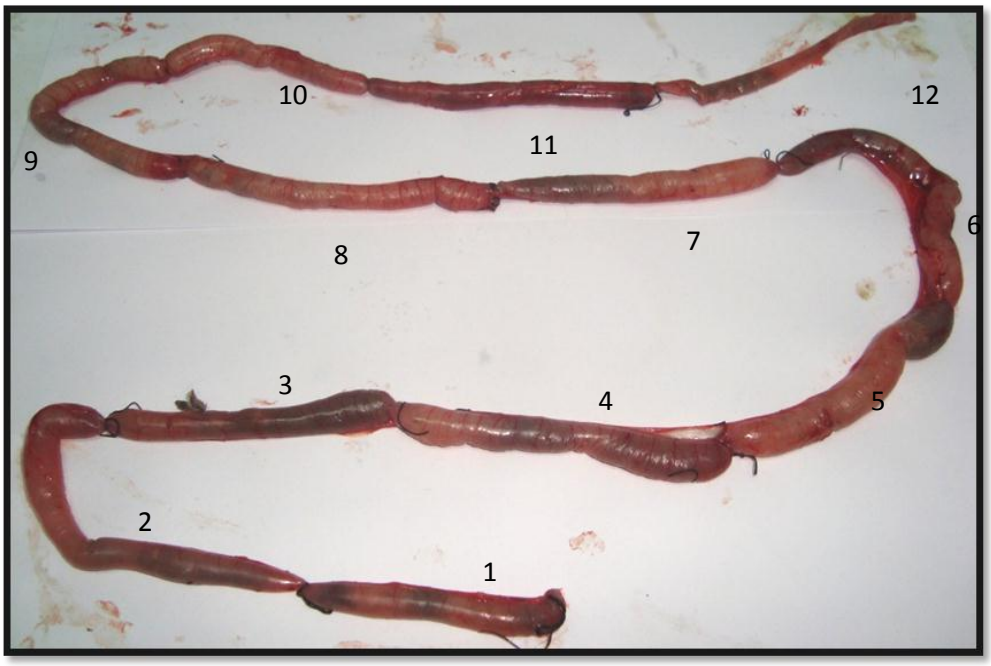
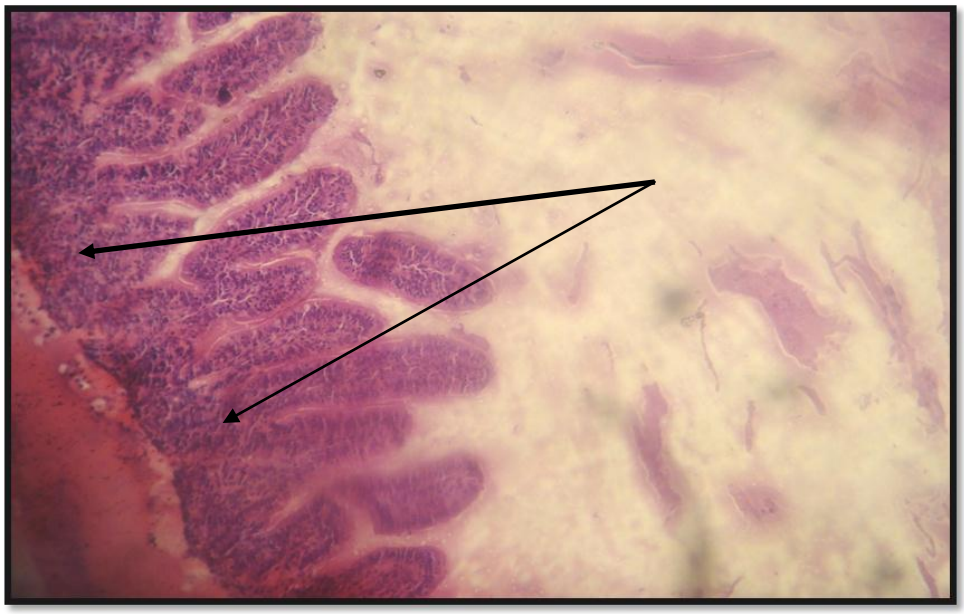


Fig (1) Ligated segments of rabbit ileal loop after injection with crude preparations of staphylococcal enterotoxin (SE) produced under different growth conditions. 1 – 6, SE produced at pH 8; 7-11, SE produced at pH 4 –there was change to a brownish colouration with less fluid accumulation than the previous pH; 12, segment inoculated with sterile saline (control).

Cell-free culture supernatants (crude toxin preparations) of the *S.aureus* strains caused fluid accumulation when injected into rabbit ileal segments, indicating enterotoxin activity. Dilatation index (DI) values ranged from 0.2 to 0.48. Moreover, there was a dark-reddish colouration of the positive ileal loops (Figure 1) and the aspirated fluid from such segments appeared bloody.

Histopathological changes in sections collected from the rabbit ileum were characterized by circulatory disturbances and inflammatory changes. Sections of the intestine collected from untreated (control) rabbits showed mucosae (including glands) and submucosae with normal histomorphology (Figures 2), while sections from rabbit's ileum inoculated with crude toxin preparations showed moderate to severe haemorrhage, erosion and inflammatory cells, In addition, there was distortion and shift of villi with the presence of some intestinal glands in mucausal area (Figures



3, 4, 5).

Fig (2) Section of control rabbit ileum showing normal villus (arrow) and intestinal gland. 125X H&E.

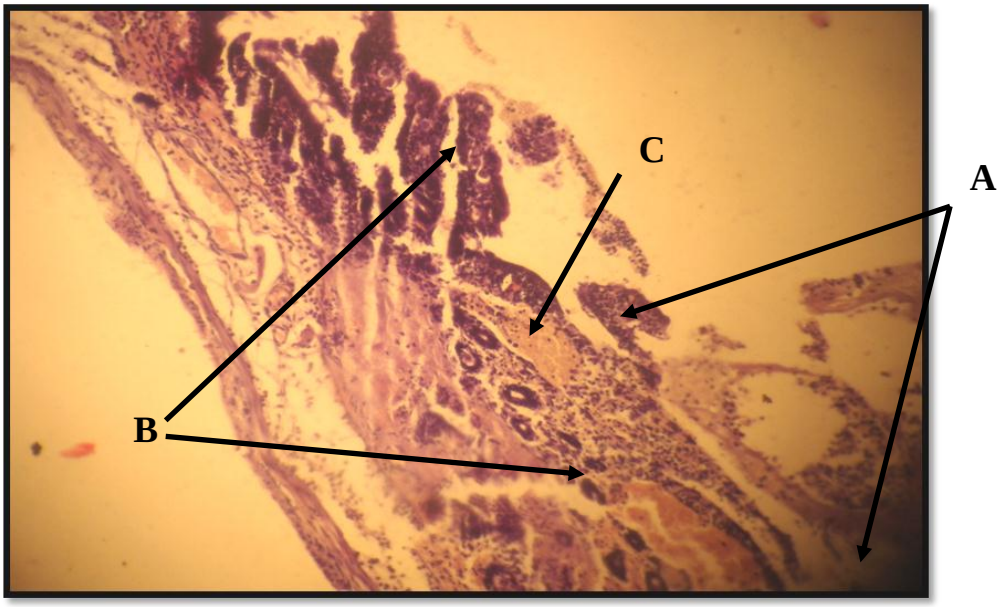


Fig (3) Section of rabbit ileum inoculated with crude staphylococcal enterotoxin ph 4, showing A) Erosion in the mucausal layer B) presences of intestinal glands and the some of the villi which shows destruction and sloughing C)Oedema 125X H&E

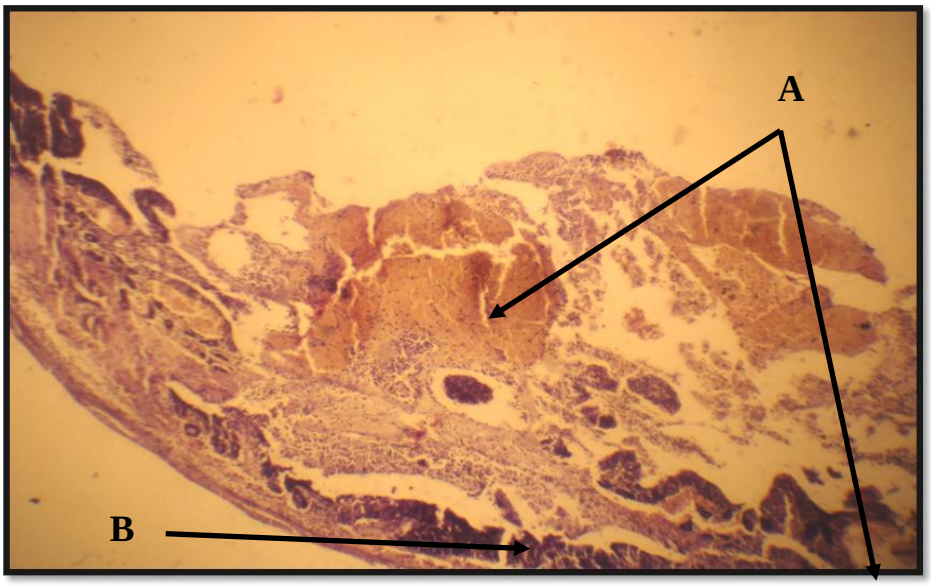


Fig (4) Section of rabbit ileum inoculated with crude staphylococcal enterotoxin ph 8, showing A) large areas of hemorrhage in the wide area of mucausal erosion and complete absence of the villi B) moderate inflammatory reaction. 125X H&E.

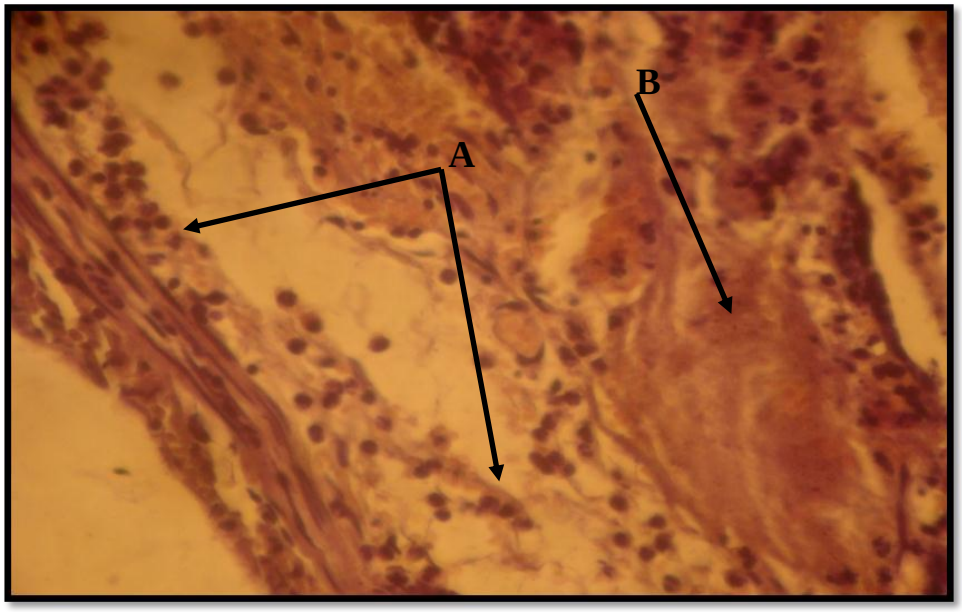


Fig (5) Section of rabbit ileum inoculated with crude staphylococcal enterotoxin ph 8, A) showing the infiltration of inflammatory cells in the mucausal region most of them of acute form (neutrophils) , B) Oedema, 500X H&E.

DISCUSSION

In the present study the crude toxin preparation of enterotoxigenic *S.aureus* can elicit positive ileal loops of the rabbits with dilatation index (DI) values that ranged from 0.2 to 0.48 . Moreover, there were dark-reddish colorations of the positive ileal loops and the aspirated fluid from such segments appeared bloody. This result agreed with (7) who found the crude toxin preparation of enterotoxigenic *S.aureus* can elicit positive ileal loops of the rabbits with the dilation index (I D) that ranged from 0.2 to 0.57 (ml/cm).

Koupal and Deibel, (9) recorded the culture supernatants of the enterotoxigenic *S.aureus* can elicit positive ileal loops of the rabbits with dilation index(I D) ranged from 0.52 to 57ml/cm.

The accumulation of the fluid in the intestine occurs because the intestinal epithelial cells form a barrier between the luminal contents and the sub epithelial region and SEs act as superantigen which causes down regulate of intestinal barrier function and increase epithelial permeability (10).

Histopathological changes in sections collected from the rabbit ileum were characterized by circulatory disturbances and inflammatory changes these include, moderate to severe haemorrhage, erosion, inflammatory cells, In addition, there was distortion and shift of villi with the presence of some intestinal glands in mucausal area.

Similar findings were obtained by (7) with the exception that degenerative and necrosis were not detected in the present study ,this may be contributed to the strains variation among different *S.aureus* isolates .(11, 5). Bhunia, (1) documented the SEs elicit damage to the intestinal epithelial cells resulting in the destruction of intestinal villi and inflammatory changes.

Also similar findings were obtained by Kuroishi *et al.*, (12) who elucidated mechanisms by which SEC induced inflammatory changes in bovine mammary glands. The SEC-inoculated mammary glands exhibited interstitial inflammation, with epithelial cell degeneration and the migration of polymorphonuclear neutrophils.

Although our study describes histological changes in a rabbit model, there is documented evidence that the clinical syndromes in some animal models simulate human enterotoxicosis (13).

اختبار فعالية وامراضية المكورات العنقودية الذهبية الفارزة للذيفان المعوي نوع C بطريقة الأمعاء المعقودة في الأرانب

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الخلاصه

اختيرت ثلاث عزلات موجبة لفحص البلمرة المتعدد (تمتلك ألجين المسؤول عن إفراز الذيفان المعوي نوع C) وقيمت قدرتها على إنتاج الذيفانات المعوية وإحداث التغيرات النسيجية باستخدام طريقة الأمعاء المعقودة للأرانب ولوحظ إن الذيفان الخام لهذه العزلات يسبب تجمع سوائل دموية في العقد اللفائفية للأرانب كما أظهرت المقاطع النسيجية وجود نزف شديد وتاكلات وتجمع خلايا التهابية فضلا عن تشوهات شديدة وانحرافات في الغدد المعوية

أظهرت النتائج إن الذيفان المعوي للمكورات العنقوديه يؤدي لحدوث حالات من التقيئ والإسهال الذي ينتهي بغضون 24 ساعة، كذلك يحدث اضطرابات أكثر خطورة مثل الالتهاب ،تضرر الأنسجة والصدمة السمية.

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