Adherence ability of *Pseudomonas aeruginosa* to intestinal epithelium of Rabbit

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الخلاصة

تضمنت الدراسة الحالية اختبار (15) u.gm من *Ps. aeruginosa* في قابليتهـا على الالتصاق بخلايا الغشاء الظهاري للأمعاء وقدرتها عل تمزيق حواجز الدفاع للأمعاء ضد الميكروب.

أكدت دراستنا على إحداث إصابة فعلية وذلك بحضانة المعلق الحاوي على خلايـا الغشاء الظهاري لأمعاء الارانب بتركيز 10^7 خلية / مل من معلق جرثومي بتركيـز 10^7 × 5 وحدة تكوين للمستعمرة / مل من الوسط الزرعي مما أدى ذلك إلى ظهور ظاهرة الالتصاق بينها وحدوث التمزق للحواجز الظهارية في الأمعاء.

ABSTRACT

This study investigated the adherence ability of fifteen isolates of *Pseudomonas aeruginosa* to intestinal epithelium, by inducing in vitro infection of incubating suspension of rabbits intestinal epithelium cells of 10^7 cell/ml concentration with 5 X 10^7 cfu/ml suspension of *Ps. aeruginosa* and detect the occurrence of the adherence between them and the altering of function with the disrupt of the intestinal epithelial barrier.

INTRODUCTION

Pseudomonas aeruginosa considered as apportunistic pathogen which is the main cause of infections-related mortality among the critically ill these infections arise as a results gastrointestinal flora (1,2). In spide of even in the absence of established extraintestinal infections and bacteremia, the presence of virulent strains of *Ps. aeruginosa* can be the major source of systemic septicemia and death amoung immuno-compromised (3,4). Among the many characteristics of highly successful pathogens that infect and harm their host is the ability to sense and responded to changes in their local microenviroment, that microenviroment state that topically signal a pathogen to responded with enhanced virulence are physicochemical properties such as changes in

PH, redox state and osmolarity of host cell contact itself has been shown to activate specific virulence regulatory pathway in several pathogens of clinical importance including *Ps. aeruginosa* (5,6). Therefore the aim of this study is to investigate a major mechanism of the lethal effect of intestinal *Ps. aeruginosa* its ability to adhere and disrupt the intestinal epithelium barrier.

MATERIALS AND METHODS

A) Bacterial isolates:

15 isolates of *Ps. aeruginosa* was obtained from patients of different ages suffering from abdominal pain in Al-Zahrawii hospital from October to December 2006, inoculated on nutrient broth and brain heart infusion broth and incubated at 37C° For 24 hr., , then kept at 4 C° For 4-6 days up to use.

B) Intestinal epithelium cells

These cells were obtained from adult rabbits (private breed) according to Knutton method (7,8) by taking aparts of small intestine especially duodenum from secattered rabbits then these parts were washed externally and internally by cooled phosphate buffer solution [(PH: 7.2), 4C°], then opened longitudinally, dipping in cooled
PBS for washing, scraping mucosal surface by scalple for obtaining the epithelium cells then transported in test tubes and centrifugated in cooled centrifuge at 1000 rpm for 2 min in 4C°, the precipitin were taken and filtered through nylon peies for elimination the foreign tissues and crystals for obtaining the suspension of intestinal epithelium cells only. The concentration of cells was 10^7 cell / ml.

C) Adherence

Adherence ability was done by using kuntton method (9) by adding 1 ml suspension of extracted intestinal epithelium cells, the concentration was 10^7 cell/ml to 2 ml of *Ps. aeruginosa* bacterial suspension of 5 X 10^7 cfu/ml concentration (8), the mixture incubated for 1 hr at 37C°, then the cells washing 3 times by PBS in cooled centrifuge at (1000 rpm, 4C°) for 2 min, then a few drop of sediment was put on glass slides, staining by Giemsa's stain for 30 min and exam with oil emersion lens under light microscope.

RESULTS AND DISCUSSION

The result of this study showed that the isolats of *Ps. aeruginosa* which are used is pathogenic and its pathogencity to intestinal epithelium involving the adherence and disruption of the epithelial barrier (picture 1,2). This result agreed with (10, 11, 12, 13).

This phenomenon belonged to the bacteria itself or the product of the bacteria (enzymes or toxin) and these factors aid the appearance of this ability at the same time the epithelial cell resist the barrier dysregulating effects by release of mucus, Immunoglobulin A (IgA) and other defensins (14,15). And there was apical side of intestinal epithelium is highly resistant to various cytolytic exoproducts of *Ps. aeruginosa* including exotoxin A and elastase (16, 17).

As the motility and adhesion to host cells are important factor to appear to predict virulence (18), so the bacteria are fully capable of change their virulence phenotype in direct response to host illness (19, 20).

Other study have identified a virulence – related attachment factor in *Ps. aeruginosa* , the PA-I lectin L adhesion, which plays a key role in adherence and distruption of the intestinal epithelial barrier, the PA – I lectin is capable of causing a significant permeability defect that allows paracellular flux of know cytotoxins of Ps. aeruginosa including exotoxin A and elastase (16), the PA – I lectin is 13.000 Da protein that is located within the cell cytoplasm when bacteria are grown in nutrient – rich media under ideal laboratory conditions, yet under conditions of physiological stress, such as occurs within the intestinal tract following surgical injury, expression of the PA-I lectin is increased and the protein relocated to the bacterial cell surface (21, 22). Another study suggest that this organism behaves like a classic opportunist, switching virulence genes on and off in response to selected environmental condition, although it is well established that environmental condition such as PH, redox state and nutrient composition can activate virulence gene expression in bacteria through a variety of membrane – bound biosensor kinases, (23). Also from the standpoint of the evolutionary fitness of the microbe, however, it is logical the pathogen might recognize the biochemistry of the host cell stress, because possessing a system that recognizes host susceptibility would allow for a more accurate assessment of the costs versus benefits of host invasion and cytotoxin effects of bacteria are often judged by their ability to adhere and alter the barrier cells, other shown that bacterial adherence alone can significantly alter the permeability of the intestinal epithelium to pro-inflammatory luminal macromolecules (24, 25).

The stress also consider an important factor for appearance adherence and disruption of the intestinal epithelial barrier by invading opportunistic microbial pathogens as Ps. aeruginosa (26,27). Finally the centrifugation and washing by PBS deleate the cells non adheres after infection and pathogeneses this agreed with our results and (28).

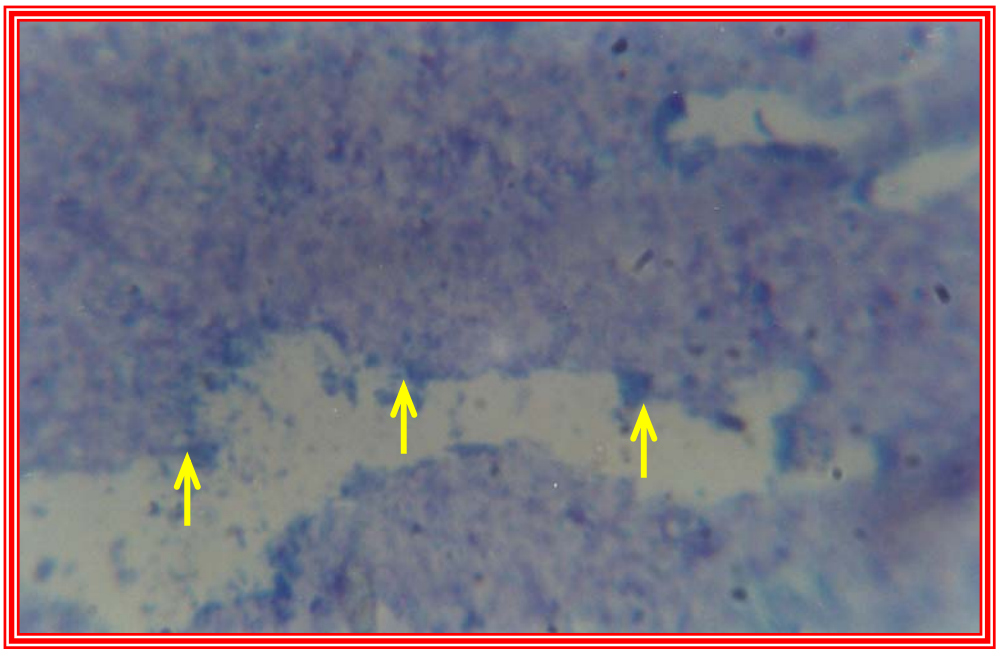

Figure 1:- Picture show intestinal epithelium involving the adherence and disruptions of the epithelium barrier in section of rabbit intestine, Giemsa's Stain, X1000.

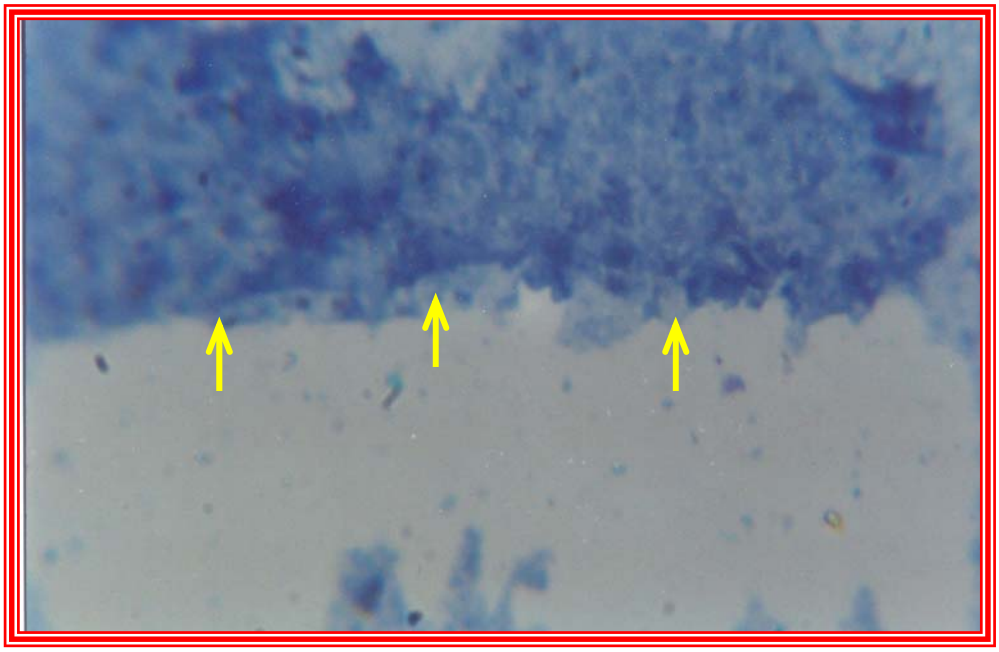

Figure 2:- Picture show intestinal epithelium involving the adherence and disruptions of the epithelium barrier in section of rabbit intestine, Giemsa's Stain, X1000.

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