Isolation of Enterobacteraerogenes from urine of patient with urinary tracts infection and studying pathogenicity of Enterobacteraerogenes in mice

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الخلاصة:

جريت الدراسة لعزل جراثيم الانتيروبكترايريجنوسا من ادرار المصابين بالتهاب المجاري البولية ، تم جمع 150 عينة ادرار وزرعت على اكار الماكونكي واستخدمت الفحوصات الكيموحيويةلتأكيد تشخيص الجرثومة ، بعدها استعملت اربع مجاميع من الفئران المختبرية لتحديد إمراضيه الجرثومة وذلك بحقن هذه الحيوانات بتراكيز مختلفة من الجرثومة الحية ($10^{8,}10^{9}$ and 10^{10} CFU/ml) على التوالي بينما تركت المجموعة الرابعة كسيطرة ،وتم استخلاص الجدار الخلوي من جرثومة الانتيروبكترلمعرفة دوره الحقيقي بالامراضية ، اظهرت النتائج بان هناك عزل جرثومي عالي من الحيوانات المحقونة بالجرعة العالية وان الجراثيم الحية ومستخلص الجدار الخلوي سبب نفس التغيرات المرضية الشديدة للأعضاء الداخلية مقارنة بحيوانات السيطرة .

Abstract:

The present study aimed to isolate *Enterobacteraerogenes* from urine of patient with UTI and showing its pathogenicity in mice, a total 150 urine specimens were collected and cultured on MacConky agare then tested biochemically and with API- 20 to confirm diagnosis of *Enterobacter aerogenes*, after that injected 4 groups of mice with ($10^{8,}10^{9}$ and 10^{10} CFU/ml) respectively, while fourth group consider as a control group, also extracted the cell wall from *Enterobacteraerogenes* and used four groups of mice to injected with different concentration(300, 200 and 150 µ/ml) of extracted cell wallrespectively, while fourth group consider as a control group. Results shows that 11 isolates of *Enterobacter* were obtained from urine and out of 11 isolates 8 isolates were belong to *Enterobacter aerogenes*. Bacterial isolation from internal organs showed very heavy isolation of Bactria in dose 10^{10} CFU/ml as compared with other dose, histopathological changes of organs for mice injected with live bacteria and extracted cell wall revealed same the tissue damage as compared with control groups.

Introduction

These organisms are Gram-negative capsulated bacilli and are similar to *Klebsiella* species with large round mucoid colonies, but can bedifferentiated by a few tests such as motilityand urease tests (1). The organisms are distributed in water, soil, sewage, dairy products and vegetables, are part of the commensal enteric flora and usually are not pathogenic (2). *Enterobacteraerogenes*, *E. Cloacae* and *E.sakazakii*are commonly encountered *Enterobacter* spp. in most clinical specimens (3) However, somestrains are known to produce shig-like toxin *Enterobacter* species have also beenassociated with nosocomial infections and avariety of opportunistic infections involvingthe urinary and respiratory tracts, and cutaneous wounds, enterobacter organisms cause significant morbidity and mortality. They can also cause communityacquired infections resulting in endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis and ophthalmic infections Risk factors for nosocomial infections include hospitalization for more than 2 weeks (4). In *Enterobacter* bacteria LPS is one of the major structural and

immunodominant molecules of the outer membrane. It consists of three domains: lipid A, core oligosaccharide (5). Lipid-A plays a major role in sepsis. Lipid-A, which is an endotoxin, is the major stimulus for the release of cytokines which are the mediators of systemic inflammation
and its complications (6) *Enterobacter* species are resistant to most antibiotics, especially the cephalosporins. Their resistance to _-lactam antibiotics, chloramphenicol, quinolones and tetracyclines have
been well documented (7). Reports abound in the literature on the increasing resistance of *Enterobacter* species to the penicillins and all
generations of cephalosporins, and their emergence in clinical specimens. The aim of study was to know role of *Enterobacter* cell wall in pathogenisis.

Materials and methods:

1-specimens collection:

Hundred and fifty urine specimens were collected from patient aged between 14 and 66 years (mean age of 34 years) attended to Al hussian hospital in AL samawa city from July to October 2013
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2-Isolation and identification:

urine samples were centrifuged for 15 minutes(3000 cycle/minutes) then from sediment will take a loopful for culturing on macConky agar. They were incubated for 24hours at 37 C then certified enterobacter growth by staining the bacteria with Gr stain and by biochemical tests (catalase, VP, citrate utilization , TSI with CO2 production and negative for H2S, oxidase, indol and MR) also using the APi 20 according to Cowan (8)

3-Pathogenicity of *E. aerogenes* in mice:

The preparation of the bacterial suspension and the counting were made using Macfarlands tubes according to procedure described by Baron (9). Four groups(5 mice for each group) of mice were used, first group was infected with 1×10^{8} CFU/ml, second group infected with 1×10^{9} CFU/ml and third group infected with 1×10^{10} CFU/ml, while the fourth group as a control group and given phosphate buffer saline, after bacterial inoculation, bacterial isolation from the internal organ were carried from the infected mice at day 7 post infection, the organ samples were cultured on brain heart infussion agar and incubated at 37c° for 2 days. The results recorded according to the density of the bacterial growth (8).

Bacterial cell wall Extraction:

1-Cell wall was extracted according to Hirschfeld (10). The carbohydrate content in the extracted cell wall was estimatedaccording to Westphal and Jann(11) and the protein content was determined according to Biuret procedure Henry, *et al.*,(12).

.Measurement of the LD50 of extracted cell wall:

Four groups(5 mice for each group) of mice were used , inoculated intraperitonialy with of extracted cell wall(300, 200 and 150 µ/ml) each group, while control groups injected with PBS. After one week LD50 was measured according to Read and Muench (13)

pathological study:

macroscopic examination (gross): postmortem examination were done for infected mice. The macroscopic features was recorded to detect any abnormal gross changes in the internal organs, including location, color, size, shape, consistency and appearance of cut section (14).

Histopathological examination:

Specimens(1*1*1cm) were taken from infected internal organs (spleen, liver, lung, and kidney). the tissues were kept in 10% formalin immediately after removal, then a tissue section were done to observe the histopathological under light microscope.

Results

Twelve samples were positive for enterobacter, out of 11 samples 8 were positive for *E. aerogenes* when cultured on macConky agar where lactose ferment on macConky agar, with smooth, round and mucoid colonies, enterobacter were positive for catalase, VP, citrate utilization and give acid results on TSI with CO2 production and negative for H2S, oxidase, indol and MR, all strain were motile as well as all strains unproduced for hemolysin on blood agar. Results of APi- 20 showed in Figure (1) and table (1) .

Figure(1) Results of API -20 of *E. aerogenes*

Table (1) showed results of APi-20 test

Orthonitropheny l- β-D-galactopyranoside	**ONPG**	Coler less
Arginine dihydrolyase	**ADH**	yellow
Lysine decarboxylase	**LDC**	red
Ornithine decarboxylase	**ODC**	red
Citrate utilization	**CIT**	green
Hydrogen sulfide	**H2S**	-
Urease production	**URE**	–
Tryptophan deaminase	**TDA**	yellow
Indole production	**IND**	pink
Vogas proskaur	**VP**	red
Gelatin liquefactions	**GEL**	-
Glucose	yellow	
Mannitol	yellow	
Inositol	yellow	
Sorbitol	yellow	
Rhamnose	yellow	
Sucrose	yellow	
Melibiose	yellow	
Amygdalin	yellow	
Arabinose	yellow	

The Protein concentration of extracted cell wall was 8.37 mg/ml and CHO of extracted cell wall was 0.17 mg/ml, while the LD50 of extracted cell wall show only 2 animals dead in first group that inoculated with 300µ/ml , while other animals showed emaciation, loss of appetite, dullness and weakness.

From results showed that isolated bacteria from internal organ were very heavy in animals inoculated with 1×10^{10} CFU/ML, heavy in animals inoculated

with1×10^9CFU/ML and moderate to mild in animals inoculated with1×10^8CFU/ML respectively, table(2).

Table(2) colonial growth of *E. aerogenes* isolated from internal organs of infected mice

Group	No.	spleen	Liver	Kidney	Lung
I	1	++	++	++	++
	2	++	++	+	-
	3	+++	-	-	+
	4	++	+	+	++
II	1	++++	+++	+	+++
	2	++++	+++	++	++
	3	+++	++	+++	+
	4	++++	+	-	+++
III	1	++++	+++	++	++++
	2	++++	++++	++	++++
	3	+++	++++	+	++
	4	++++	+++	+	++
IV	1	-	-	-	+
	2	-	-	-	-
	3	+	-	-	-
	4	-	-	-	-

+++ = moderate (11-15) colony

++++ = heavy (16-20) colony

+++++ = very heavy (20-more)

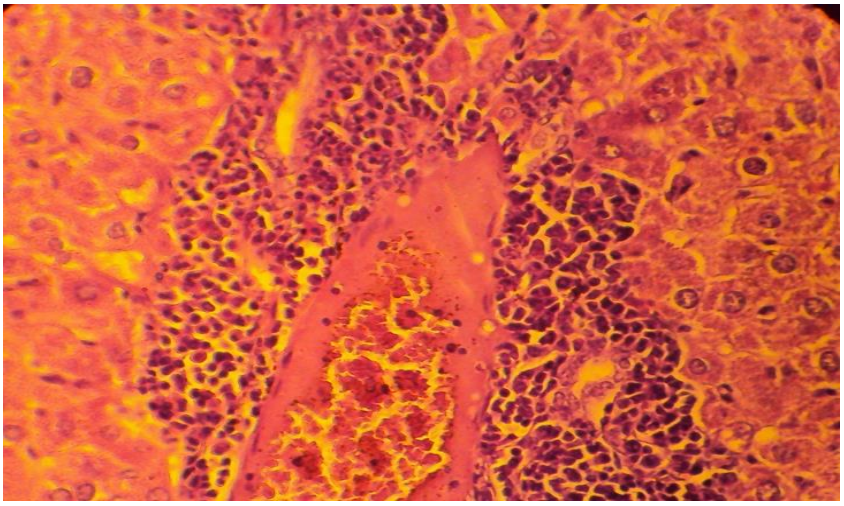
- = negative (0) colony

+ = very mild (1-5) colony

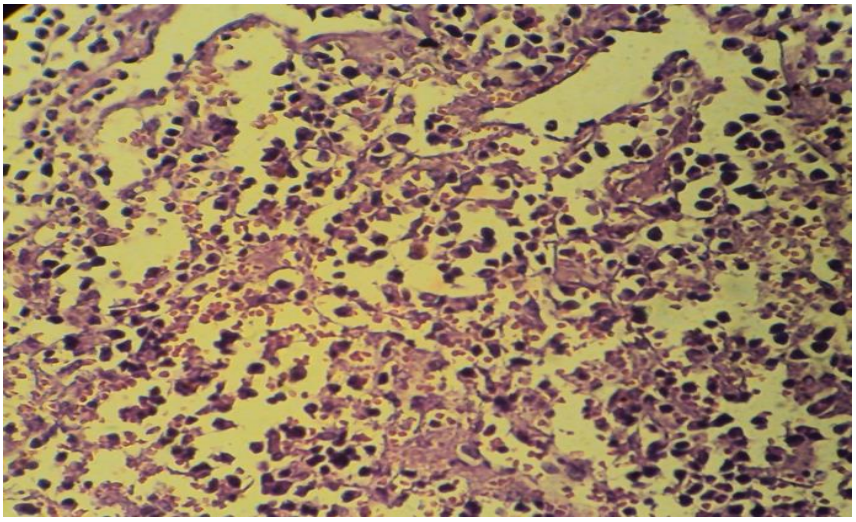
++ = mild (6-10) colony

Histopathological examination of animal infected with Enterobacter spp showed inflammatory cells infiltration in the interstitial tissue of lung and in the lumen of the alveoli mainly macrophages, lymphocytes with few neutrophil (figur 1) also there is severe centrilobular congestion and hepatocellular necrosis of liver the lumen of the blood vessels contain inflammatory cells mainly, neutrophils and macrophages(figure 2). While changes in spleen were acute congestion of the red pulp, infiltration of macrophages, plasma cells and few neutrophils throughout white and red pulp as well as depletion of the splenic follicle and deposition of amyloid like substance.

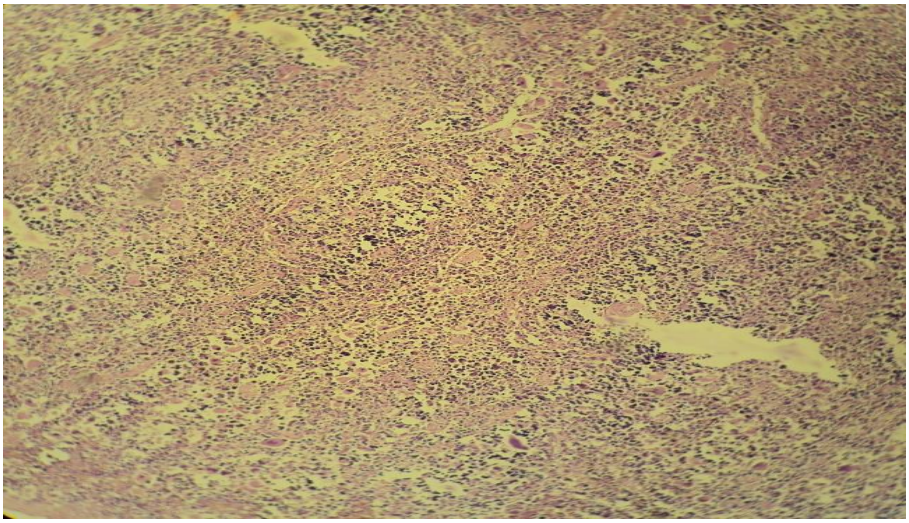
Histopathological changes in animals injected with extracted cell wall were extended in sinusoid, edema, degeneration and hemorrhage in liver tissue, while spleen tissue were showed enlargement of white pulp with accumulation of giant cells, neutrophil and lymphocytes (figure 3).



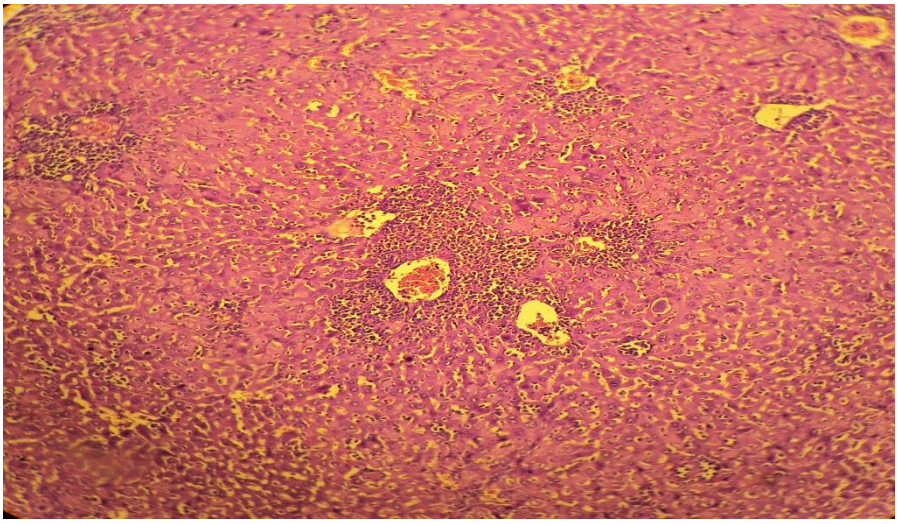
Figure(2)Section of liver of mice infected with*E. aerogenes* shows inflammatory cells infiltrationand hepatocellular necrosis of liver (H&E).



Figure(3) section of spleen of mice infected with*E. aerogenes* shows depletion of the splenic follicle and deposition of amyloid like substance(H&E).



Figure(4) Section of liver of mice injected with extracted cell wall shows degeneration and hemorrhage in liver tissue(H&E).



Figure(5) Section of spleen of mice injected with extracted cell wall shows enlargement of white pulp with accumulation of giant cells(H&E).

Discussion

The study showed *E. aerogenes* in urin samples It is known to be associated with a variety of opportunistic infections (15) growth culture of Enterobacter like that reported by (9) *E. aerogenes* shows negative for urease test which can differentiate it from E. *Cloacae* as a (3). From results shows very heavy bacterial isolated from internal organs of mice injected with and heavy bacterial isolated from mice injected with as a compared with control groups and animals injected with small dose and revealed the role of live bacteria in invasiveness and causes multiplication in internal organs as that reported with(16), Enterobacter can produce extracellular enzymes that have role in pathogenesis. Attachment properties may be important in the firm or maintenance of bacterial infections. Adhesins are often also hemagglutinins(HA) and may or may not be located on fimbriae. Most strains of *Enterobacter* produce a mannose sensitive hemagglutinin (MS-HA) associated with type 1 fimbriae, i.e., thick, channelled fimbriae of external diameter 7 to 8 nm. These fimbriaecan be coated by type 1 fimbrial antiserum against *E. cloacae* 035 but not by type 1 fimbrial antiserum against *Klebsiellapneumoniae*K55/1. No other hemagglutinin and fim,Aerobactin was first isolated from a strain of *E. aerogenes*(then called "*Aerobacteraerogenes*") Aerobactin and cloacin DF13 bind to the same receptor sites located in the outer membrane(17),also the results were in agreement with histopathological study were show sever histopathological changes include infiltration of inflammatory cells with necrosis of internal organs also animals injected with extracted cell wall showed serve tissues changes of organs The lipopolysaccharide from *E. agglomerans*(commonly found in cotton dust) can bind to the pulmonary lipid-proteinaceous lining material (surfactant) and alter its surface tension properties. Thiolas (18) said that the binding in the lung may change the physiological properties of surfactant and be a possible mechanism for the pathogenesis of by ssinosis, an occupational respiratory disorder caused by the inhalation of cotton dust.

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