Study the pathogenicity of *Enterobacter cloacae* in rats that isolated from diarrheatic buffalos calves in Babylon province

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Abstract

The study was aimed to isolate *Enterobacter cloacae* from feces of buffalo calves suffering from diarrhea and shows its pathogenicity in rats, 150 fecal samples were collected and cultured directly on MacConky agar then tested biochemically and with EPi 20 test to confirm diagnosis of *Enterobacter cloacae*. After that injected 4 groups of rat with ($10^{6,}10^{7}$ and 10^{8} CFU/ml) respectively, while the fourth group not treated and consider as a control group, also extracted the cell wall from *Enterobacter* and used four groups of rat to injected with different concentration (150, 250 and 350 µ/ml) of extracted cell wall respectively, while fourth group consider as a control group. Results shows that 10 isolates of *Enterobacter* were obtained from stool and out of 10 isolates 7 isolates belong to *Enterobacter cloacae*. Bacterial isolation from internal organs shows very heavy isolation of bacteria in dose 10^{8} CFU/ml as compared with other dose, histopathological changes in organs (liver and spleen) of animals which injected with live bacteria and extracted cell wall reveal severe changes as compared with control groups.

Key words: *Enterobacter cloacae*, buffalo diarrhea, pathogenicity, rats.

دراسة إمراضية جرثومة الانتيروبكتركلويكا المعزولة من عجول الجاموس المصابة بالإسهال في محافظة بابل

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الخلاصة

أجريت الدراسة لعزل جراثيم الانتيروبكتركلويكا من براز العجول المصابة بالإسهال ، تم جمع 150 عينة إسهال وزرعت مباشرة على اكار الماكونكي واستخدمت الفحوصات الكيموحيوية وتم استخدام نظام Epi لتأكيد وتشخيص الجرثومة ، بعدها استعملت اربع مجاميع من الجرذان المختبرية لتحديد إمراضية الجرثومة وذلك بحقن هذه الحيوانات بتراكيز مختلفة من الجرثومة الحية ($10^{6,}10^{7}$ and 10^{8} CFU/ml) على التوالي بينما تركت المجموعة الرابعة مجموعة سيطرة ، وتم استخلاص الجدار الخلوي من جرثومة الانتيروبكتر لمعرفة دوره الحقيقي بالامراضية ، اظهرت النتائج الحصول على 10 عزلات لجرثومة الانتيروبكتر منها 7 عزلات تعود لنوع الانتيروبكتركلويكا اثبت العزل الجرثومي من الاعضاء الداخلية (الكبد و الطحال) بان هناك عزل جرثومي عالي من الحيوانات المحقونة بالجرعة العالية وان الجراثيم الحية ومستخلص الجدار الخلوي سبب درجات عالية في الشدة من التغيرات المرضية النسيجية للأعضاء الداخلية مقارنة بمجموعة السيطرة .

الكلمات المفتاحية: جرثومة الانتروباكتر ، الجاموس ، الاسهال ، الامراضية ، الجرذان.

Introduction

Enterobacter is Gram-negative capsulated bacilli forms large round mucoid colonies on nutrient agar. It resembles to *Klebsiella* species with large round mucoid colonies, but can be differentiated by a few tests such as motility and urease tests (1). They are facultative anaerobic, catalase positive, citrate positive, indole negative and oxidase negative. They ferment glucose and lactose with the production of acid, and are sucrose

positive. The organisms are distributed in water, soil, sewage, dairy products and vegetables. They are a part of the commensal enteric flora and usually are not pathogenic (2). *Enterobacter aerogenes*, E. *Cloacae* and *E. sakazakii* are commonly encountered *Enterobacter* spp. in most clinical specimens. *E. cloacae* infected buffalo and causing diarrhea (3). These three species are differentiated by urease test and pigment production. *E. cloacae* are urease positive while the other two are negative (3). *E. sakazakii* produces yellow pigment which differentiates it from the other two species. However, some strains are known to produce Shiga-like toxin. *Enterobacter* species have also been associated with nosocomial infections and a variety of opportunistic infections involving the urinary and respiratory tracts, and cutaneous wounds (4). *E. sakazakii* which is a pigmented strain. *E. cloacae* have been encountered in several cases of meningitis, bacteremia and sepsis in human and animals (5). It has also been associated with outbreak of necrotizing enterocolitis associated with the strain in powdered milk formula, and fatality rate is as high as 75 %. (6). Enterobacter organisms cause significant morbidity and mortality. They can also cause community acquired 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 (7).

Materials and methods

Hundred and fifty fecal samples were collected from buffalo calves suffering from diarrhea in Babylon province.

1-Culture:

Fecal samples were cultured directly on MacConky agar at 37C°/24hrs. for isolation of bacteria. Then Enterobacter growth was identified by staining the bacteria with Gram stain, biochemical tests and by using the Epi 20 test (8).

2-Pathogenicity of bacteria:

Preparation of the bacterial suspension from the isolated bacteria was made using McFarland tubes (9). Four groups (5 rats for each) of rats by s/c methods were used, first group was inoculated with 1×10^6 CFU/ml, second group infected with 1×10^7 CFU/ml and third group infected with 1×10^8CFU/ml, while the fourth group as a control group and given Phosphate Buffer Saline, after bacterial inoculation the rats were sacrificed and the many swabs were taken from (liver and spleen), bacterial isolation from the internal organ were carried from the animals at day 7 post infection, the samples cultured on Brian Heart Infusion Agar and incubated at 37c° for 2 days.

3-Extraction cell wall of bacteria:

1-The cell wall was extracted according to (10), after that evaluated the carbohydrate content in the extracted cell wall according to (11), and measure the protein content according to (12).

2. Measurement the LD50 of cell wall extracted:

Four groups (5 mice for each group) of mice were used, inoculated intraperitonialy with (350, 250 and 150 µ/ml) concentration for each group, while control group injected with phosphate buffer saline. After one week LD50 was measured according to (13).

4-Pathological study:

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

5-Histopathological examination:

Specimens (1cm) were taken from internal organs include spleen, liver, lung, and kidney. the tissues were kept in 10% formalin solution immediately after removal and the histopathological changes were observed under light microscope according to (14).

Results

Out of 150 fecal samples, ten samples were positive for Enterobacter. Out of the ten, 7 samples were positive for *E. cloacae* 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 strains were motile as well as all strains unproduced for hemolysin on blood Agar. Protein concentration of extracted cell wall was 5.97 mg/ml and CHO of extracted cell wall was 0.20 mg/ml, also when determine the LD50 of extracted cell wall show only 2 animals dead in first group that inoculated with 350µ/ml, while other animals showed

Table (1) shows results of *E. Cloacae* isolation from internal organs of infected rats.

Group	No.	Spleen	Liver	Kidney	Urinary bladder
I	1	+++	++++	+	+++
	2	++	++	+	+
	3	++	+	++	-
	4	++	+	+	++
II	1	+++++	++++	++	++++
	2	++++	+++	+++	+++
	3	+++	+	+++	+
	4	++++	-	+	+++
III	1	+++	++++	+++	+++
	2	+++	++++	+++	++++
	3	+++	++++	++	+++
	4	++++	+++	++	++
IV	1	+	-	-	-
	2	-	-	-	-
	3	_	-	-	-
	4	-	-	-	-

+++ = moderate (11-15) colonies, ++++ = heavy (16-20) colonies, +++++ = very heavy (more than 20) colonies.

emaciation, loss of appetite, dullness and weakness. Results showed that bacterial isolation from internal organ were greatly heavy in animals inoculated with 1×10^{8}CFU/ml, heavy in animals inoculated with1×10^{7}CFU/ml and moderate to mild in animals inoculated with1×10^{6}CFU/ml respectively, table (1). Histopathological examination of animal infected with Enterobacter showed inflammatory cells infiltration in the interstitial tissue of lung and in the lumen of the alveoli mainly macrophages, lymphocytes also few neutrophils in less, as well as there was severe centrilobular congestion and hepatocellular necrosis of liver, the lumen of the blood vessels contain inflammatory cells mainly neutrophils and macrophages (fig.1). While the 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 (fig. 2). Histopathological changes of animals injected with extracted cell wall were extended in sinusoid, edema, degeneration and hemorrhage in liver tissue (fig. 3), while spleen tissue were showed enlargement of white pulp with accumulation of giant cells, neutrophil and lymphocytes (fig. 4).

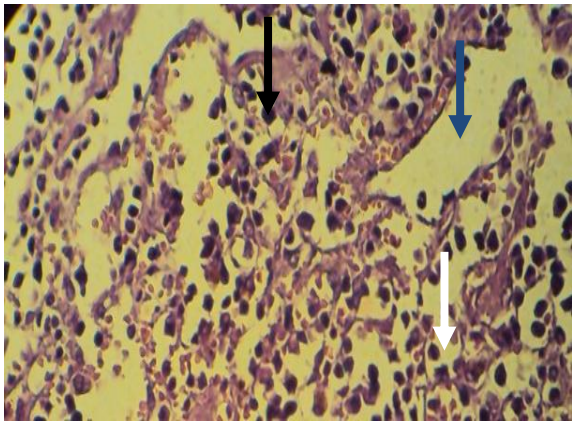

Fig.(1): Cross section of rat liver infected with *E. Cloacae*, Shows infiltration of inflammatory cells (macrophages) (black arrow), hepatocellular necrosis of liver (white arrow), and edema (blue arrow). (H&E) 400X.

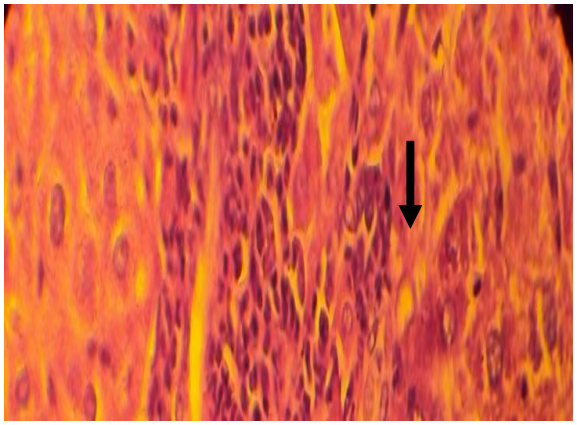

Fig. (2): Section of rat spleen infected with *E. Cloacae* shows depletion of the splenic follicle and deposition of amyloid like substance (black arrow) (H&E) 400X.

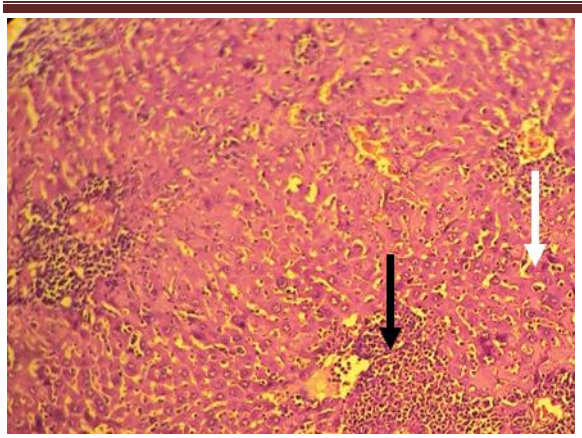

Fig. (3): Section of rat liver injected with extracted cell wall shows degeneration and hemorrhage in liver tissue (white arrow), and inflammatory cells (black arrow). H&E (100X).

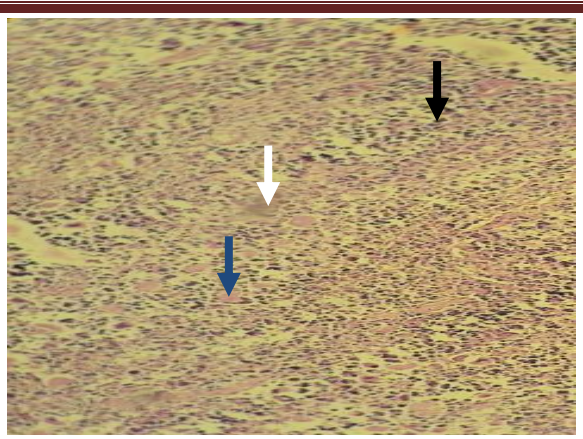

Fig. (4): Section of rat spleen injected with extracted cell wall shows enlargement of white pulp (white arrow) with accumulation of giant cells (blue arrow) and inflammatory cells (black arrow) (H&E) 100X.

Discussion

E. Cloacae are isolated from diarrheatic fecal samples. It is known to be associated with a variety of opportunistic infections (15). The growth culture of *Enterobacter* seen in this study resembles that reported by (9). *E. Cloacae* are positive for urease test which can differentiate it from *E. aerogenes*. Results declare heavy bacterial isolates from internal organs (liver, spleen, and lung) of rats injected with more than 1×107CFU/ml as a compared with control group and animals injected with small dose of bacteria, therefore reveal the role of live bacteria in invasiveness and multiplication in internal organs as that reported by (16), also *Enterobacter* can produce extracellular enzymes that have role in pathogenesis. Adhesive properties may be important in the establishment or maintenance of bacterial infections. Adhesive enzyme often hemagglutinins (HA) 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, channeled fimbriae of external diameter 7 to 8 nm. These fimbriae can be coated by type 1 fimbrial antiserum against *E. cloacae* 035 but not by type 1 fimbrial antiserum against *Klebsiella pneumonia* K55/1. Aerobactin is 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). The histopathological results show severe histopathological changes including infiltration of inflammatory cells with necrosis of internal organs; also animals injected with extracted cell wall showed severe tissues changes of organs. The lipopolysaccharide from *E. Cloacae* (commonly found in cotton dust) can bind to the pulmonary lipid-proteinaceous lining material (surfactant) and alter its surface tension properties. This binding in the lung may change the physiological properties of surfactant and be a possible mechanism for the pathogenesis of byssinosis, an occupational respiratory disorder caused by the inhalation of cotton dust (18).

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