

RESEARCH ARTICLE

Experimental Transmission of *Enterobacter cloacae* from Fishes to Wounds of Skin by Using balb/c Mice

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Abstract

Often, housewives are injured in the hands, which may expose them to infection, possibly with different diseases, when they are exposed as is the case in this study, which was dependent for isolation, identification of *Enterobacter cloacae* from different healthy fishes and experimental infection for bacteria in mice. The thirty clinical specimens were collected from fishes is diagnosed according cultural and biochemical tests as well as the central health laboratories/enterobacterial unit/Bagdad. The Murine Model of Wound *E. cloacae* Infection were done with 2×10^5 ID₅₀ CFU/ml and the bacteria was isolated from infected mice group to different organs with high prevalence result of 100% percent from intestine, duodenum and blood, the spleen and liver give percentage 83% from bacteria with smallest prevalence from heart 66% and brain 50% respectively, the pathogenicity will be study and all infected group give positive results.

Keywords: *Enterobacter cloacae*, Fishes, Skin.

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INTRODUCTION

The family *Enterobacteriaceae* have members of genus *Enterobacter* such as *E. cloacae* are act as opportunistic human pathogens; This organism is an opportunistic pathogen, which means that the disease targets compromised patients such as the young, old, or those that have a severe disease such as human immunodeficiency virus¹. Nosocomial infections are most frequent from these bacteria, which means it may be contracted from the result of being hospitalized or debilitated patients, and investigation of *Enterobacter cloacae* infections at a small animals veterinary teaching hospital². A National Nosocomial Infection Surveillance System (NNIS) study found that *Enterobacter* accounts for 5 to 11% of all nosocomially transmitted blood products, wound that have been isolated from hands of personnel, and respiratory tract and urinary tract infections³. Many genera of *Enterobacteriaceae* such as *Escherichia*, *Enterobacter*, *Klebsiella* and *Serratia* have been implicated in a broad range of clinical syndromes bacteremia and infections of skin and soft tissues as well as fruits, vegetables, and devices such as a hot water treatment tank, respiratory tract, urinary tract, bone and joints, central nervous system, gastro-intestinal tract, and other organs⁴. *Enterobacter cloacae*, *Enterobacter sakazakii*, and *Enterobacter hormaechei* have caused sepsis and a severe form of necrotizing meningitis in neonates⁵. *Enterobacter spp.* are facultative anaerobic gram – negative bacilli means they contain two cell membranes, 80% are capsulated, most species are uses its peritrichous flagella for movement and have class 1 fimbriae¹. *Enterobacter cloacae* and *Enterobacter aerogenes* are part of the normal flora of the gastrointestinal tract and are found in a high proportion of sewage sample, at concentrations of up to 10⁷organism g-1¹. In extra intestinal infections *Enterobacter* are involved, which are known to possess virulence associated characteristics, they have the ability to adhere to and invade eukaryotic cells, which can cause toxins to run in the tissues and blood stream, once outside the gastrointestinal tract, they would take advantage of being able to chelate iron to survive and spread within the host⁷. *E. cloacae* infections have the highest mortality rate compared to other *Enterobacter* infections⁸.

MATERIALS AND METHODS

Samples collection: A total 30 different specimens were collected from ten *Tilapia* and *Liza abu* fishes bought from locale market in Alqasim city during December 2017 (IRAQ), A different samples from (mucus of skin, viscera fluid, gill, kidney, muscle, eyes and blood) were cultured in nutrient agar after that transferred to selective media XLD,SS media for Morphological characteristics of colonies as primary identification of *Enterobacter cloacae* according to the criteria of^{9,10}.

Preparation of bacteria inoculum

Samples of bacteria tested by gram stain for studying the microscopic properties under the oiled lens of light microscope, for ensuring diagnosis we sent to the central health laboratories/enterobacterial unit/Bagdad.

Grow the bacteria at 37°C with brain heart infusion broth for 12 hr. then collect bacteria by centrifugation (3000 rpm/min for 15 min, 20°C). Wash bacteria three times in an equal volume sterile PBS and harvest by centrifugation. Resuspend bacteria two- fold dilution in PBS to attain a final concentration of 2×10⁵ colony-forming unit(CFU)/ml.

Infectious dose (ID50) calculated by the two-fold dilutions by applied in six mice group for used later in Murine Model of Wound Infection.

Preparation of Mice for infection

Twelve male 1month old mice (balb/c) examined with negative culture *E. cloacae* in feces. Later, used in Murine model of wound infection divided into two groups the first group contain six mice for test and the second group contain six mice as control group¹¹.

Mice are shaved by Casanova® hair remove spray to inoculation bacteria, all mouse procedures are performed under local lidocaine anesthesia.

Pinch the skin by Miltex® standard biopsy punch of the prepared site of infection, inoculate the animals subcutaneously with 0.05 ml of 2×10⁵ live *E. cloacae* or sterile saline. Monitoring the course of infection on 2-3 days archives for 6 days.

The infected mice were dissected and the samples was taken from many organs (blood, brain, heart, spleen, intestine and duodenum) for isolated bacteria. The control group were also tested from bacteria.

The statistical analysis

Statistical analyses were carried out using chi-square tests of the SPSS software version 9.0 for windows (Levesque 2007) to compare the rate of isolation of bacteria among different organs. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation of associated bacteria

The results were specified of thirty samples of *Enterobacter cloacae* from fishes give positive culture and appeared under the microscope in oil emersion 100× magnification (Picture No1). Salmonella Shigella (SS) agar the bacteria appear as small pink colonies, that were round, dark center, slightly mucoid and 2-3 mm in diameter, with regular edges. (Picture No2). The *E. cloacae* was isolated later from anatomical infected mice appeared in a nutrient agar as a yellowish pale, that were large flat mucus colonies with irregular edges. and the control group were gives negative results.

Table 1. *Enterobacter cloacae* isolated from internal organs (n=48) of mice.

Isolated <i>E. cloacae</i> bacteria	No. of positive samples and prevalence (%) (n=60)	Total No. (%)
Intestine	6 (60)	(100%)
Duodenum	6 (60)	(100%)
Blood	6 (60)	(100%)
Spleen	5 (50)	(83%)
Liver	5 (50)	(83%)
Heart	4 (40)	(66%)
Brain	3 (30)	(50%)
eye	NI	NI

Legend: NI- Not identified

Table 2. Pathogenicity test of *E. cloacae*

Group	Rout of inoculation	Number of mice inoculation	Number of mice infected within 48hrs.	Inter-pretation
Test	subcutaneous	6	6	P
control	subcutaneous	6	0	NP

Legend: P=Pathogenic, NP= Nonpathogenic.

Application the murine test

The Murine model of wound *E. cloacae* infection mice were injection with live *E. cloacae* or sterile saline show picture No3 by: Remove hair from dorsal side of mice, Euthanized mice 1.5×1.5 cm-tissue full thickness punch surgical wound covered with plaster, Inject subcutaneously with 0.05 ml of 2×10^5 (CFU) *E. cloacae* under plaster.

Isolation of bacterial from internal organs of mice: The *E. cloacae* isolated from the internal organs of infected mice were identified in (Table 1).

Prevalence of bacteria in different internal organs of mice: From the collected samples (n=6), *E. cloacae* was isolated (100%) percentage from 6 mice intestine, Similarly, the overall prevalence of duodenum, and blood organs whereas *E. cloacae* was found in 5 cases (83%) of spleen and liver, also the bacteria could be isolated from heart (66%) cases, the brain give (50%) from total number, respectively. The results have been notable that all seven different organs could be isolated the bacteria. No *E. cloacae* could be isolated from eyes samples. The control group give negative result in culture media. Intestine, duodenum, and blood was the most prevalent region in all types of samples in this study (Table 1).

Pathogenicity test of *E. cloacae* in mice: Pathogenicity test of *E. cloacae* was calculated throw the murine test when inoculation bacteria for mice (Table 2). The experimental mice were infected with positive grow in culture media within 48 hrs and no clinical signs noted, whereas the control mice give negative result. Thus, the isolates were categorized as pathogenic. Enterobacter cloacae have been considered as opportunistic pathogens involved in a broad array of infections, the study focused on explaining the contribution of transmission activity, as well as the analysis of incidence of light microscope rod- shape bacterial



Fig. 1. Gram- negative bacilli of *Enterobacter cloacae* bacteria



Fig. 2. Pink, round and black center bacteria from mucus, viscera, and gill samples cultures in SS agar.

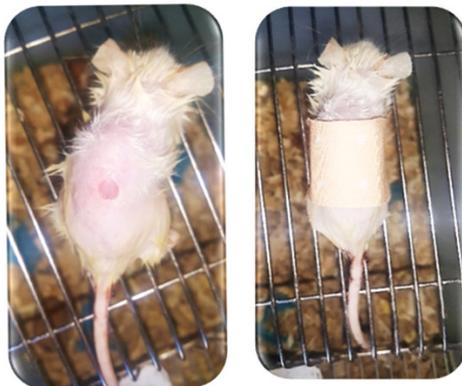


Fig. 3. Murine test: a/ 1.5×1.5 cm-tissue full thickness Miltex® standard biopsy punch surgical skin wound. b/ plaster covered wound.

compression with (Sylwia, et al. 2009) that studies of cell morphology in a scanning electron microscope (SEM) with needle-like structures cell. *Enterobacter*, joins the genera *Klebsiella* and *Serratia* in the tribe *Klebsiellae*¹² Colonies of *Enterobacter* strains may be slightly mucoid. In the present study, eight different internal organs (intestine, duodenum, blood, spleen, liver heart, brain and eyes) of mice were could be isolated bacteria from it. This is in line of findings by Azza H *et al.* (2012)¹³ when isolated from *Mugil capito* fish, considering all the 48 samples, *E. cloacae* was highly prevalent (100%) in intestine, duodenum, and blood, whereas highly prevalent in spleen and liver (83%)¹⁴. Studied the prevalence of *E. coli* in broiler feces and found 65% prevalence which is lower than that of the present study.¹⁵ Reported a 30.55% prevalence of *Salmonella* spp. In poultry meat which is lower than that of the present study. The pathogenicity test for all the isolated *E. cloacae* revealed that all. were pathogenic.¹⁶ reported that more than one predisposing factors such as environmental and managerial factors (housing, climate etc), imbalance nutrition and immune status of the poultry might play roles in developing diseases while harboring the potential pathogenic bacteria. A mouse model can be used to investigate a number of factors related to the pathogenesis of skin and soft tissue infections, including the contribution of specific bacterial molecules to disease with virulence strain, and agree with¹⁷ that understand staphylococcal pathogenesis as a human pathogen when determine many factors like environmental changes and produce a variety of elements that make to virulence by used a mouse as model.¹⁸ skin infections are caused by a taxonomic diversity of infectious microorganisms that include fungi, viruses.

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