



RESEARCH ARTICLE

STUDY OF BIOFILM AND SWARMING OF *PROTEUS MIRABILIS* ISOLATED FROM URINARY TRACT INFECTION FROM WOMEN

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ABSTRACT

The study included the collection (50) urine sample, and through the results of the transplant bacteriological the circles MacConkey agar and blood agar and central Eosin Methylene Blue, diagnosis phenotypic and biochemical, were obtained (10) isolation belonging to the type *proteus mirabilis* (20%). The results of the investigation of some virulence isolates factors explained, including the ability to produce Haemolysine. The results showed that all isolates *proteus mirabilis* do not have the ability to produce hemolysin on central blood Agar, was to investigate the ability of bacteria to produce the Swarming, and the results showed that all isolates *proteus mirabilis* have the ability to produce these, the results showed that all isolates have the ability to produce biofilm and Swarming 100%.

INTRODUCTION

Proteus mirabilis genus back to the family gastrointestinal (Enterobacteriaceae), which is characterized as a negative for the gram stain, ranging in width between (1 - 0.3) micrometer, while the length ranges from (6 - 0.6) micrometer, and features such bacteria as an analyst for the blood and do not produce gas H₂S and more types of them the ability to consume urea, to be colonies and pink, containing the capsule and non-fermented to sugars lactose and glucose, sucrose, immobile, and anaerobic optional, Barred shape, producing acid into glucose (Sharmeen, 2012). The bacterium *proteus mirabilis* opportunistic human pathogens and resistance to the process of phagocytosis to contain them on the capsule (Umeh, 2006). These bacteria cause many diseases, particularly acquired diseases in hospitals, including urinary tract and respiratory tract and wounds operations and meningitis, and infections of the middle ear and infections of the mucous membranes soft and necrosis of the nose and injuries of the lungs (Kumar, 2010). Bacteria of *proteus mirabilis* has a number of virulence factors that refers to the pathogenesis including antigens portfolio and factors adhesion and produce endotoxins such as multi-saccharide adipose advantage of these bacteria resistant to antibiotics broad-spectrum, as unable to resist an anti-beta-lactam through the production of enzymes beta-lactam working on shattering episode of beta-lactam and make molecules ineffective vital. Adding to the seriousness of these

enzymes for mutations in the genes responsible for encrypting it to turn it into enzymes and broad-spectrum resistance to antibiotics modern B-lactam antibiotics, including the advanced generations of Cephalosporins (Keynami, 2007). Newly announce globally disease called super bacteria caused by the bacterium *proteus mirabilis*, then was followed by the discovery of injuries in India and Pakistan, the United Kingdom and the United States of America, Canada and Japan, which are spread between the source in India and other countries as a result of transport, travel, the disease leads to the emergence of resistant many antibiotics, especially beta-lactam antibiotics (Pillai, 2011).

And the lack of studies related to these bacteria came to this study aims to:

Isolation and *proteus mirabilis* diagnosis of patients with different clinical infection and learn about the epidemic this isolated bacteria from patients in the city of Baquba and the investigation of different virulence of the bacterium *proteus mirabilis* factor.

MATERIALS AND METHODS

Collection of specimens

All specimens were collected from patients admitted to Baquba Teaching Hospital Wide-mouth containers were used. These were sterilized by hot-air oven at 180°C for 1 hour.

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Culture technique

Clinical specimens were inoculated onto 5% blood agar and MacConky agar for the isolation of enteric gram-negative rods (Pinsky, 2009).

Culture characteristics

For the identification of different enteric gram-negative bacilli, the colonial appearance on simple solid media was studied, after 24 hours incubation at 37°C. Was diagnosed colonies scrupulous emphasis was placed on the colonies non fermented sugar lactose.

Antimicrobial Susceptibility Test

The most widely used method for susceptibility testing was the disc agar diffusion method. It had the advantage of being simple, economical, and reproducible. The procedure, which was accepted by the National Committee for Clinical Laboratory Standard (NCCLS) was employed, as described by (Freeman, 1989).

Preparation of culture medium

Muller-Hinton medium was employed. The medium was cooled to (45-50)C and poured into Petridishes on a level surface to a depth of 4mm. When the medium was hardened, the Petridishes were placed in the incubator at (15-30) minutes to allow excess moisture evaporate.

Preparation of inoculum

With a sterile wireloop, the tops of (4-5) isolated colonies of the test organism were picked from the original culture and introduced into a sterile test tube containing 4ml of nutrient broth. The broth was incubated at 37C for about (2-6) hours. The growth was adjusted to a MacFarland (No.0.5) turbidity standard using sterile saline or nutrient broth.

Inoculation of plates

A sterile cotton wool swab was dipped into suspension and rotating the swab firmly against the side of the tube above the level of fluid. The swab was streaked in three direction onto the dried surface of a Muller-Hinton plate until the plate was completely and uniformly covered.

Investigate some virulence factors in bacteria *proteus mirabilis*

Congo-red method

PROCEDURE:

- Deparaffinize and hydrate to water.
- Mayer's Hematoxylin for 10 minutes.
- Wash in tap water until blue.
- *Working sodium chloride solution, microwave, 20 power, 45 seconds.
- Allow slides to sit in solution for 2-5 minutes.
- 5 Place directly into Working Congo red solution, microwave, 20 power,
- For 45 seconds. Allow slides to sit in solution for 2-5 minutes.

- Dehydrate rapidly in absolute alcohol, 10 dips, 3 changes.
- Clear in xylene, coverslip with Permount.
- *Conventional Method:
- 4. Working sodium chloride solution, room temperature, 20 minutes.
- 5. Place directly into Working Congo red solution for 1 hour (Collee, 1996).

Swarming

Use this test to detect susceptibility of isolates under study on the phenomenon of possession swarming conducted this test using the method of circles solid agar and followed the way (Liaw, 2000) and his group as follows:

- Vaccinated blood agar containing way spots, as Vaccinate dish center (10) M of airborne bacterial.
- Incubated dishes degree 37M° for a period of 18 hours.
- Emergence of traffic spikes (swarming) indicate that the result is positive. (Harajly, 2010).

RESULTS AND DISCUSSION

Isolate and diagnose the bacteria *Klebsiellaspp*

Microscopic examination

Collected 50 samples bacteria have been isolated from people with different pathogens of both sexes and various ages from hospitals in Baquba, microscopic examination showed that 22 isolated shaped rod surrounded of capsule, and there are single or body pairs, or short chains, and to distinguish them from the remaining species were adopted Culture Details and biochemical test.

Biochemical tests for bacteria

Biochemical tests on local isolates belonging to the genus *proteus mirabilis* conducted was diagnosed *proteus mirabilis* based on phenotypic characteristics and microscopic and biochemical tests.

Table 1. Biochemical tests and microscopic to diagnose bacteriaproteus mirabilis

Proteus mirabilis	Testes
-	Gram stain
+	Motility test
-	Oxidase test
+	Catalase test
-	Indol production test
+	Methyl red test
-	Vogesproskauer test
+	Citrate utilization test
+	H ₂ S production test
+	Gas

Distribution of isolates by the position of the injury

Classified isolates and the number of 10 isolated by infection positions after being diagnosed means foregoing, and notes from the Table (2) The largest percentage of positive isolates were within lactation samples (20%) Lactation 20%. The results showed that the number of isolates of bacteria in samples *proteus mirabilis* lactation (10 isolates, 20%) The

findings are compatible with the findings of the (10) as the proportion *Proteus mirabilis* in samples lactation (21.8%).

Table 2. The number of isolates and their proportions according to sites of injury

Percentage	Positive percentage	Number of isolates	Isolates type
%20	10	50	urine

Investigate Virulence Factors

The present study included the investigation of a number of pathogenic bacteria associated *Proteus mirabilis* factors.

Swarming production

The investigation of the ability of bacteria to produce the swarming, and the results showed that all isolates *proteus mirabilis* have the ability to produce 100% (Al-Charrakh, 2011). Swarming movement by the whip, which is a appendages filamentous-like hair Hair - like appendeces helical Helical hollow stand out on the cell surface, owns bacteria *Proteus mirabilis* Aswata "oceanic the more than 100 whip peripheral site (Burnely, 2000).

Biofilm production

The results showed that all isolates have the ability to produce biofilm 100%, since all black colonies emerged with crystallinedensity dry and this is the positive result, and this percentage came compatible with the findings of the (Montanaro, 1999) as percentage of production *proteus mirabilis* biofilm and in large quantities 97.3%, and did not agree with the findings of the (Freeman 1989) as percentage producing biofilmand in large quantities 52%, and also the ratio came close relatively with the findings of the (Montanaro, 1999) and the use of red method of Congored as the proportion of the production of its Isolates biofilm amounted to 83%, and is the red way Congored modalities favorite to detect the production of bacteria layer viscous as not affected heavily cultivated bacterial and more sensitive to the use of Congo red is responsible for a multi-layer dab saccharide that serves as the base material for biofilm (Pillai, 2011). There are several possible environmental factors that affect the production of the mucous layer of the bacteria using this method, including oxygen, temperature, and other conditions as possible to give different results (Götz, 2002).

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