Swarming motility Patterns of *Pseudomonas aeruginosa* isolated from Otitis media

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Abstract
Pseudomonas aeruginosa presents three types of motility: swimming , twitching and swarming. The latter is characterized by rapid and coordinated group movement over a semisolid (viscous) surface resulting from morphological differentiation and intercellular interactions. A striking feature of *P. aeruginosa* swarming motility is the formation of migrating tendrils producing colonies with complex fractal-like patterns. In this study we reported an evidence that *P. aeruginosa* can spread on semisolid surfaces and expose swarming motility and to investigate the swarming motility patterns of these bacteria isolating from Otitis media, we culturing thirty clinical isolations of these bacterium on the swarming agar plate containing 0.5% agar. Our results reveals that 12 (40%) of studied isolates were detected dendritic swarming pattern , and 18 (60%) of these isolates were ring swarming pattern, besides that seven of *P. aeruginosa* total isolates were able to reveal both patterns of swarming motility when sub cultured on new swarming agar media, the results showed that the site of bacteria isolation don't influence and determine a swarming motility patterns of this bacteria, where, there are many studies on the same bacteria isolated from different infection sites which show the same swarming motility patterns according to this study. However, the mechanisms responsible for the dendritic and ring swarming patterns are largely unknown. Swarming motility may be an important factor in the pathogenesis of *P. aeruginosa*.

Introduction
Bacteria use a variety of motility mechanisms to colonize environments , including flagella-dependent swimming and swarming, and flagella-independent, twitching, gliding, and sliding. Of these motility mechanisms, the least investigated is sliding motility, which Henrichsen [1] defined as surface translocation produced by expansive forces in the growing colony combined with special surface properties to lower the friction between the cells and substrate. Harshey [2] points out that sliding motility is a passive mode of translocation for spreading over surfaces.

Swarming motility is a multicellular phenomenon involving the coordinated and rapid movement of a bacterial population across a semisolid surface [3]. It is widespread among flagellated bacteria, including Salmonella, Vibrio, Yersinia, Serratia, and Proteus [4,5]. Swarming is highly dependent on bacterial cell density, nutrient growth medium, and surface condition moistness[6]. In addition to physical changes such as an increase in the number of flagella or cell elongation, swarmer cell differentiation results in substantial alterations in metabolic bias and gene expression, indicating that swarming represents a complex lifestyle adaptation in response to particular medium conditions rather than merely a form of locomotion [5,7].

swarming motility may contribute in pathogenesis, since movement over surface may enable bacteria to migrate over, adhere to, and disperse from, sites of infection [8]. Swarming may protect pathogens from macrophages as swarm cells were shown to have enhanced resistance to engulfment [9]. Finally, toxin secretion is often co-regulated with swarming motility [5].

Otitis external is characterized by inflammation of the ear canal, with purulent ear drainage. It can be quite painful, and cellulitis can extend into adjacent soft tissues. A common from is associated with swimming in water that may be contaminated with aerobic, Gram-negative organisms such as Pseudomonas species [8].

Pseudomonas aeruginosa is a ubiquitous Gram-negative rod-shaped bacterium responsible for many infections among immunocompromised hosts, burned patients and individuals suffering from cystic fibrosis. Besides well known swimming and twitching motilities, this bacterium is capable of another type of migration called swarming. This complex type of motility is usually defined as a rapid and coordinated translocation of a bacterial population across a semi-solid surface [9]. In addition to flagella, swarming of *P.aeruginosa* requires the release of two exoproducts, rhamnolipids (RLs) and 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), which act as wetting agents and chemotactic-like stimuli [10]. The best studied bacterial social behaviour is the formation of attached communities called biofilms. Besides playing a role in swarming motility, RLs and HAAs are implicated in many aspects of biofilm development [8]. Interestingly, swarmer cells a range of bacteria, including P. aeruginosa and Salmonella typhimurium, display enhanced resistance to a variety of antibiotics [10], a well-known feature of the biofilm way of life. A complex relationship exists between swarming motility and biofilm development [11]. Swarming of P. aeruginosa is often typified by a dendritic coloial appearance. It possesses three types of movement depending on medium viscisity, namely, swimming in aqueous environment, twitching on solid surfaces or interfaces, and swarming on semisolid, viscous media , such as those containing 0.4 to 0.7% (wt/vol) agar [9,11].

Material and Methods

Culture media: the following cultures were used: Agar-Agar, Blood agar base, Brain Heart Infusion, MacConky s agar, Nutrient agar and Nutrient broth (Himedia/India). Cetrimide agar and Swarming medium (Structural media).

Sampling, Isolation and Identification

A total of thirty clinical samples were collected from ear infections that are suspect as causes Otitis media, from out patients admitted to Tikrit Teaching Hospital in Tikrit City.

Collected samples were cultured onto nutrient, blood and MacConky s agar for primary isolation. Non-lactose fermented colonies were selected and cultured onto Cetrimide agar, then incubated overnight at 37 °C for refreshment and demonstration of their ability for blood hemolsis [10,11]. Isolated bacteria were stained with Gram s stain method, and exramined under the microscope [12].

The selected colonies were cultured onto 0.03% Cetrimide agar and incubated at 37°C for 24 hours. The suspected colonies were identified by positive reaction to oxidase, IMVC, growth at 42 °C and 4 °C [10,11].

Swarming motility assay

The medium

Bacto Agar.....2 gm
Nutrient Broth.....3.2 gm
D.W.....400 ml

After autoclaving, filter- sterilized 10% (w/v) D-glucose in distilled water was added to get a final concentration of 0.5% (w/v) [13].

The method

Cells were grown overnight in nutrient broth and diluted in nutrient broth medium to an OD 600 of 0.02. Two - microliter aliquot of the diluted culture was inoculated onto the surface of a swarming plate and incubated overnight at 37°C. The ability of isolates to swarm was assessed by the distance of swarming from the central inoculation site[13].

Results and Discussion

Isolation and Identification of *P. aeruginosa*

All bacterial isolates were cultured selectively using cetrimiede agar medium and according to microscopic characteristics, cultural and biochemical tests. Presumptively, these colonies were identified as *P. aeruginosa*. The selected colonies were tested for oxidase and catalase production and sub-culture on MacConkey s agar to obtain pure culture for further diagnosis investigations.

thirty isolates were identified as *P. aeruginosa*.` All these isolates produced pyocyanin (blue pigment), which is a dignostic feature mentioned by [14]; and all isolates were able to grow at 42°C but not at 4°C , theses criteria were used for the identification of *P. aeruginosa* from other species [15]. Under microscopic it appears as gram negative, rod-shape, and occur as single, pairs, or in short chains[10,16]. In addition all studied isolates were produced alginate (mucoid appearance)[17].

Swarming Motility of P. aeruginosa Isolates

P. aeruginosa is capable of two well-known types of motilities: swimming motility, based on the flagellum, and twitching motility, relying on type IV pili. A third less well-understood motility behavior, swarming, which consists of a rapid and coordinated translocation of a bacterial population across a semisolid surface. In this study we reported an evidence that *P. aeruginosa* can spread on semisolid surfaces and expose swarming motility. When cells are inoculated at the surface of agar, regular colonies are formed initially. Later on, cells at the periphery or rim of the colonies undergo differentiation to from long, multinucleate, aseptate, hyper-flagellated swarm cells due to activation of flagella production and repression of cell septation [18,19,20]. Figure -1 reveal that 12 (40%) of studied isolates were detected dendritic swarming pattern, and 18 (60%) of these isolates were ring swarming pattern, in addition to that 7 (23.3%) of total isolated reveal both patterns of swarming motility when sub-cultured on new swarming medium. We conclude that a site of bacteria isolation don't influence and determine a swarming pattern, where, there are many studies on the same bacteria isolated from different infection sites which show the same swarming motility patterns according to our research. However, the mechanisms responsible for the dendritic and ring swarming patterns are largely unknown [21,22].

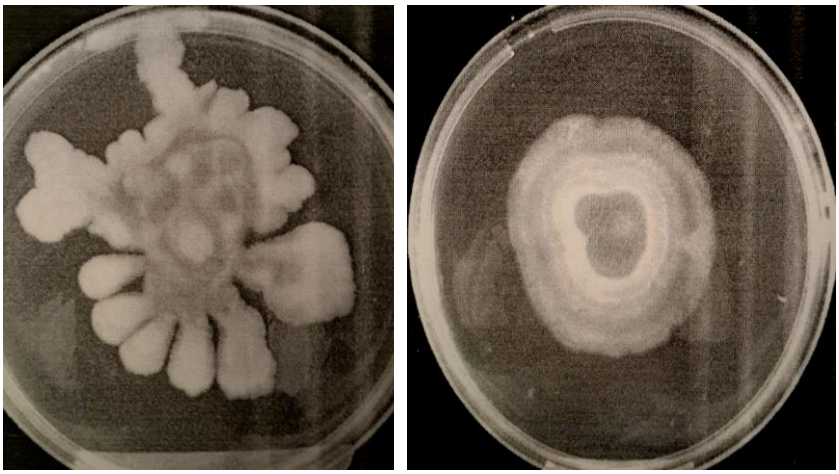

Figure-1. Left picture reveal the denedritic swarming pattern, and right reveal ring swarming pattern.

Conclusions

1. *P. aeruginosa* isolated from Otitis media were able to show two types of swarming motility; denedritic and ring patterns.
2. Some *P. aeruginosa* isolate were able to change from the first to the second swarming patterns.
3. Site of bacteria isolation don't influence and determine a swarming motility patterns of *P. aeruginosa*.
4. Swarming motility may be an important factor in the pathogenesis of *P. aeruginosa*.

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أنماط حركة العج (الأحتشاد) لبكتريا *Pseudomonas aeruginosa* المعزولة من ألتهابات الأذن الوسطى

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الملخص

بكتريا الـ *Pseudomonas aeruginosa*قادرة على إظهار ثلاثة أنواع من الحركة هي : السباحة والاهتزاز وحركة العج. النوع الأخير وصف بأنه حركة سريعة متناسقة على أسطح الأوساط الزرعية شبة الصلبة (اللزجة) ينتج عنها التمايز الشكلي ناتج عن تفاعل الخلايا البكتيرية فيما بينها. الصفة المميزة لحركة العج التي تظهرها بكتريا *P. aeruginosa*هي تكوينها لهجرة عن المركز ناتجة عن تكون مستعمرات معقدة تظهر على شكل نمط هندسي متكرر. وللبرهنة على قدرة هذه البكتريا المعزولة من التهابات الأذن الوسطى بإظهار حركة العج على أسطح الأوساط شبه الصلبة وهل لموقع العزل تأثير على ذلك, تم زرع ثلاثين عزلة من بكتريا *P. aeruginosa* على وسط swarming agar plateللكشف عن هذا النوع من الحركة (swarming motility) يحتوي على 0.5% من مادة الأكار. أظهرت النتائج إن 12(40%) كانت قادرة على إظهار النمط الشجيري من حركة العج , و18 (60%) كانت قادرة على إظهار النمط الحلقي من حركة العج , بالإضافة إلى ذلك فأن سبعة من إجمالي العزلات كانت قادرة على إظهار كلا النمطين الشجيري والحلقي . كذلك أظهرت النتائج إن ليس لموقع عزل هذه البكتريا أي تأثير في تحديد نمط حركة العج. حيث إن هناك عدة دراسات على نفس البكتريا معزولة من مواقع إصابات مختلفة توافقت نتائجها مع نتائج الدراسة الحالية حيث أظهرت نفس الأنماط الحركية التي أظهرتها عزلات الدراسة الحالية ,على أية حال فأن الميكانيكيات المسؤولة عن أظهار أنماط حركة العج غير مفهومة لحد الآن. وقد يساهم هذا النوع من الحركة في زيادة أمراضية هذه البكتريا.