



# The Effect of Some Antibiotics, Natural and Chemical Compounds on Swarming Motility of *Proteus mirabilis*

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تأثير بعض المضادات الحيوية والمركبات الطبيعية والكيميائية على حركة الانتحال

لبكتيريا *Proteus mirabilis*

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## ABSTRACT

**Background:** *Proteus mirabilis* known for its motility, swimming and swarming motility and involvement in various infections, these two types of motility are important for tissue colonization, thus increase the pathogenicity of the bacteria.

**Materials and Methods:** Twenty-one isolates from different clinical and animal sources belong to *P. mirabilis* were collected. The isolates identified using biochemical tests and the Vitek-2 compact system. Swimmer and swarmer cells media used to detect the effect of antibiotics, sodium azide and tannic acid.

**Results:** Sodium azide inhibited swimming more effectively than swarming, with higher concentrations (0.1%) showing the greatest inhibition. Tannic acid also inhibited swimming and swarming, with greater effects on swimming cells, particularly from clinical isolates. Swarming motility, especially in animal-derived isolates, exhibited resistance to tannic acid. Additionally, the isolates showed varying resistance levels to antibiotics.

**Conclusions:** This study demonstrated that, *P. mirabilis* exhibit different responses to antibiotics, sodium azide and tannic acid according to the type of cells whether it was swimmer or swarmer. Sodium azide and tannic acid inhibit swimming more than swarming cells. Antibiotics resistance patterns was differ according bacterial isolate and type of antibiotic. These finding could be used in future to enhance the activity of antibiotics especially those used to treat gram-negative bacterial infection.

**Key words:** *Proteus mirabilis*; Tannic acid; Sodium azide; Swarming; Swimming.



## **INTRODUCTION**

Hauser first discovered *Proteus* in the year 1885, after isolation from putrefied meat. The genus *Proteus* includes many species such as *mirabilis*, *vulgaris* and *zenkeri* according to the ability to liquefy gelatin [1]. The bacteria *P. mirabilis* belongs to the family enterobacteriaceae are gram negative rods, motile by flagella and gain its energy from fermentation and respiration [2]. *P. mirabilis* are isolated from variety of sources such as soil, water and clinical samples [3] and they are part of the large intestine of flora of human, also they are present in many animals according to their nature of life [4]. *P. mirabilis* acquire many virulence factors responsible for its pathogenicity [5]. The enzyme urease which is an important virulence factor hydrolyze ammonia leading to increase urine pH leading to the formation of struvite stones [6]. The phenomenon of swarming defined as rapid migration of bacterial cells as a group in order to enhance colonization of rich nutrient environment. Swarming require cellular signaling, a system known as quorum sensing (QS) used by bacterial cells to communicate with each other. This system relies on chemical signaling molecules known as auto-inducers, which are acyl-homoserine lactones (AHL) in gram-negative bacteria, which are passively diffuse through their cell membrane. Additional to chemical signals, physical signals represented by flagella movement rapidly, both lead to differentiation of cells from swimmer to swarmer. Swimmer cells, which are the vegetative cells of *P. mirabilis*, when they are transported to firm surfaces, they turn to hyper-flagellated elongated rods [5], [7], [8]. Migration of swarmer cells forms concentric zones within few hours, and then they enter to unification state followed by loss of differentiation to swimmer cells. The process continuously repeated to form a centric region in around the agar center [9]. Many studies referred that swarming phenomenon could be eradicated by many substances such n-acetylcysteine [6], Glycerol [10], Fatty acids [11], purified resveratrol [12] and the use of 1-6% agar [13]. Also the swarming could be enhanced by using amino acids, which serves as source of carbon leading to increase growth rate [14, 15]. The present study aimed to detect effect of sodium azide, Tannis and antibiotics against on swimmer and swarmer cells of different *Proteus mirabilis* isolates.

## **Materials and Methods**

### **Isolation and identification of *P. mirabilis***

Up to Twenty-one of *Proteus* isolates gathered from different sources. Clinical samples represented by urine, wound swabs and sputum were handled [27], briefly each sample were cultured on MacConkey and Blood agar and incubated for overnight at 37°C. animal sources isolates represented by Chicken faeces, Dog Rectal and Cat Rectal were also culture on MacConkey and Blood agar for overnight at 37°C, after following the methods of handling of each sample [27]. Primary identification depends on Morphological and Biochemical tests [15], followed by identification by using Vitek-2 compact system.

### Swimmer and Swarmer Cells Test

A full loop of each tested isolate cultured on swimmer and swarmer cells media as mentioned in [17].

### Sodium azide Effect on swimmer and swarmer cells

Three concentrations (0.1, 0.01 and 0.001)% of Sodium azide were added to swimming and swarming media [17] to determine its activity against swimmer and swarmer cells.

### Tannic acid effect on swimmer and swarmer cells

Tannic acid added at different concentration 0.001%, 0.01% and 0.1% to swimming and swarming media [17] to estimate their activity against swimming and swarming cells.

### Antibiotics effect on swimmer and swarmer cells

Kirby-Baure method as mentioned in [18] carried out to detect the activity of antibiotics on swimming and swarming cells by using swimming and swarming media mentioned in [17].

Up to Twenty-one isolates of *Proteus* identified according to morphological characterization, followed by many biochemical tests, which include oxidase, catalase, Indole, Methyl red, Vogues Proskauere and Citrate utilization [19, 20]. The results of Biochemical tests showed that only 18 isolate were *P. mirabilis* and it was confirmed by using the automated Vitek -2 compact system with accuracy of (93-99)%. The number percentage of isolates distribution shown in figure (1).

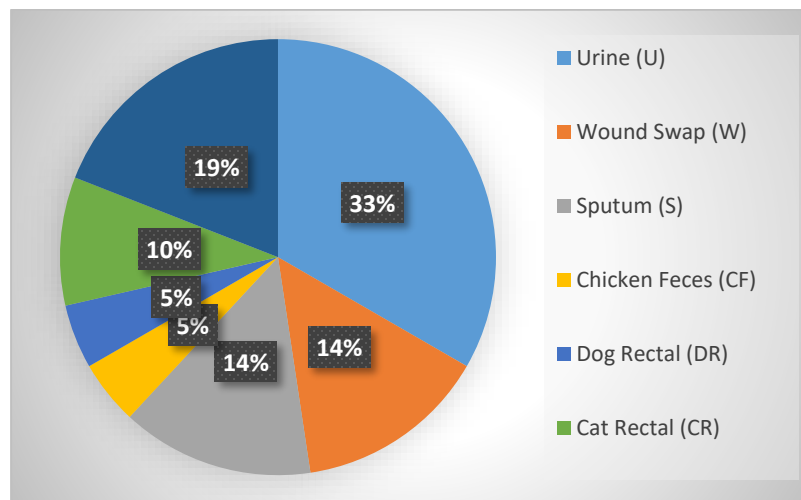


Figure (1) Distribution percentage isolates

### Swimmer and Swarmer Cells Test

The result revealed that all swimmer cells were motile, and the calculated diameter was 26 to 80 mm, while the swarmer cells motility diameter ranged from 10 to 35 mm.

### Sodium azide effect on swimmer and swarmer cells

The results revealed that Sodium azide eradicate swimmer and swarmer cells. As displayed within figure (2), the lowest concentration (0.001%) was inefficient to inhibit both of clinical and animal sources isolates, while at the concentration of 0.01%, eradication of swimmer cells was differ as isolate source differ. The isolates S1, CF1 and DR1 reaches the highest inhibition ratio



ranged from 85 to 87.5%, while it was 62.5% for W isolate and 68.5% for CR1 isolate and it was 50% for U1. The highest concentration represented by 0.1% which inhibit swimmer cells and ranged from 81.4 to 88.7%. As shown in Figure (3) sodium azide effect was variable on swarmer cells depending on the source of isolate. All of the tested concentrations inhibit swarmer cells of CF1 isolate; also, none of the tested concentrations inhibits CR1 isolate. The lowest concentration (0.001 %) inhibit swarmer cells of S1 isolate and the inhibition ratio was to 6.5 %, while it was not active against the other isolates. The inhibition ratio ranged from 2.1 to 43.7% at the concentration 0.01 % against U1, W1 and S1, while for DR1 isolate, it was 12.5 %. Also the 0.1 % concentration reaches its maximum effect against CF1 isolate with a ratio of 75.3%, while in U1 , W1 and DR1 the ratio was (50.9,57.5 and 25) %.

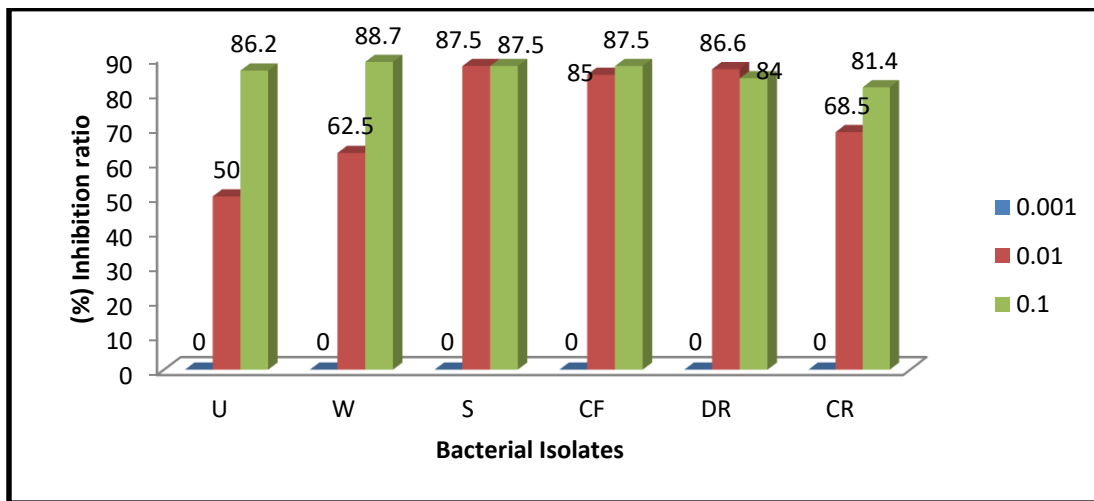


Figure (2) Sodium azide effect on swimmer cells of *P. mirabilis* clinical isolates Urine (U), Wounds (W) and Sputum (S) and Animal sources isolates Chicken Feaces (CF), Dog Rectal (DR) and Cat Rectal (CR)

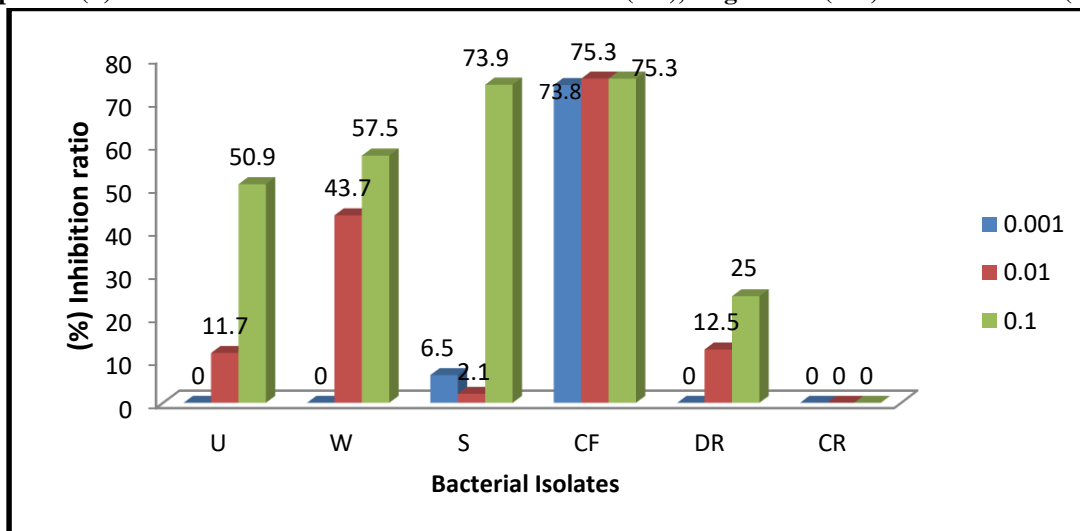


Figure (3) Sodium azide effect on Swarmer cells of *P. mirabilis* clinical isolates Urine (U), Wounds (W) and Sputum (S) and Animal sources isolates Chicken Feaces (CF), Dog Rectal (DR) and Cat Rectal (CR)



These results showed that swimmer cells of *P.mirabilis* were inhibited more than swarmer cells by and the inhibition rate depend on isolate source whether it was clinical or from animal source. As referred in [21] study, sodium azide at different concentration blocks *P.mirabilis* swimmer and swarmer cells motility. Also in a different study of [22], pointed that supplementing of the media with sodium azide at 0.005% concentration leads aerobic respiration poisoning as result from its activity on Tri-carboxylic Acid cycle genes. In a another study, [23] conduct that bacterial flagella formation could be blocked by using chemical compounds, which effect on bacterial cells motility, while [24] suggest that the differentiation from swimmer to swarmer cells could be discourage by chemical compounds and thus lead to block swarming phenomenon. These finding give a clear vision of possibility of using sodium azide salt as a supplement for antibiotics to improve their activity against Varity types of infections.

**Tannic acid effect on swimmer and swarmer cells**

The results exhibited tannic acid ability to eradicate swimmer and swarmer cells. As presented in figure (4), all of the concentrations inhibit swimmer cells. The lowest concentration 0.001% shows an inhibition rates ranged from 56.1 to 75.6% in U1, W1 and S1 isolates, while fro the animal sources isolates, it ranged from 41 to 56.3%. The concentration 0.01% inhibition rate was the same in all isolates and ranged from 83.2 to 87.5%. The highest concentration 0.1%, showed the highest inhibition rate 89% against U1 isolate, while it was 83.3 and 78.7% against W1 and S1 isolates, respectively. The animal sources isolates inhibition rate ranged from 81.3 to 86.2%.

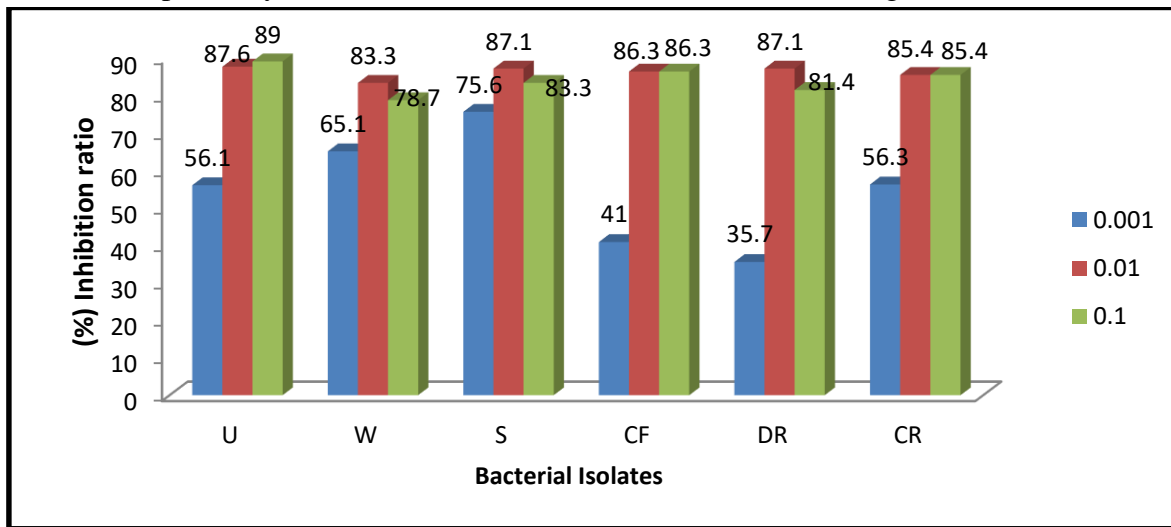


Figure (4) Tannic acid effect on swimmer cells of *P. mirabilis* clinical isolates (U, W and S) and Animal sources isolates (CF, DR and CR)

As shown in Figure (5) swarmer cells motility inhibited. All concentrations were unsuccessfully effective against CR1 isolate. The lowest concentration 0.001% was incapable to inhibit U1 isolate swarmer cells, while the inhibition ratios ranged from 31.2 to 61.5% for W1, S1, CF1 and DR1 isolates. At 0.01% concentration, the maximum inhibition ratios scored for W1, CF1 isolates, and it was 84 % and 81.4 %, respectively. The inhibition ratio was 68.6% for U1 isolate

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and 73.9% for S1 isolate, while it was 37.5% for DR1 isolate. The isolates U1, W1, S1 and CF1 inhibition ratio ranged from 72.4 to 82.4% at the concentration 0.1 %, while it was 18.7% for DR1 isolate. The results of tannic acid effect showed that, it was effective against swimmer cells more than swarmer cell according to source of bacterial isolate and tannic acid concentration. Additionally, *P. mirabilis* swarmer cells isolated from animal sources were more resistance than the clinical isolates to tannic acid. According to Smith [25] results, the adding of tannic acid to culture media with concentration of 0.00001% to 0.1%, may be responsible for blocking swarming, when it binds with outer membrane phospholipids without any harmful effect on growth or cell signaling system. These results represented by using tannic acid, which is a natural compound at specific concentration, may limits the infection of *P. mirabilis*. Additionally it may be added also at specific concentration to antibiotics to enhance their activity.

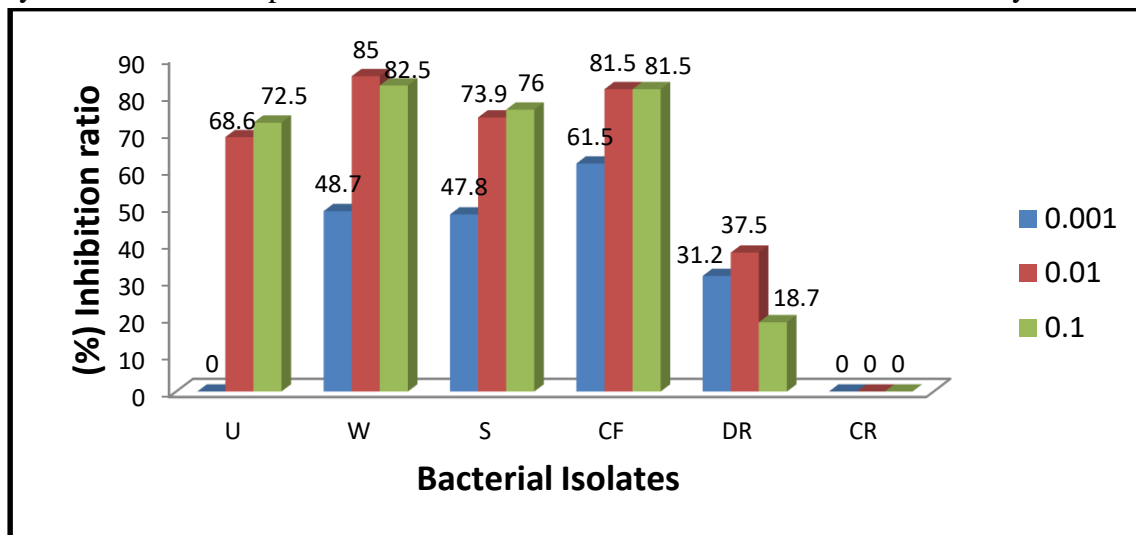


Figure (5) Tannic acid effect on swarmer cells of *P. mirabilis* clinical isolates (U, W and S) and Animal sources isolates (CF, DR and CR)

### Antibiotics effect on swimmer and swarmer cells

The resistance of *P.mirabilis* isolates towards four types of antibiotics (Penicillin, Tetracycline, Nitrofurantoin and Cefoxitin) tested by Kirby-Baure method. The results showed that all swimming and swarming cells were (100) % resistant to the antibiotic Penicillin (P), while the resistance rate of swarming cells was 83.3% for the antibiotic Tetracycline (TE). The resistance of swimming cells to the antibiotic Nitrofurantoin was (100)%, while the resistance rate of swarming cells was 55.5%. As for the antibiotic Cefoxitin (FOX), the resistance rate was 5.5% for both swimming and swarming cells, as shown in Figures (5, 6, 7, 8, 9, 10), it showed variation in the resistance of bacterial isolates to antibiotics.

The results in Figure (6), (7) and (8) show that the swimming cells of the clinical and animal isolates of *P.mirabilis* under study were resistant to Penicillin (P) at a very high rate, except for the isolates U7, DR, S1, S2, W2 and CF, whose inhibition diameters reached (21, 21, 5, 9, 7, 19) mm, respectively. The isolates U1, U2, U3, U5, CR1, DR, H.en1 and H.en3 were highly resistant





to Tetracycline (TE). The isolates U4, U6 and U7 had TE inhibitory diameters of (8, 7 and 8) mm, respectively. The isolates W1, W2, S1, S2 and CR2 had TE inhibitory diameters of (7, 8, 8, 9 and 8) mm, respectively. The isolates CF and H.en2 had inhibitory diameters of (6 and 9) mm, respectively. The isolates U1, U3, U5, U6, W1, CR1, CR2, DR, CF, H.en1 and H.en3 were highly resistant to Nitrofurantoin (F), while the isolates U2, U4 and U7 had a diameter of inhibition to Nitrofurantoin of (6, 10, 7) mm, respectively, while the isolates W2, S1 and S2 had a diameter of inhibition of (6, 9, 9) mm, respectively, while the isolate H.en2 had a diameter of inhibition of (9) mm. Most of the *P.mirabilis* isolates under study showed sensitivity to Cefoxitin (FOX) as the diameter of inhibition of swimming cells ranged between (19-29) mm, while the diameter of inhibition of isolates U5 and CR1 was (13, 17) mm, respectively.

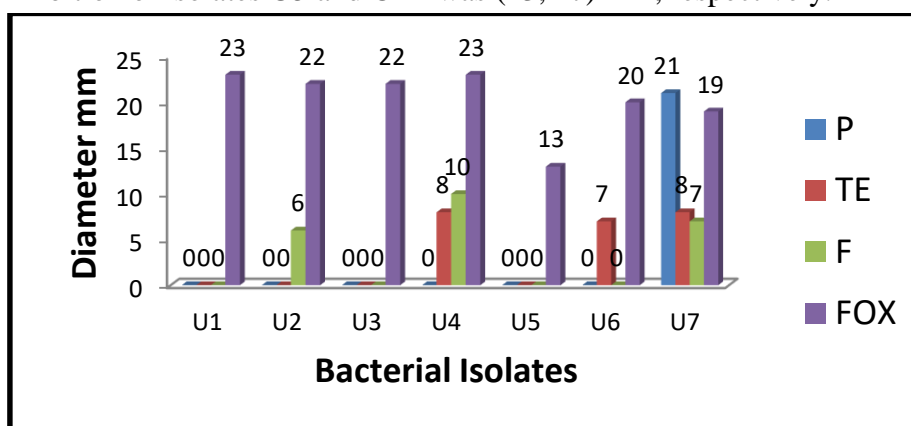


Figure (6) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on swimmer cells of *P. mirabilis* Urine (U) isolates

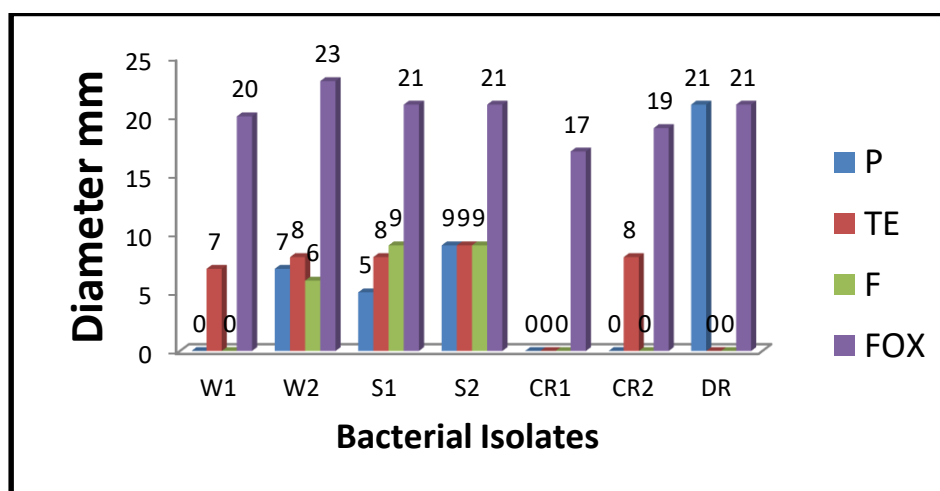


Figure (7) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on swimmer cells of *P. mirabilis* clinical isolates Wounds (W), Sputum (S) and animal sources isolates Cat Rectal (CR) and Dog Rectal (DR)

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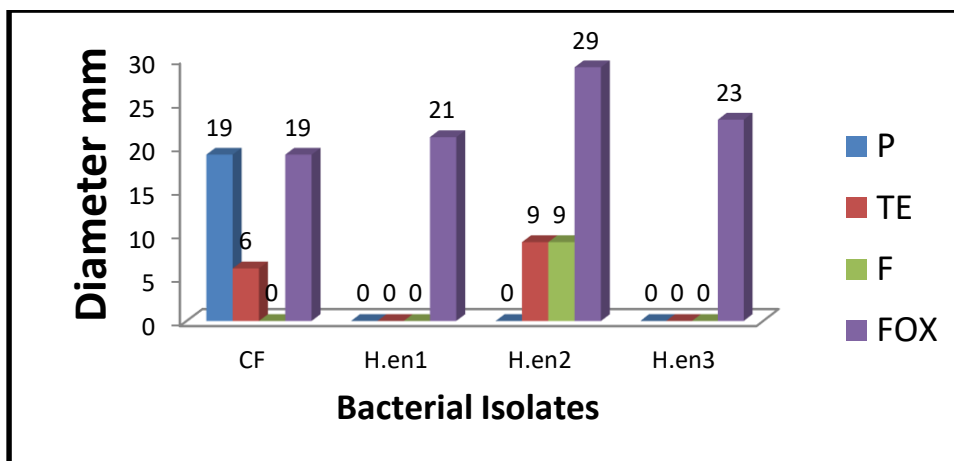


Figure (8) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on swimmer cells of *P. mirabilis* animal sources isolate Chicken faeces (CF) and Hospital environment (H.en)

As for the swarming cells, Figures (9, 10, 11), show their resistance to antibiotics. The germ cells of the isolates U1, U2, U3, U4 and H.en3 were highly resistant to the antibiotic Penicillin (P). The inhibition diameters of isolates U5, U6 and U7 reached (7, 9, 23) mm, respectively. As for isolates W1, W2, S1 S2, CR1, CR2, DR, their inhibition diameters reached (12, 15, 9, 9, 15, 11, 23) mm, respectively. While the inhibition diameters of isolates CF, H.en1 and H.en2 reached (20, 7, 11) mm, respectively. The isolates U3, CR1 and CF were highly resistant to Tetracycline (TE), the diameter of resistance of isolates U1 and U2 was (10, 6) mm, respectively, while that of isolates U4, U5, U6, U7 and S2 was 8 mm, the diameter of isolates W1 and S1 was (11) mm, while that of isolates W2, DR, H.en2 and H.en3 was (12, 9, 15, 12) mm, respectively, while that of isolates CR2 and H.en1 was 7 mm. The isolates U1, U2, W1, S2 and DR were highly resistant to the antibiotic Nitrofurantoin (F), the inhibition diameter of isolates U5, U6 and U7 was (18, 15, 14) mm, respectively, while the isolates U3 and U4 were (17) mm, while the isolates W2, S1, CR1 and CR2 were (25, 12, 13, 14) mm, respectively, while the isolates CF, H.en1, H.en2 and H.en3 had an inhibition diameter of (13, 23, 17, 15) mm, respectively. The isolates of *P.mirabilis* bacteria under study showed sensitivity to the antibiotic Cefoxitin, as the inhibition diameter ranged between (22-29) mm, while the inhibition diameter of isolates U5 and W2 was (17, 7) mm, respectively.



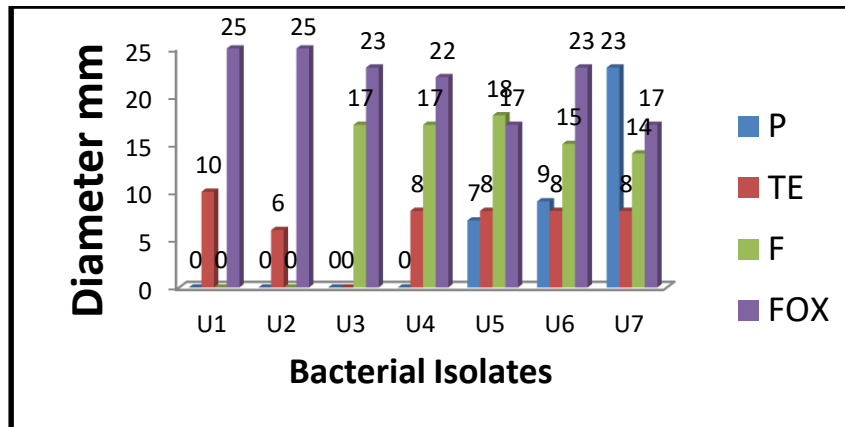


Figure (9) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on Swarming cells of *P. mirabilis* Urine (Urine) Isolates

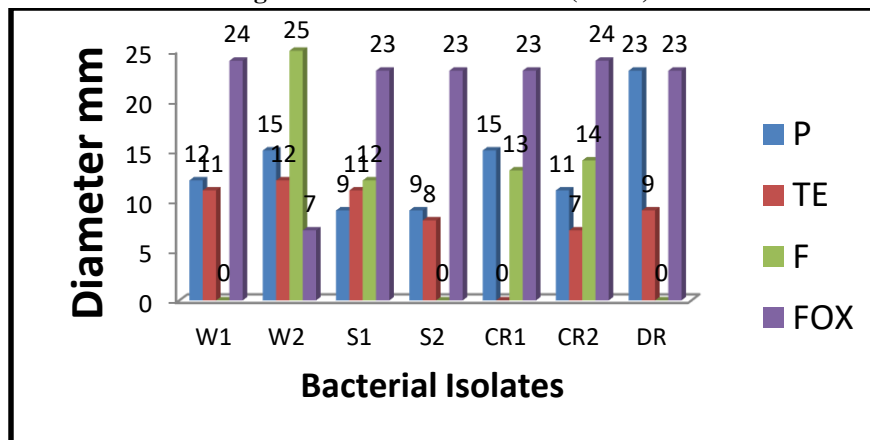


Figure (10) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on swarmer cells of *P. mirabilis* clinical isolates Wounds (W), Sputum (S) and animal sources isolates Cat Rectal (CR) and Dog Rectal (DR)

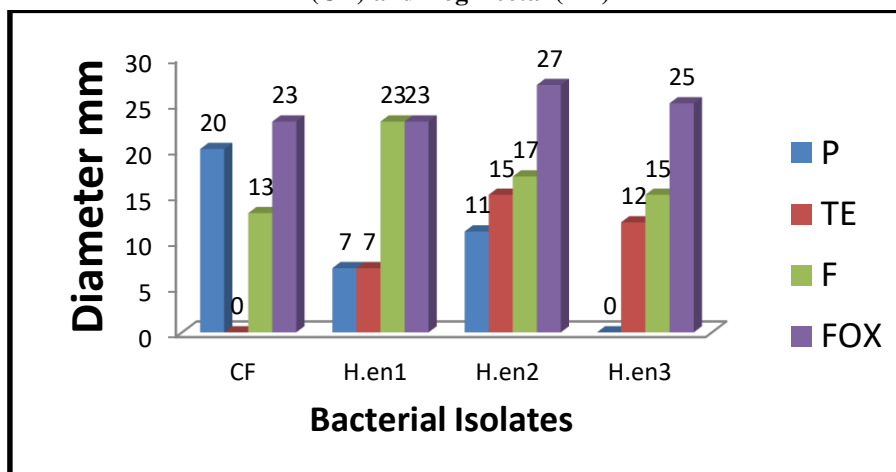


Figure (10) antibiotics Penicillin (P), Tetracycline (TE), Nitrofurantoin (F) and Cefoxitin (FOX) effect on swarmer cells of *P. mirabilis* animal sources isolate Chicken faeces (CF) and Hospital environment (H.en)



It is also noted that antibiotics have different effects on swimming cells and swarming cells. This difference may be due to the source of isolation, in addition to the resistance of thalli cells to antibiotics more than swimming cells. The results of the current study agree with [26] who indicated that *P.mirabilis* bacteria isolated from urine, wounds and burns are resistant to the antibiotics Tetracycline and Cefoxiti, Hussein [27] indicated that *P.mirabilis* bacteria isolated from chicken feces, dog rectum and cat rectum were sensitive to the antibiotic Cefoxitin.

### Conclusions:

*P. mirabilis* showed Variety of responses according to the type of cells which was simmer or swarmer to the tested compounds and antibiotics and this differences in cells response was depends on type of cell, type of compound or antibiotics and concentration. Both of swimming and swarming cells were inhibited by Sodium azide and tannic acid. Additionally, the resistance patterns of the tested Antibiotic varied according to the type of antibiotic.

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### Conflict of interests.

There is no conflict of interests.

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## الخلاصة

**المقدمة:** تعرف بكتيريا *Proteus mirabilis* بقدرتها على الحركة متمثلة بالخلايا السباحة والخلايا الانثيالية وهي مسؤولة عن أنواع مختلفة من العدوى، اذ تعتبر هذه الحركتين مهمتين في استعمار الانسجة وبالتالي تزداد امراضية البكتيريا.

**طرق العمل:** جمعت 21 عزلة بكتيرية من مصادر سريرية وحيوانية مختلفة عائدة لبكتيريا *Proteus mirabilis*. شخضت جميع العزلات باستخدام الاختبارات الكيموحيوية ونظام الفايتك -2. استخدم الوسط الزراعي الخاص بالخلايا السباحة والخلايا الانثيالية للكشف عن تأثير المضادات الحيوية، ملح ازيد الصوديوم و حامض التانيك.

**الاستنتاجات:** أظهرت هذه الدراسة أن البكتيريا *P. mirabilis* تظهر استجابة مختلفة للمضادات الحيوية وأزيد الصوديوم وحامض التانيك وفقاً لنوع الخلايا سواء كانت سباحة أو متكثلة. أزيد الصوديوم وحامض التانيك يثبطان السباحة أكثر من الخلايا المتكثلة. تختلف أنماط مقاومة المضادات الحيوية وفقاً للعزلة البكتيرية ونوع المضاد الحيوي. توصلت الدراسة الى امكانية استخدام هذه المواد في المستقبل لتعزيز نشاط المضادات الحيوية وخاصة تلك المستخدمة لعلاج العدوى البكتيرية السالبة لصبغة كرام.

**الكلمات المفتاحية:** السباحة، الانثيالي، ملح ازيد الصوديوم، حامض التانيك، بكتيريا *Proteus mirabilis*.