Study the Phenotypic and Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Isolated Clinically from Baghdad Hospitals

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ABSTRACT

Background: *Pseudomonas aeruginosa*, is negative to gram stain, takes a rod shape, and is strictly aerobic; it is considered the most effective bacteria in nosocomial infection. *P.aeruginosa* is an opportunistic pathogen bacterium that can cause serious infections in humans who are immune compromised, such as urinary tract infections, skin, ear infections, and others. **Methods**: Antibiotics sensitivity test and biofilm formation assay were performed on clinical isolates diagnosed as *P. aeruginosa*. **Results**: Fifty-seven isolates were diagnosed as *P. aeruginosa* by characteristic in culture media, biochemical tests, and API 20E. Forty isolates were involved in our study ten from each source. Antibiotic susceptibility tests were performed for forty clinical isolates of *P. aeruginosa* by the disk diffusion method against some antibiotics belonging to different groups and the results revealed that bacteria are multi-drug resistant (MDR) as well, revealed that most compound that has activity against *P. aeruginosa* were Imipenem, Piperacillin, and Ceftazidime. Biofilms were quantified and the *P. aeruginosa* reflected a high ability to produce biofilm. All isolates used in this study formed biofilm with differences in the thickness of the formed layer. **Conclusion**: in this study, we concluded *P. aeruginosa* is one of the most common gram-negative bacteria involved in hospital infections causing opportunistic infection because they have intrinsically and acquired resistance to several antimicrobial agents and produce several exoproducts that are implicated in the pathogenesis of *P. aeruginosa* infections.

Keywords: Antibiotics susceptibility test, Biofilm, *P. aeruginosa*
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1-Introduction

Worldwide nosocomial infections can be substantial to load for the economy and health (1). *Pseudomonas aeruginosa*, is negative for gram stain, takes the rod shape, and is considered as strictly aerobic. (2) It is considered the most effective bacteria in nosocomial infection. *P.aeruginosa* is an opportunistic pathogen bacterium that can cause serious infections in humans who are immune compromised (3,4). The ability of *Pseudomonas* to produce different virulence factors and metabolic substances is considered a challenge for any therapy and drugs used in clinical and hospitals (5), Moreover, *P. aeruginosa* has inherent numerous types of drug resistance genes along with their ability to acquire antibiotic resistant genes from other types of bacteria (6,7,8)

Biofilm production is considered one of the most important virulence factors that play an important role in the pathogenicity of many organisms such as *P.aeruginosa*. The bacteria can communicate by biofilms. The mode of biofilm is predominant for bacteria in different environments. Bacteria usually can grow in a biofilm better than its capability to grow in microcolonies (aggregate in the form of thousands of cells) because microbes that form biofilm can assist each other to resist a wide range of antibiotics (9). The bacteria within biofilm can transfer to the surface after attachment of one cell to another, this movement of bacteria can done by twitching motility to make clumps of microbes cells (10).

2-Materials and Methods

Sample Collection:

One hundred twenty-one clinical samples (ear, wound, UTI, burn) were taken from patients at AL-Yarmouk Hospital and Ghazi Al-Hariri Hospital in Baghdad city.

***Pseudomonas aeruginosa* diagnosis:**

The swabs from samples were cultured on MacConkey, Blood, and Cetrimide agar then incubated at thirty-seven Celsius for twenty-four hours in an aerobic condition. Bacteria were characterized depending on their morphological characterization on different agar mediums along with their biochemical tests (11), more conformation was done using the API 20E kit.

Antibiotics sensitivity Test

This test was done using a modified Kirby-Bauer procedure according to (12) and as the following:

1. From an overnight culture plate, a few bacterial colonies were picked up by a sterilized inoculating loop and emulsified in 5ml of sterile normal saline until a turbidity equivalent to the 0.5 McFarland standards was achieved.

2. A sterile swab was dipped into the inoculum tube, and any excess fluid was expressed against the side of the tube.

3. The surface of a Mueller-Hinton agar plate was inoculated by bacterial colonies. The whole surface of the plate was streaked with the swab, after that the plate was rotated through a 45º angle and streaked the whole surface again. Finally, the plate was rotated another 90º and streaked once more.

4. After a few minutes, the seven antimicrobial discs which are listed in Table (1), were placed on the surface of the inoculated plate.

5. The plates were incubated at 37ºC for 18-24 hours. After incubation, the plates were examined for the presence of an inhibition zone of bacterial growth around the antimicrobial discs.

Table (1)**:** The Antibiotics used in this study

Antibiotic	**Disc content(µg)**	**Abbreviation**
Pipracillin	100	PRL
Imipenem	10	IMI
Ceftazidime	30	CAZ
Gentamicin	10	GM
Ticarcillin	30	TC
Ciprofloxacin	5	CIP
Ticarcillin-Clavulanat	75/10	TIM

Assay of Biofilm formation

Biofilm is detected by ninety-six microtiter plates and then quantified as mentioned by (13).

Strains were grown overnight at 37°C in Trypticase soy broth (TSB), and overnight culture was diluted into (3:300) with fresh media. 300µl from the dilution is added to the well plate. The 96-well plate was covered with a lid and incubated at 37°C for 24 hours. After the incubation period, the wells were shaken out to remove the unattached bacteria and then were rinsed twice in water and shaken out the excess water by tapping the plate on paper towels Subsequently, 300 µl of Crystal violate (CV) stain (at 0.1% concentration) was added to each well and to control un-inoculated well then the plate was let sit to 10-15 minutes. The excess stain was shaken out into the waste container and the plate was rinsed twice. In sequence, to quantify the biofilm, 300µl of 30% glacial acetic acid was added to biofilm wells and to the negative control well (media with crystal violet stain). Plates were allowed to sit at room temperature for 10-15 minutes. Then, the solubilized crystal violet stain was pipetted up and down gently to equally mix just before transferring 300 µl from each well to a 96-well flat-bottomed plate. Finally, the plate was read by a spectrophotometer at an absorbance of 490 nm.

Biofilm is divided into three parts according to the mean absorption results as follows:

- Weak biofilm layer: When the absorbance values are equal to or more than cut-off values for control.
- Moderated biofilm layer: When the absorbance values are equal to or more than twice the cut-off values for control.
- Strong biofilm layer: When the absorbance values are equal to or more than four times cut-off values for control.

Statistical analysis: Percentage method.

3-Results

One hundred twenty-one samples were collected. Samples and swabs from different sources were cultured on different media for the diagnosis of *P.aeruginosa*. These samples involved (32%) wound swabs followed by UTI samples with 33 %, Burn swabs 29%, and ear swabs with 17% as shown in Table (2).

Table (2)**:** Samples sources and the percentage

Source of sample	Number	Percentage	Total
Ear	21	17%	121
Wound	38	32%	
UTI	33	27%	
Burn	29	24%	

Collected samples were cultured on agar media Cetrimide which is considered a selective media for *P.aeruginosa* at 37C° for twenty-four hours, fifty-seven isolates showed positive result growth on Cetrimide agar forming green pigment as in figure(1). Several biochemical tests were done to assist and confirm the diagnosis. Fifty-seven isolates were positive for motility, oxidase, catalase, and gelatin liquefaction, and also showed positive results for the Simmon citrate test, while the negative results were for urease, indol, methyl red, and Vogus proskaure. According to biochemical test results, fifty -seven isolates can be diagnosed as *P. aeruginosa* as in Table (3). API 20E system was done to all of these isolates as illustrated in figure (2), and it gave the same results confirming the identifications as *P. aeruginosa*.

Table (3)**:** Frequency of *P. aeruginosa* in different clinical samples

Samples source	Samples number	Isolates no. and percentages	Isolates no. and percentages for gram stain
Ear	21	15(71.4%)	6(28.6%)
Wound	38	12 (31.6%)	26(68.4%)
UTI	33	11(33.3%)	22(66.7%)
Burn	29	19(65.5%)	10(34.5%)
Total	121	57(47.1%)	64(52.9%)

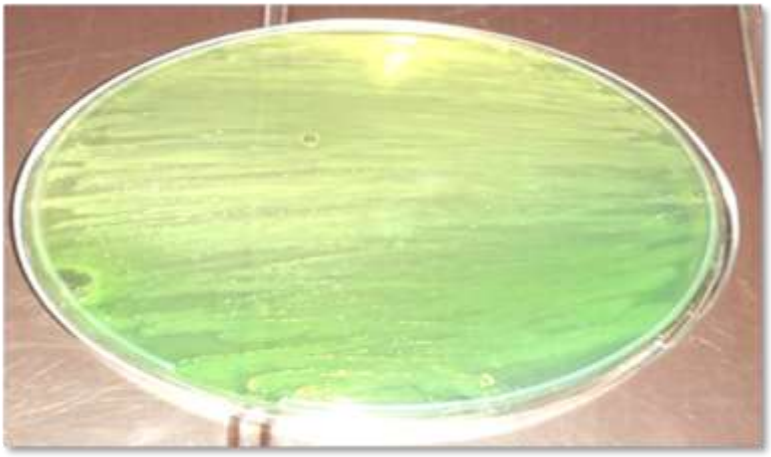

Figure (1): the culture of *P. aeruginosa* grown on Cetrimide agar after overnight incubation at 37C°.

Figure (2): API 20E system tests for detection of *P. aeruginosa*

Antibiotic susceptibility tests were performed for forty clinical isolates of bacteria *P.aeruginosa* from different sources by the disk diffusion method against different antibiotics. This test revealed 19(47.5%) of these isolates were multidrug-resistant (MDR) and "resistant to three or more antimicrobial classes", these MDR isolates show resistance to more than one antimicrobial group: Aminoglycosides, Cephems, Penicillin, Penems, β-Lactams, and Quinolones. These isolates showed a different resistant ability to each antibiotic as illustrated in Figure (3), and the percentage of resistant were: for penicillin group: Pipracillin 17.5% (burn (2.5%), wound (10%), ear (0%), UTIs(5%)), and Ticarcillin 42.5% (burn(10%), wound(7.5%), ear(15%), UTIs(10%)). β-Lactams/lactamase inhibitor combinations group: Ticarcillin-Clavulanat 70% (burn(15%), wound (20%), ear(20%), UTIs(15%)). Cephens group: Ceftazidime27.5% (burn (12.5%), wound (5%), ear (0%), UTIs (10%)). Percentage of Penems group: Imipenem7.5% (burn (2.5%), wound (2.5%), ear (0%), UTIs (2.5%)). Aminoglycoside group: Gentamicin 42.5% (burn (15%), wound (2.5%), ear (12.5%), UTI (12.5%)). Percentage of Quinolones group: Ciprofloxacin57.5% (burn(2.5%),wound(15%), ear(22.5%), UTI(17.5%)). The antibiotic sensitivity test revealed the most active compound against *P.aeruginosa* was Imipenem, followed by Pipracillin then Ceftazidime Table (4) and Figure (4).

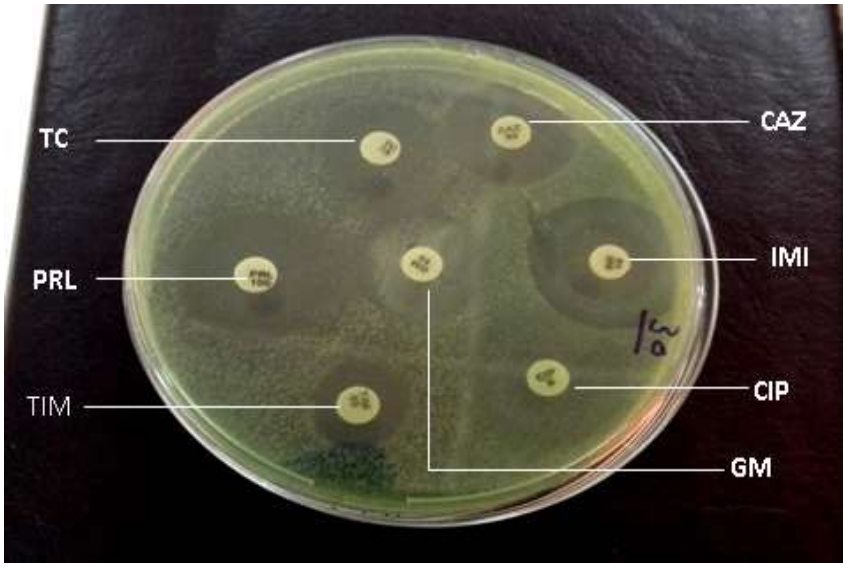

Figure (3): Antibiotic susceptibility test for *P. aeruginosa* against different antimicrobials

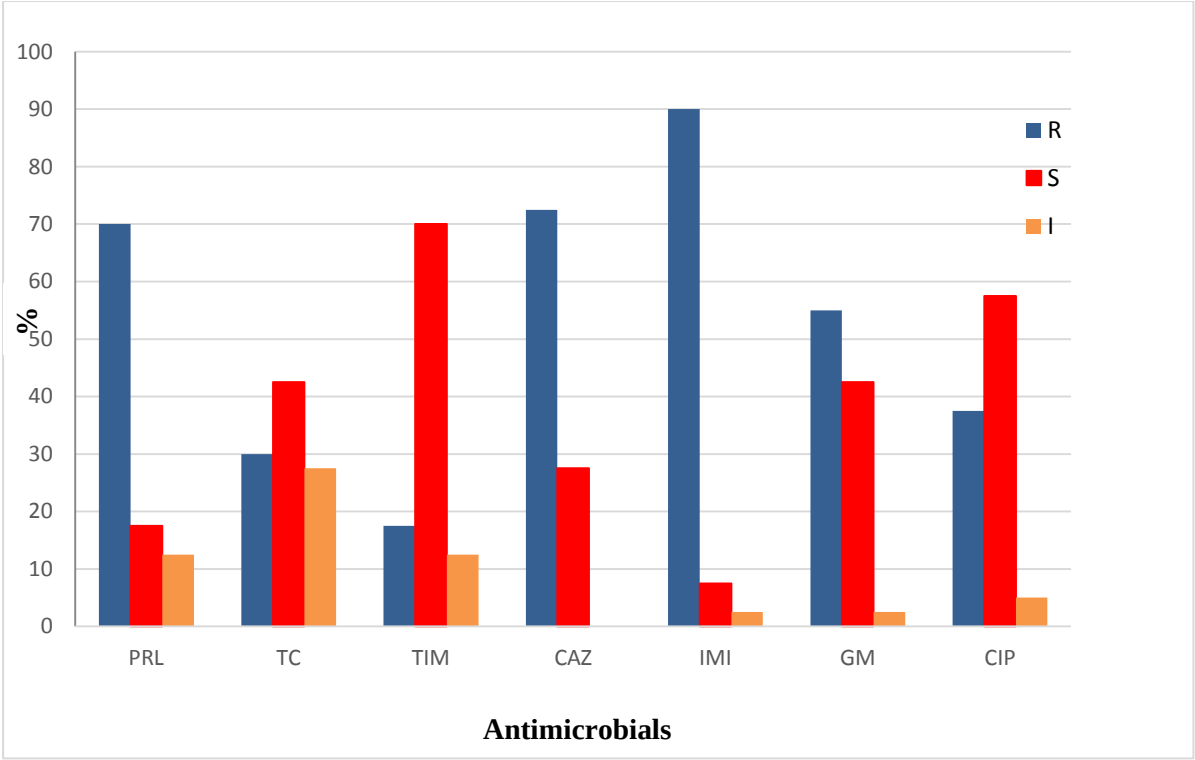

Figure (4): The percentage of antibiotic susceptibility test for *P.aeruginoa*

TABLE (4): ANTIBIOTIC SUSCEPTIBILITY RESULTS OF *P.AERUGINOA*

Isolation Source	Number	Piperacillin	Ticarcillin	Ticarcillin - Clavulanic acid	Ceftazidime	Imipenem	Gentamicin	Ciproflaxine
Burn	1	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	RESIST	SENSITIVE
	2	SENSITIVE	SENSITIVE	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE
	3	INTERMEDIATE	RESIST	RESIST	SENSITIVE	RESIST	RESIST	RESIST
	4	SENSITIVE	RESIST	RESIST	RESIST	SENSITIVE	RESIST	SENSITIVE
	5	SENSITIVE	SENSITIVE	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE
	6	SENSITIVE	SENSITIVE	RESIST	RESIST	SENSITIVE	RESIST	SENSITIVE
	7	INTERMEDIATE	RESIST	RESIST	RESIST	SENSITIVE	RESIST	SENSITIVE
	8	SENSITIVE	SENSITIVE	INTERMEDIATE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
	9	RESIST	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	SENSITIVE
	10	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
Wound	11	RESIST	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	SENSITIVE
	12	RESIST	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
	13	SENSITIVE	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
	14	SENSITIVE	SENSITIVE	INTERMEDIATE	RESIST	RESIST	SENSITIVE	RESIST
	15	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	16	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	17	RESIST	RESIST	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST
	18	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	19	RESIST	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
	20	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	INTERMEDIATE	SENSITIVE	RESIST
Ear	21	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	22	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	23	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	24	SENSITIVE	INTERMEDIATE	INTERMEDIATE	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	25	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	26	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	27	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	28	SENSITIVE	SENSITIVE	INTERMEDIATE	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	29	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
	30	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
Urinary tract infections	31	INTERMEDIATE	INTERMEDIATE	SENSITIVE	RESIST	SENSITIVE	INTERMEDIATE	RESIST
	32	SENSITIVE	INTERMEDIATE	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	33	INTERMEDIATE	INTERMEDIATE	INTERMEDIATE	RESIST	SENSITIVE	RESIST	INTERMEDIATE
	34	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST
	35	SENSITIVE	RESIST	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	36	RESIST	RESIST	RESIST	SENSITIVE	SENSITIVE	RESIST	RESIST
	37	SENSITIVE	SENSITIVE	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	INTERMEDIATE
	38	SENSITIVE	SENSITIVE	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	39	INTERMEDIATE	RESIST	RESIST	SENSITIVE	SENSITIVE	SENSITIVE	RESIST
	40	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE	RESIST	SENSITIVE
Total	S%	70%	30%	17.5%	72.5%	90%	55%	37.5%
	R%	17.5%	42.5%	70%	27.5%	7.5%	42.5%	57.5%
	I%	12.5%	27.5%	12.5%	0%	2.5%	2.5%	5%

Biofilm-producing ability was investigated in forty clinical isolates of *P.aeruginosa* , all isolates were able to produce biofilm with variation in the thickness of the formed layer, of these(15%) have strong biofilm forming ability distributing between the isolates from burn 3(50), wound 2(33.3%) , and UTIs 1(16.7%) isolates. While 18(45%) isolates were moderate and 16(40%) were weak producer.

4-DISCUSSION

P. aeruginosa is an important nosocomial pathogen in many medical centers throughout the world and can act as a nosocomial pathogens (14). The huge mortality rate is linked to hospital-acquired *P. aeruginosa*. It caused a broad spectrum of infections in burn, wound, ear, urinary tract, respiratory and gastrointestinal tract, eyes, as well as with other sites. The highest percentage of *P. aeruginosa* infections was observed in ear infections, so this bacterium can be considered the major agent of nosocomial infections in the ear followed by burn infection, then UTIs, and finally in wounds. *P. aeruginosa* was the causative agent for 66.6% of burn infections as shown by (15) which is similar to this study's results, while there are differences in the percentage of. *P.aeuginosa* isolated from wounds with (16) who mentioned that *P. aeruginosa* can cause infection in only eight% of wound infections. On the other hand, results of a research conducted in Iraq by (17) showed that *P.aeuginosa* was the causative agent for 68.7%. of otitis media. In this study, the percentage of resistance to Imipenem show similarity with another study conducted by (18) while the percentage of resistance to Ceftazidime differs from a study by (19) in which the percentage was 57.5%. In the present study the percentage of resistance for Piperacillin corresponding to previewed studies by (20) in which the percentage was 20%. Gentamicin percentage resistance revealed similar percentage to study by (21) isolated from burn and wound infection and was 45%. Ciprofloxacin resistance percentage different from another study by [18] was 38%. Biofilms detect in over 65% of nosocomail infection and eighty% of total number of microbial infections. *P. aeruginosa* isolates were able to produce high quantity of biofilm and consider one of the most important virulence factor which play important role in the pathogenecity of *P. aeruginosa*. Biofilm was investigated in forty clinical isolates of *P. aeruginosa* involved in this study, all isolates show the ability to produce biofilm with a difference in thickness of formed layer which ranging from strong, moderate to weak this result agree with study by (22) which revealed that percentage of biofilm resulted from *P. aeruginosa* was (100%), and another study by (23) revealed the percentage of biofilm produced by *P. aeruginosa* isolated from burn and wound was (95%), while disagree with the study by (24) which shown that the amount of biofilm produced by *P. aeruginosa* was (47%).

5-CONCLUSION

From 121 samples, 57 of (ear, wound, UTIs,and burn) isolates were *P. aeruginosa*. Seven antibiotics sensitivity test results revealed that the most active compound against *P. aeruginosa* was Imipenem, followed by Pipracillin and Ceftazidime. *P. aeruginosa* reflect high ability to produce biofilm. Each infection source taken in this study has been considered a good environment which provides bacteria with optimal conditions for biofilm formation.

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دراسة الصفات المظهرية وحساسية المضادات للزائفة الزنجارية المعزولة سريريّا من مستشفيات بغداد

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الخلاصة

الخلفية: الزائفة الزنجارية ، سلبية لصبغة جرام، تأخذ الشكل العصي، وتعتبر من الكائنات الهوائية البحتة. البكتيريا الأكثر تأثيراً في عدوى المستشفيات .وهي بكتيريا ممرضة انتهازية يمكن أن تسبب التهابات خطيرة لدى الإنسان الذي يعاني من ضعف المناعة، مثل عدوى المسالك البولية، وعدوى الجلد والأذن، وغيرها. **الهدف من البحث**: هدفت هذه الدراسة إلى الكشف عن عزلات بكتيريا الزائفة الزنجارية المقاومة للمضادات الحيوية المختلفة، وقياس تكوين الأغشية الحيوية في هذه العزلات السريرية .**المواد وطرق العمل:** تم أخذ مئة وواحد وعشرون عينة من مرضى في مواقع مختلفة من الالتهابات (الأذن، الجرح، التهاب المسالك البولية، الحروق) من مستشفيات اليرموك وغازي الحريري في بغداد خلال الفترة من تشرين الثاني 2016 الى شباط 2017. تم إجراء اختبار الحساسية وفحص تكوين الأغشية الحيوية على العزلات السريرية التي تم تشخيصها على أنها زوائف زنجارية.**النتائج:** تم تشخيص سبعة وخمسون عزلة على أنها زائفة زنجارية من خلال خصائصها في الوسط الزرعي والاختبارات الكيموحيوية وAPI 20E. أربعون عزلة شملتها دراستنا عشرة من كل مصدر. تم إجراء اختبار الحساسية للمضادات الحيوية لأربعين عزلة سريرية من بكتيريا الزائفة الزنجارية بطريقة الانتشار القرصي ضد بعض المضادات الحيوية التي تنتمي إلى مجموعات مختلفة وأظهرت النتائج أن البكتيريا مقاومة للأدوية المتعددة (MRD) كذلك، وكشفت عن أكثر المركبات التي لها نشاط ضد الزائفة الزنجارية هي إيميبينيم، بيبراسيللين، سيفتازيديم. تم قياس كمية الأغشية الحيوية و الزوائف الزنجارية عكست قدرةعالية على إنتاج الأغشية الحيوية. جميع العزلات المستخدمة في هذه الدراسة شكلت غشاء حيوي مع وجود اختلافات في سمك الطبقة المتكونة.**الاستنتاج:** في هذه الدراسة استنتجنا إلى ان الزوائف الزنجارية هي واحدة من أكثرانواع البكتيريا السالبة لصبغة جرام شيوعًا في عدوى المستشفيات المسببة للعدوى الانتهازية لأنها تمتلك مقاومة ذاتية ومكتسبة لعدد من المضادات الحيوية وتنتج عددًا من المنتجات الخارجية المتورط في التسبب في العدوى التي تسببها الزوائف الزنجارية.

الكلمات المفتاحية: اختبار الحساسية للمضادات الحيوية، الأغشية الحيوية، الزوائف الزنجارية.