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, take the rod shape and considered as strictly aerobic. The most affect bacteria in nosocomial infection. *P.aeruginosa* is an opportunistic pathogen bacterium that can made serious infections in human who are immune compromised, such as urinary tract infection, skin, ear infection, and others.**Methods**: Antibiotics sensitivity test and biofilm formation assay were performed on clinical isolates diagnosis as *P. aeruginosa*. **Results**: Fifty-seven isolates were diagnoses as *P. aeruginosa* by characteristic in culture media, biochemical tests and API 20E. Forty isolates involved in our study ten from each source. Antibiotic susceptibility test performed for forty clinically isolates of *P. aeruginosa* by the disk diffusion method against some antibiotics belong to different groups and the results revealed that bacteria is multi-drug resistance (MRD) as well, revealed the most compound that have activity against *P. aeruginosa* were Imipenem, Pipracillin, and Ceftazidime. Biofilms were quantified and the *P. aeruginosa* reflected high ability to produce biofilm. All isolates used in this study formed biofilm with differences in thickness of 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 resistant to a number of antimicrobial agents and produces a number of exoproducts which 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 occur as substantial to load for the economics and health (1). *P. aeruginosa*. *Pseudomonas aeruginosa*, is negative to gram stain, take the rod shape and considered as strictly aerobic.(2) the most affect bacteria in nosocomial infection. *P.aeruginosa* is an opportunistic pathogen bacterium that can made serious infections in human who are immune compromised (3,4). The ability of *Pseudomonas* to produce different virulence factor and metabolic substances consider as challenge for any therapy and drugs used in clinicals and hospitals (5). *P. aeruginosa* has inherent resistance to numerous drug classes (6). The risk factors for the drug-resistant of *P. aeruginosa* are due to the role of virulence of bacteria, and also to the other substances (7).The mix of bacterial-associated factor (original and new insertion antimicrobial resistance, expression of virulence factors , prevalence and persistence in the hospitals environments), and the variation in the host sensitivity influence and the infection from outside (8). *P. aeruginosa* is almost show resistance to many antibiotics, because of original resistance specific the mutations, acquired of resistant determinant and impermeability. *P. aeruginosa* isolates were able to produce high quantity of biofilm and consider the one of the most important virulence factor which play important role in the pathogenicity such as *P.aeruginosa*. the bacteria can communicate by biofilms. The mode of biofilm is predominant in actuality for bacteria in different environments. The discrete characteristics in old and mature biofilms were few. Bacteria usually can grow in a biofilm larger than its capability to grow in microcolonies (aggregate in a form of thousands of cells). The microbes that grows in Biofilm-grown can assist each other to resist wide range of antibiotics (9). The bacteria can transfer to the surface After it began attachment of one cell to , this movement of bacteria can done by twitching motility to make clumps of microbes cells (10).

2-Materials and Methodes

Sample Collection:

One hundred twenty one of clinical samples of (ear, wound, UTI , burn) were taken from patients comes to 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 hour in an aerobic condition. The characteristic of bacteria detection depending on the results of culture in the different agars, biochemical tests (11), more conformation were done using API 20E kit.

Antibiotics sensitivity Test

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

1. From an overnight culture plate, few bacterial colonies were picked up by 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 inoculums tube, 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 few minutes by a sterile forceps, the seven antimicrobial disc Table (1), was placed on the surface of the inoculated plate. The disc was pressed gently into a full contact with the agar.

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

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

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

Assay of Biofilm formation

Biofilm detect by ninety six microtiter plates then quantified as mention by (13) and as the following:

Strains were grown overnight at 37°C in reach media (TSB), after then the dilute overnight culture (3:300) into fresh media. 300µl from the dilution is added to well plate. The 96-well plate was covered with a lid and incubate at 37°C for 24 hours. After 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 trapping 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 control well (no bacterial cell just stained 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 prior to 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 the 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 cut off values for control.
- Strong biofilm layer: When the absorbance values are equal to or more than four time cut off values for control.

Statistical analysis: Percentage method.

3-Results

One hundred twenty one samples were collected. The smear taken from sources were cultured on different media for diagnosis of *P.aeruginosa*. These samples involved ear twenty one (17%), wound thirty eight (32%), UTIs thirty three (27%) and burn twenty none (24%) 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 cultured on agar media Cetrimide which consider as selective media for *P.aeruginosa* at 37C° for twenty four hours , fifty seven isolates show positive result growth on Cetrimide agar forming green pigment figure(1). Number of biochemical tests were done to assist and confirm diagnosis. Fifty seven isolates were positive for motility, oxidase ,catalase, gelatin liquefaction, and also show positive results in Simmon citrate test, while the negative results were for urease , indol ,methyl red, and Vogus proskaure. According to biochemical test results we can conclude that these 57 isolates can diagnosis as *P. aeruginosa* Table(3). API 20E system was done to all these isolates figure (2), and its 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%)

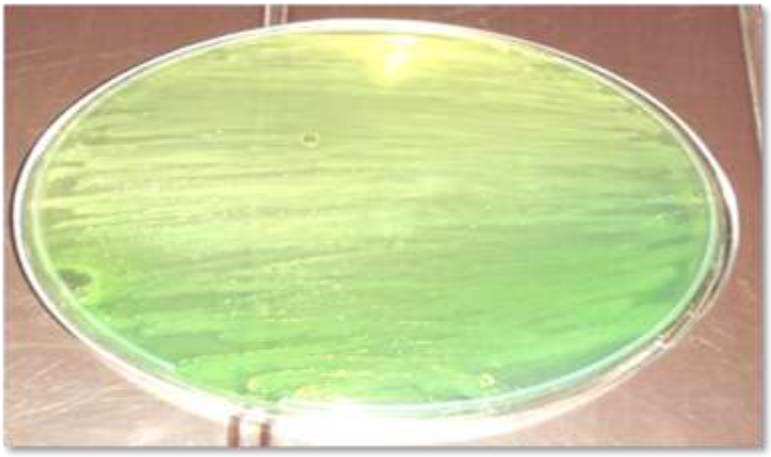

Figure (1): show the culture of *P. aeruginosa* grow on Cetrimide agar after overnight incubation at 37C° .

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

Antibiotic susceptibility test performed for forty clinically isolates of bacteria *P.aeruginosa* from different source by the disk diffusion method against different antibiotics. This test revealed 19(47.5%) of this isolate was multidrug-resistant (MDR) "resistant to three or more antimicrobial classes", these MDR isolates show resistance to more than antimicrobial groups: Aminoglycosides, Cephems, Penicillins , Penems, β-Lactams, and Quinolones. *P. aeruginosa* identified in this show different ability to each antibiotic 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).

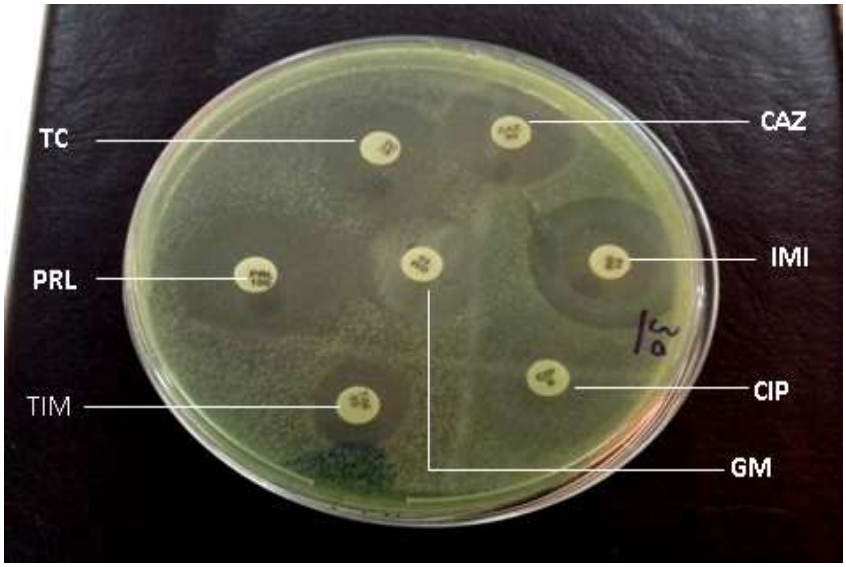

Figure (3): Antibiotic susceptibility test for *P. aeruginosa* against different antimicrobials

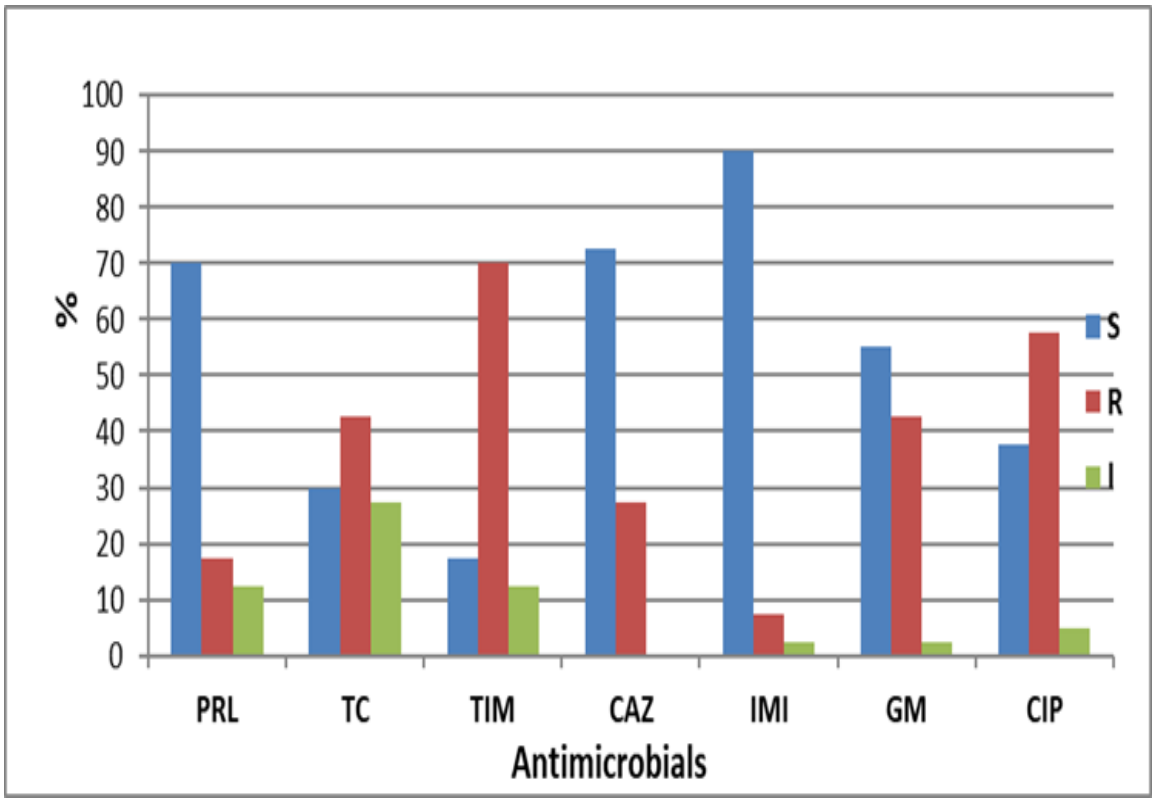

Figure (4): The percentage of antibiotic susceptibility test for *P.aeruginoa*

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 was investigated in forty clinical isolates of *P.aeruginosa* , all isolates show ability to produce biofilm with difference in thickness of formed layer. The isolates source used show differences in thickness of layer, from 40 isolates 6(15%) formed strong layer distributing between the isolates from burn 3(50), wound 2(33.3%) , and UTIs 1(16.7%) isolates. 18(45%) isolates formed moderate layer while 16(40%) produced weak layer distributing between four sources.

4-DISCUSSION

Pseudomonas aeruginosa is important nosocomial pathogens in many medical centers throughout the world. *P.aeuginosa* can act as a nosocomial pathogens (14). The huge mortality rate linked with hospital-acquired *P. aeruginosa*. It is cause 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 infection, so this bacterium can be considered the major agents of nosocomial infections in ear followed by burn infection, then UTIs and finally in wound. (15) agree with our results of burn infections as indicated 66.6%. Results of wound are not confirmed with (16) who mention that *P. aeruginosa* can cause infect in about eight% of wound infection. In research in Iraq by (17) show similarity with our results that revealed the percentage of *P. aeruginosa* isolated from otitis media was 68.7%. In our study the percentage of resistant for Imepenem show similarity with other study by (18) who the percentage was 9.6 %, while the percentage of resistance of Ceftazidime different from study by (19) who the percentage was 57.5%. In the present study the percentage of resistant for Pipracillin 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 agreement 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 resulted from *P. aeruginosa* isolated from burn and wound was (95%), while disagree with the study by (24) which shown that the amount of biofilm resulted from *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 مائة 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.