Comparative study of antibiotics resistance pattern in P. aeruginosa infection among patient from burn, wound and urine isolates in Iraqi hospitals

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Abstract:
The Pseudomonas aeruginosa considered as an opportunistic bacterium and one of the pathogen causing nosocomial infections affecting hospitalized patients. This study was performed on 90 isolates from burn, wound swabs and urine samples, 30 isolates from each source. The bacteria were isolated and Identification by used blood agar MacConkey agar, Nutrient agar, HiFluore Pseudomonas Agar Base and Cetrimide agar. Traditional biochemical tests were done. The VITEK-2 System for Identification of P. aeruginosa was used to prove a final identification. The susceptibility of the 90 isolates was tested by using a vitek2 compact system (kit VITEK 2 AST-GN, No. 222 cards. The biofilm ability of formation to act as a factor of virulence along with (MTP) method were used. The results indicated that, most isolates were capable to form biofilm. Higher P. aeruginosa percentage rate of isolates was from burin swabs (69.8%), all isolates were resisted strain, fallow by urine culture (60%) and wound swabs (49.2%). Most isolates of P. aeruginosa showed high prevalence of the antibiotic-resistance to wide range of antibiotics that has been used for treatment, especially, in burn followed by wound and urine. Furthermore, bacteria isolate in case of wound and urine infection from inpatient (hospitalized patients) were found to be highly resistance to almost all antibiotic used in this study while compared with the isolated from outpatient and there were no sensitive strain isolates from hospital. This study highlighted that should found control programs for mitigation P. aeruginosa circulation among patients admitted into hospitals, especially in burn centers.
Keywords: Pseudomonas aeruginosa; antibiotic sensitivity; burn; urin and wound.

1. Introduction

Gram-negative Pseudomonas aeruginosa bacterium having a rod shaped pathogen and found to be the one of leading infections of nosocomial affecting patients admitted into hospitals, which has the potentiality to cause acute infections, example of these infections are those occurs in case of cancer patients during the chemotherapy period, and infection in patients diagnosed with cystic type fibrosis otitis externa, burn wound infection, patients diagnosed with chronic wounds of diverse etiology and patients who are immunocompromised or vulnerable. In these kinds of infections, congregate of bacteria took place in biofilms. Therefore, cannot be eradicated by traditional antibiotic cure or through the responses of the immune system. of the hosting in the previously mentioned patients. the infection of P. aeruginosa has been seen to be mostly associated with sever mortality and morbidity [1, 2, 3, 4, 5] .Moreover, it has been noted that, the capability to form a biofilms by P. aeruginosa subjected to different conditions has the effect of reducing antibiotic efficiency to treatment the patient, and as a consequence, resulting in increasing the infectious chronic diseases [6].It is worth mentioning that, biofilms composed of microbes that located in a dense- packed slowly-growing micro-colonies integrated in a biopolymer matrix described as passive produced protective. In this life-regime, the levels of resistance of the microbes considered the highest to our todays set of antibiotics and the immune system [7]. The Biofilm mechanism of working is in helping the attachment of microorganism on the surfaces in order to get protected from drying process, defense hosting, and both physical or/and chemical biocides [8]. Thus, biofilms considered to be the most major driver of infections [5]P. aeruginosa shows different mechanism of antibiotic resistance for multiple set of available used antibiotics, giving it the chance to develop its multi resistance capability , resulting in the difficulties faced in treating infections [9] Producing several enzymes by bacteria can deactivate the both carbapenems and beta-lactams including metallo-b-lactamases (MBLs) and beta lactamases extended spectrum (ESBLs) which was considered as P. aeruginosa one of the principal resistance mechanisms [10, 11] .Now adays, the worldwide crucial concern is the rises in P. aeruginosa resistance for wide range of antibiotics types caused by excessively used of antibiotic having broad-spectrum especially in the hospital most

doi.org/10.31838/hiv22.02.664 Received: 10.04.22, Revised:12.05.22, Accepted: 16.08.22

critical cases treatments wards , namely, Intensive Care Units (ICUs) and burn ward through putting controlled pressure on bacteria that most likely causing the strains of multi-drug-resistant (MDR). However, P. aeruginosa bacteria (MDR) participated in (4 to 60) percent infections of nosocomial type globally [12]Since P. aeruginosa has been classified as micro-organism with great ability of growing even under sever kind of conditions like a media having high percentage of humidity and principal nutrition. As mentioned before, and because this bacteria own big resistance to antibiotics and disinfectants, therefore, it has been found in various departments and yards of the hospital, including the staff's dresses, prepared meal, staff computer touching accessories, drainage facilities, bathroom taps, potable water , pharmacy stores and contaminated equipment of medical sector [13,14]Furthermore, P. aeruginosa bacteria transferring among the healthcare workers in the hospital' resulting in creating the contaminated environment in hospital, hands, on the other hand, is a good media of transmitting of this bacteria to infect patients [13,14,15] . Further, patient relative and visitors or contaminated surfaces could be a big source of this kind of bacteria. Besides, the hospital surrounded area can host the stains of MDR P. aeruginosa. As consequence, make it to get circulated within the hospital different yards and units. Thus, these contaminated spots could be a good source for colonization process and uprising[16,17]. This study aims to address, on the one hand, P. aeruginosa bacteria resistance characterization profile to antibiotic. on the other hand, the biofilm formation capacity strains that had been already isolated from burn, wound and urine infection for patients.

2. Materials and Methods

samples Collection

One hundred fifty-four of clinical samples were collected through the period extending from September 2020 to March 2021,154 samples from various clinical sources, Samples were taken from different ages were collected from patients under sterile conditions from hospital in Baghdad city (Ghazi-AL- Hariri Hospital, Baghdad Teaching Hospital, Burin Center), Center and Surgical sections from the Educational Al-Yarmouk Hospital and Imam Ali (Jawadr) Hospital. Identification of *Pseudomonas aeruginosa* All of the specimens were cultured by using sterile swabs on MacConkey agar appeared as a pale color because this pathogen is not fermenting lactose and it produces a diffusing green pigment in the agaon[18]blood agar gave the β type of hemolysis while some gave the γ type on blood agar after 24 - 48 hr. at 37 C growth at 42ºC and on HiFluore *Pseudomonas*-Agar-Base (has been utilized for selection of medium for *Pseudomonas aeruginosa bacteria* isolation from drains and sputum, and etc) produces a visible fluorescence, yellow-green and fluorescent pigment production under long wave UV light, All samples contain *P. aeruginosa* grow on this media. Then were selected a single colony was inoculated on Cetrimide agar that has been used as a medium in order to identify the bacteria (i.e., *P. aeruginosa*) Growth with fluorescent green Color, elevated colonies, and grape- like odor, all isolates had the ability to grow on this media with blue green pigment production and chromogenic medium to carry out other biochemical tests that confirmed the bacterial isolates identification [18]. *P. aeruginosa* isolates had been identified by furtherly characterized by performing gram staining, traditional biochemical tests including Oxidase test(positive), Catalase test (positive). VITEK-2 System compact ID GNB cards was used to prove a final identification of *P. aeruginosa.* The culture results and biochemical tests revealed that 90- isolates out of 154 samples was *Pseudomonas aeruginosa* were collected from various clinical sources. Determination of Antibiotic sensitivity by VITEK-2 compact System method

The resistant patterns of antibiotics were done by AST-GN, No. 222 cards with VITEK-2 System compact had been condected to identify the susceptibility patterns of antimicrobial accourding to the instructions of the manufacturer, i.e., (bioMerieux, France). The antibiotics are Ticarcillin, Ciprofloxacin and Colistin Ticarcillin/Clavulonic, Piperacillin, Cefepime, Ceftazidime, Meropenem, Amikacin, Gentamycin, Tobramycin, and Imipenem,.Quantitative Formation of Biofilm:

The biofilm formation capability of *Pseudomonas aeruginosa* isolates had been identified by 96- well micro -titer plate assay utilized the method of crystal violet staining. In short, those flatbottomed containing199µL of Mueller–Hinton together supplemented in 1% glucose had been inoculated with 1µL volume provided by suspended bacterium of 0.5-0.7 McFarland (1.108cfu/ml). In addition, the microplates had been put in incubation for 24 h at constant temperature of 37°C. The liquid media has been disregarded. besides, the adherent cells have been washed two times with (P-B-S), then after, wells had been dried at a temperature of 60°C for 60 minutes at least. The staining procedure started for duration of 15 minutes using volume of 150µL with 2% of crystal violet. Then, in order to discharge crystal violet stain, the microplates had been washed two times with PBS and dried by air. The biofilms dye which was lined the microplate walls was re-solubilized by volume of 150µL with 95% of ethanol. After passing a period of 5-10min, the microplate was measured by spectrophotometrically at (570 nm) using a reader microplate r of . The assay was done at least three times using fresh samples each time [19].

Statistical Analysis

In this study data have been introduced in the form of a frequency and the percentage %, whereas the test of Chi-square has been utilized for analyzing data with (P-value ≤ 0.05) considered as significant for the purpose of statistical analyzations.

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Pseudomonas aeruginosa Identification

Identification and distributions of bacteria (*pseudomonas aeruginosa*) isolates of the 154 specimens 90 were detected as *pseudomonas aeruginosa* by used multiple diagnostic methods such as, cultural, biochemical tests, vitek2 system compact to confirm the detection. The distribution of isolates according to the clinical sources is shown in Table 1, n=30 (69.8%) out of 43 burn swabs, n=30(60%) out of 50 from urine cultures, n=30(49.2%) out of 61wound swabs as showed in Table 1.

Table 1. Number of samples collected from different source and distribution of antibiotics isolations

Type of sample	No. of sample	No. (%) of P. aeruginosa isolates	No. (%) of Resistant isolates	No. (%) of Sensitive isolates
Burn	43	30(69.8%)	30 (69.8%)	0
Wound	61	30(49.2%)	20 (32.8%)	10 (16.4%)
Urine	50	30(60%)	20 (40%)	10 (20%)
Total	154	90(58.44%)	70 (45.5%)	20 (13%)

3.2. Antibiotic sensitivity Test by VITEK-2 compact System method

The results of drug sensitivity test revealed. The MDR *P. aeruginosa* isolates distribution based on the clinical sources were n= 30 (69.8%) burn swabs, all isolates were resistance, wound swabs were 20 (32.8%) resistant and 10 (16.4%) sensitive isolates, finally 20 (40%) resistant and sensitive isolates 10 (20%), as show in Table 1. Isolates resistance to most antibiotic groups including β–lactams, aminoglycosides, fluoroquinolones. Cephalosporins and Carbapenems as shows in

Table 2. shows percentage of isolates resistance to

Antibiotic Groups	Antibiotic	percentage of resistant in each Source		
		Wound	Burn	Urine
penicillin	Ticarcillin	80% (24/30)	96.7% (29/30)	86.7% (26/30)
	Ticarcillin/Clavulonic	76.7% (23/30)	90% (27/30)	80% (24/30)
	Piperacillin	66.7(20/30)	93% (28/30)	80% (24/30)
Cephalosporins	Ceftazidime	66.7% (20/30)	83.3% (25/30)	60% (18/30)
	Cefepime	60% (18/30)	73.3% (22/30)	50% (15/30)
Carbapenem	Imipenem	53.3(16/30)	76.7(23/30)	36.7% (11/30)
	Meropenem	46% (14/30)	76.7(23/30)	40% (12/30)
aminoglycoside	Amikacin	53.3% (16/30)	73.3(23/22)	46.7% (14/30)
	Gentamycin	53.3(16/30)	83.3% (25/30)	56.7(17/30)
	Tobramycin	56.7(17/30)	86.7(26/30)	63.3(19/30)
Quinolone	Ciprofloxacin	63.3% (19/30)	90 % (27/30)	60 % (18/30)
Polymyxin	Colistin	3.3 % (1/30)	6.7 % (2/30)	3.3 % (1/30)

3.3. A comparative Study of the antibiotic's sensitivity for inpatients-vs- outpatients isolates

The results of Antibiotic sensitivity were analyzed and compered for both cases of patients with wound and urinary tract infection. The cases of burn infection were not included from the previous comparisons, because there is no outpatient as all those with burn cases was admitted immediately into hospital. In this study, the antibiotics sensitivity had been investigated and compared for each antibiotic for both inpatients (hospitalized patients) and outpatients isolates as shows in Table 3,

Figure 1, Table 4, and Figure 2, shown comparison of antibiotics sensitivity test for inpatients-vs-outpatients' isolates in case of wound and urine.The bacteria isolated from inpatient (hospitalized patients) found significantly associated with highly resistance to almost all antibiotic used in this study were compared with the isolated from outpatient. The P value results of all isolates were summarized in Table 5.

Table 4. shows comparison of antibiotics sensitivity test for inpatients-vs- outpatients' isolates in case of Urine isolates

Antibiotic	% Of resistance		Rate of resistance
	Inpatients	Oupatients	
Ticarcillin	Ticarcillin	100.00	60.00
Ticarcillin/Clavulonic	Ticarcillin/Clavulonic	100.00	40.00
Piperacillin	Piperacillin	90.00	60.00
Ceftazidime	Ceftazidime	85.00	10.00
Cefepime	Cefepime	75.00	0.00
Imipenem	Imipenem	55.00	0.00
Meropenem	Meropenem	60.00	0.00
Amikacin	Amikacin	90.00	0.00
Gentamycin	Gentamycin	85.00	0.00
Tobramycin	Tobramycin	95.00	0.00
Ciprofloxacin	Ciprofloxacin	90.00	0.00
Colistin	Colistin	5.00	0.00

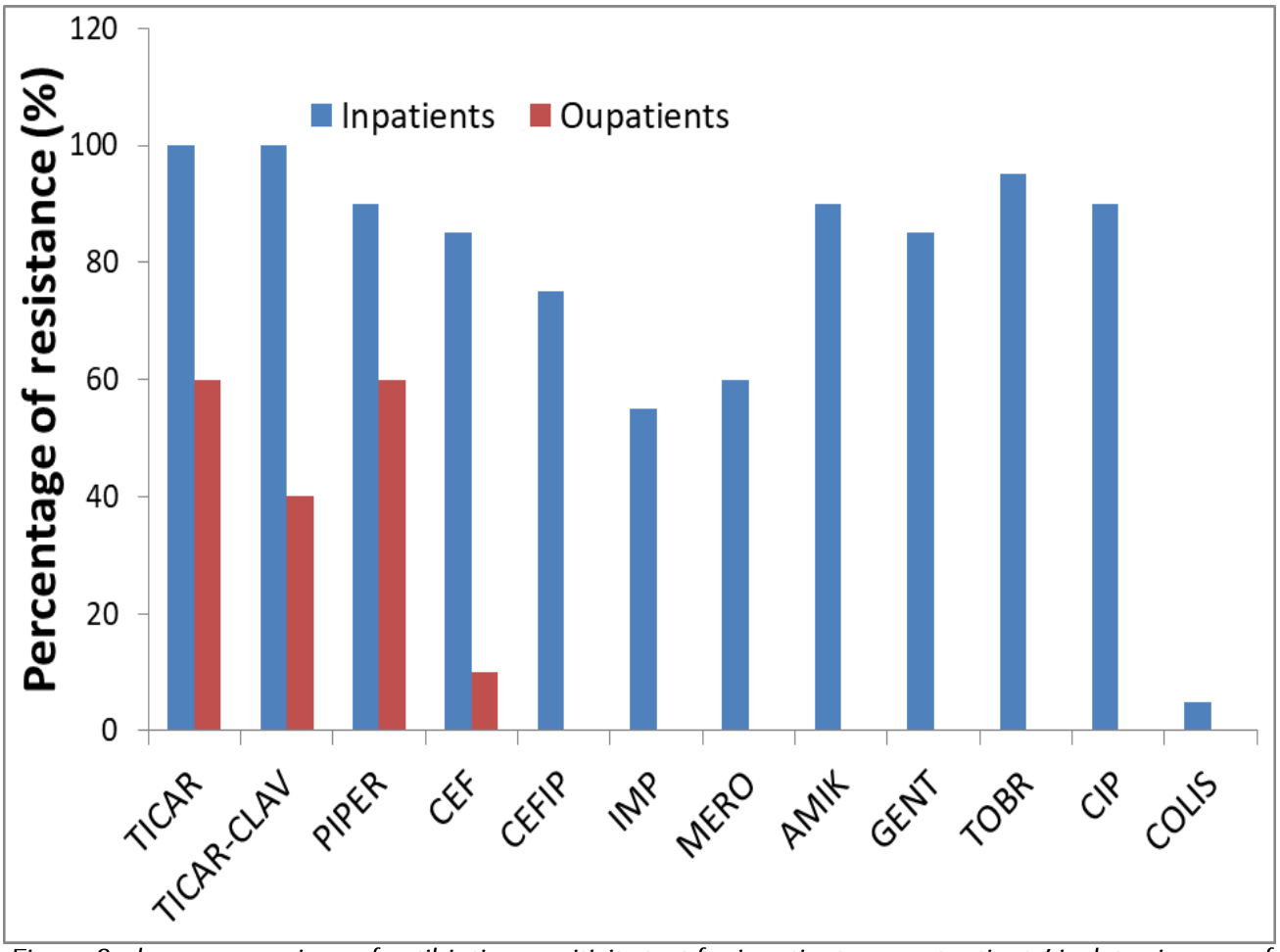

Figure 2. shows comparison of antibiotics sensitivity test for inpatients-vs- outpatients' isolates in case of urinary tract infection isolates.

Table 5. shows P value results of comparison of antibiotics sensitivity test for inpatients-vs- outpatients' isolates in Wound and Urine

Antibiotic	P value (p<0.05)	
	Wound isolates	Urine isolates
Ticarcillin	0.008	0.002
Ticarcillin-clavulonic	0.002	0.0001
Ceftazidime	0.028	0.0001
Cefepime	0.0001	0.030
Imipemen	0.0001	0.007
Meropenem	0.0001	0.007
Amikacin	0.0001	0.001
Gentamycin	0.0001	0.0001
Tobramycin	0.0001	0.0001
Ciprofloxacin	0.0001	0.0001

The effect of pipracillin and colisitin in case of wound and urine isolates found to be not significant for in patients -vs-out patients isolates

3.4. Biofilm Formation

The present research showed different ability to form biofilm by using MTP method as shows in Figure 3 (a,b,c) and Table 6 . The results show her Biofilm formation was recorded high producer of Biofilm (Strong producer) in case of burn 15(50%) followed by wound 12(40%) and 8(26%) in urine, wile the moderate Biofilm formation were in 12(40%) in burn, 11(36%) in wound and 8(26%) in urine. In case of weak Biofilm formation were 11(36%) in urine, 4(13.3%) in wound and 3(10%) in burn. As well as that 3(10%) of *P. aeruginosa* isolates in case of wound and urine infection were non-producer of biofilm.

Table 5. shows different ability to form biofilm by using MTP method

Sources of isolates	No. of isolates	Biofilm Formation			
		Strong producer	moderate producer	weak producer	non-producer
Burn isolates	30	15(50%)	12(40%)	3(10%)	-
Wound isolates	30	12(40%)	11(36.7%)	4(13.3%)	3(10%)
Urine isolates	30	8(26%)	8(26%)	11(36.7%)	3(10%)

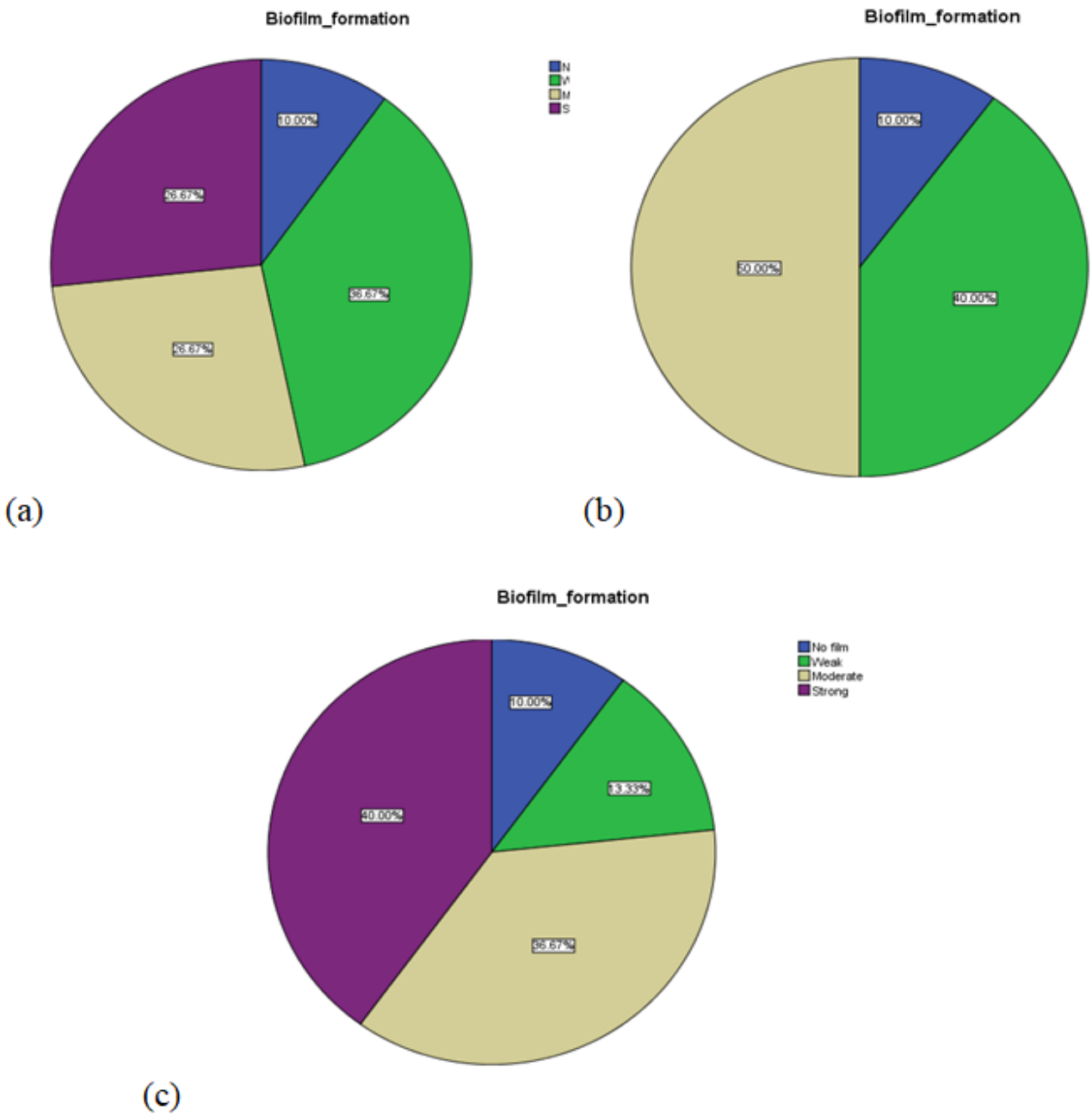

Figure 3. The percentage of Biofilm Formation in different sources :(a) urine, (b) burn and (c) wound.

4. Discussion

Authors The infections occurs as a consequences of *pseudomonas aeruginosa* are commonly attributed to health cares acquired infections and appear as main impendence to risky ill patients mostly burn patients diagnosed with a high rate of morbidity and mortality [10]

Our study reveals, *P. aeruginosa* appears in different sources (burn, wound, urine infections). in addition, *P. aeruginosa* present in greater rate than other bacteria noted in burn centers in Iraqi hospital visited for samples collection, flawing by urine and then wound isolates. This results which agrees with the findings of the study conducted by [20].They concluded that, the main bacterium colonizer in case of burn wound patients was *P. aeruginosa*, also, they reported that , in patient's burn , the risk of be infected ,could be resulted from this pathogen . Also, in anther studies found *P. aeruginosa* was highly isolated from burns samples flawing by urine and then wound infection, [10 ,22,21]. Also, these results could be explained by sepsis of burn wound, which is presently, the major cause of mortality and morbidity following the trauma of burn. The infections due to *P. aeruginosa*, impair patient recovery and cause of invasive infections in burning patients and death. burn injuries classified as sever case, lead to losing skin barrier and tissue destruction due to energy transfer, and, therefore, needs immediately medical intervention and intensive medical care for the purpose of homeostasis maintenance [22,23].

The reasons for the difference in distribution of these bacteria between those three sources are due to difference sample's sources, sample's No.,

and health care degree, including the time before patient admission into the hospital before the growth of pathogen along with the antisepsis methods for wounds or burns, antisepsis frequency, or the case of the patient previously randomly took antibiotics prior to hospitalization, rising the ability of bacteria to resist these bacteria. As a consequence, this may prolong the admission period with high isolation measures must be implemented especially in burns cases [24].As the P. aeruginosa shown to be owns the capability for producing biofilm, that is why it was considered as an important pathogen leading to a serious life threating like nosocomial infection.[25, 26].The results of our study found out that large number of isolates, were capable to produce a biofilm ,were resistant to antibiotics. Isolates were highly biofilm producer in burn, all isolated produce biofilm, follow by wound and urine. This agreed with the study which involved the detection of biofilm formation is one of reason to resist antibiotics in bacteria [27].The reason behind *P. aeruginosa* ability to withstand and survive under sever condition along with its great resistance toward the usage of multiple antibiotic were attributed to its interstice feature to form a biofilm formation,[20,29] . Further, the environmental condition of the hospital may be fully contaminated by P. aeruginosa, through transmission process of this pathogen between and from healthcare workers contacts, infected patients, visitors or contaminated surfaces. Consequently, the different areas hospital might accommodate the MDR P. aeruginosa stains give rise to the circulation of this bacteria making it easy for colonization process. [17,16] .

Our results of antibiotic sensitivity test, showed the highest resistance of *P. aeruginosa* isolates against commonly antibiotic used, occurred in case of burn wound than urine and wounds, all isolates were multi-drug resistance strains of *P. aeruginosa* and there are no *P. aeruginosa* sensitive strains found in hospitalized patients, following by urine and then wound isolates, in addition, it has been found sensitive strains of *P. aeruginosa* in both case from outpatient.

Our finding is indicated that *P. aeruginosa* is becoming resistance to commonly used of antibiotic due to excessing consumption of antibiotics exerting selected present bacteria. The current result confirmed the occurrence of MDR strains of *P. aeruginosa*, which agree with [24, 30,31] who concluded that,*P. aeruginosa* had multi resistance towards wide range of antibiotic, in their studies, especially beta-lactam antibodies.

According to our result Colistin has fewer resistant percentages, were 6.7 % (2/30) in burn, 3.3 % (1/30) in both wound and urine isolates, fallow by Amikacin were 73.3% in burn, 53.3% in wound and 46.7% in urine isolates. Cefepime were 73.3% (22/30) in burn, 60% (18/30) wound and 50% (15/30) in urine isolates Imipenem 76.7(23/30) in burn, 53.3(16/30) wound and 36.7% (11/30) in urine isolates. Meropenem were 76.7(23/30) in burn, 46% (14/30) in wound and 40% (12/30) in urine isolates. Gentamycin and Tobramycin in case of urine were 56.7%, 63.3% and wound were 53.3% 56.7% respectively, but in burn was highly resist. The penicillin antibiotic group was highly resisted in all isolates fallow by Ciprofloxacin and Ceftazidime.

Other studies revealed similar percentages for Colistin by[32]. The percentage was 6.7%. Studies by [33] found resistant percentage of Amikacin close to our results, percentage was 70.8%, the percentage found by Rahimzadeh et al. (2020) was 75%.

In previews studies for Cefepime by [35, the] percentage was 77.55%, and by[36] , the percentage was 74%.

Our rate for Imipenem close to the rate found by [37] who isolates from burn infection, the percentage was 79.2% and 57.14% resistant percentage mention by [35]

While for Meropenem, our results are near by the results of [35], the percentage was 73.5%. (Raheem 2020) the percentage was 73.68%. [39]percentage was 45%.

results of Gentamycin were agreed with [12]percentage was 82.3%, Jayanthi (2015) found resistant percentage was (81.7%).

Tobramycin results were closed to [38] isolated from various clinical specimens, the percentage was 100%, also results of resistant percentages by[41]from different clinical specimens was 50.0%.

In case of Ciprofloxacin In previews studies, by [12] percentage was 89.6% agree with our results[40], who isolated *P. aeruginosa* from clinical cases, percentage was (83.3%).

Ceftazidime our results close with study of [34] the percentage was 81.2%, the percentage was 60.4% by[12], the percentage was 82.6% by[37].

A comparative Study of the antibiotic's sensitivity for inpatients-vs- outpatients isolates had been conducted. Our results revealed that, this bacterium has highest level of resistance percentages to antibiotics was detected with penicillin group and agree with [35, 24,42].

In our study, results show the bacteria isolates in case of wound and urine infection from inpatient (hospitalized patients) were found to be highly resistance to almost all antibiotic used in this study while compared with the isolated from outpatient as shows in Table 3, Figure 1 and the Table 4 and Figure 2, the driving cause of *P. aeruginosa* high pathogenicity was the intrinsic feature of highly-resistance toward wide range of studied antibiotics, in addition to that, the development of broad range of drug resistance during the hospitalization period. .[43] .Our results agree with [44] and [45]

5. Conclusions

The results showed different ability to isolated *P. aeruginosa* from different sources. The percentage rate of *P. aeruginosa* isolates is relatively higher than the rate of isolation from urine and wound. All isolates from inpatient in burin centers were

resistances strains, while in case of urine and wound there were sensitive strains from outpatients. Most isolates of *P. aeruginosa* showed high prevalence of the antibiotic resistance to most types of antibiotics used in the present study and biofilm formation ability were observed in different sources. The penicillin antibiotic group was highly resisted in most isolates fallow by Ciprofloxacin and Ceftazidime. Colistin has fewer resistant percentages fallow by Amikacin, Cefepime, Imipenem and Meropenem. Gentamycin and Tobramycin in case of urine and wound but in burn was highly resist. Furthermore, bacteria isolate in case of wound and urine infection from inpatient (hospitalized patients) were found to be highly resistance to almost all antibiotic used in this study while compared with the isolated from outpatient and there were no sensitive strain isolates from hospital. This study highlighted that should found control programs to prevent transmission of *P. aeruginosa* between patients in hospitals, especially in burn centers.

Author Contributions

Ahmed S. Abdulamir: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation, Investigation. Jassem M. Karhoot: Supervision, Visualization, Validation, Writing- Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Ethical Clearance: The ethical research committee at scientific research by ethical approval of both health and higher education and scientific research ministries in Iraq. Conflict of Interest the authors declare that they have no conflict of interest.

Acknowledgments

We extend our thanks and gratitude to all patients who cooperated with us to collect samples and conduct tests. We also thank the medical and nursing staff at Baghdad city (Ghazi-AL- Hariri Hospital, Baghdad Teaching Hospital, Burin Center), Center and Surgical sections from the Educational Al-Yarmouk Hospital and Imam Ali (Jawadr) Hospital, for their assistance in completing this study.

Conflicts of Interest

The authors declare no conflict of interest.

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