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Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Burns and Wounds Infections in Hospitals of Baghdad

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ABSTRACT

The aims of this study is to shed light on *Pseudomonas aeruginosa*, since it is considered as the main source of medical clinic diseases and is manifestly resistant to most germ-killing drugs. The rise of Multidrug Strokes of Resistance (MDR) was recorded on the planet and represented an incredible test in the administration of diseases related to this organism. Significant work was carried out on strains of the majority of other diseases of this species in nations. However, little attention was given to the resistance profiles of strains in general isolated from the Middle East, and to the genetic diversity of isolates isolated from burns and wounds in Baghdad-Iraq patients. Potential risk factors of acquiring Multidrug-Resistant isolates and leading clonal growth in clinics and community settings still ambiguous. The transversal examination was done to risk factor detection related which carrying Multidrug Resistance *Pseudomonas aeruginosa* among the people in wounds, and antimicrobial susceptibility pattern among all the isolates was analyzed as well. The prevalence of *Pseudomonas aeruginosa* was 47% in 95 isolates from burns and wounds of 200 patients. The most significant prevalence of antimicrobial-resistance was observed for (Gentamicin) (92.63%),(Amikacin) (86.31%), (Ciprofloxacin) (85.26%), and (Piperacillin) (65.26%). While Colistin the most potent resistance-prevalent antimicrobial drug, was 2.10%. Relatively, more resistance against antimicrobial was recorded in comparison with other researches.

Keywords: Aeruginosa; Carbapenems Resistance; Resistance Carriage of Aeruginosa

INTRODUCTUION

It is worth noting that *Pseudomonas aeruginosa* is medically the most significant among all pseudomonas species. It is a rod negative grams, and the main cause of nosocomial infection. In fact, the microorganisms are opportunistic pathogens and cause serious Infections in mammalian species, animal and plant[1]. This organism is the most common life-threats cause infections, which mostly have higher rates of mortality in the variety of P.aeruginosa infections in burn and wound infection[2].

Over the last several decades, *Pseudomonas Aeruginosa* had become one of the most common causes of nosocomial infections related to increased mortality rates. In soil and aquatic environments, *Pseudomonas aeruginosa* could thrive and colonize the surface of humans, animals, and plants.[3] This living being has the ability to prosper greatly in a damp environment. Furthermore, in this way, clonal might spread from claiming reason for persistency nosocomial infections in the doctor's facility surroundings. Indeed, past investigations were archived *P.aeruginosa*, made the predominant reason for nosocomial infections .[4] Pathogenicity of *P.aeruginosa* is regulated by its own ability to generate a wide variety of virulence factors, which is supported by its inherent resistance to environmental change and xenobiotics including antimicrobials, heavy metals, and disinfectants.[5] These

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factors have been shown to permit the pathogens to create effective persistence, colonization, and invasion within the host organisms [6,7].

Pseudomonas aeruginosa infections were a medical problem, hard to cure, due to lower susibtablites to many antibiotics (Multidrug-Resistant) and a significant risk of resistance during treatment.[8] Carbapenem is a class of β-lactam antibiotics, a wide range of antimicrobial activity, for the most structure of β-lactamases makes them highly resistant. Carbapenems involve imipenem and meropenem, which may be considered medical choices to cure the Psedomonas aeruginosa infections [9,10]. The synthesis and distribution of modified β-lactamases were the main cause of antimicrobial resistance in *P.aeruginosa*. This resistance strategy is in the plasmid integron and is therefore easily spread through species by the transference of horizontal genes[11,12]. During the infection cycle, the *P.aeruginosa* genomic expression profile is tightly controlled and involved the activation of processes known as Quorum Sensing (QS) mechanisms[13,14].

Significantly, the bla_{NDM-1} is only an enzyme that interrupts the β-lactam ring amide bond and creates resistance to β-Lactam antibiotics in the main class.[15] bla_{NDM-1}(NewDelhi Metallo-β-lactamase-1) are genes coding for NDM-1.[16] In *P.aeruginosa*, genetic elements such as Class 1 and Class 2 have also been recorded for resistance to β-lactam. As well as, Fluoroquinolone and aminoglycoside, antimicrobial groups, provide a huge capacity to be used to treat infections with *P.aeruginosa*.

MATERIALS AND METHODS

Clinical isolation and identification

Due to getting burn and wound swabs from Patients in hospitals of Baghdad such as (Al-Kindi Teaching Hospital, Al-imam Ali Hospital, Al-Sader General Hospital), therefore, a successive irregular testing procedure was utilized in this cross-sectional investigation and was taken six months from December 2019 to May 2020. Altogether, 200 members were enrolled in this examination, where 200 of burns and wound swabs samples were acquired. 156 (78%) of these samples were adults, and 44 (22%) were children. The median age was about 28 years. Patients with burns and wounds have been recognized in the therapeutic and careful emergency section. Burn and wound swab from patients were transported by Cary-Blair media within two to three hours of collection in a cool box to the laboratory. Sampling codes for samples were transferred to all test samples. Patient information was collected to illustrate demographic data including sex, age, antimicrobial sources, and accessibility. Bacteria were isolated from burn and wound swabs directly on different culture media to isolate and characterize the bacteria. The identification was done by standard laboratory methods, and Vitek 2 compact (BioMerieux). *P.aeruginosa* isolate suspension was prepared from pure cultures growth of bacteria cultivated on specific media containing trypticase soya agar with 5% sheep blood (bioMerieux) and incubated for 24 hours at 35 °C. In 2.5 mL of 0.45% sodium chloride solution, bacterial cells were suspended. A DensichEk (bioMérieux) was used to modify the suspension used in the system VITEK 2 to a McFarland standard of 0.5. [1]

Susceptibility testing for antimicrobial

Susceptibility testing for antimicrobial was performed on Mueller Hinton agar (Oxoid) using Kirby–Bauer disk diffusion technique[18]. To achieve a confluent lawn the standard for McFarland turbidity 0.5 was uniformly distributed over the Mueller Hinton plates. Including antimicrobial disks; gentamicin (GEN 30 μg), amikacin (AMK30 μg), Ciprofloxacin (CIP 10

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µg), piperacillin (PIP 10 µg), ceftazidime (CAZ 30 µg), aztreonam (AZT30 µg), imipenem (IMP 10 µg), colistin sulphate (COS 25 µg) levoflaxacin (LEV 5 µg) and meropenem (MEM 10 µg). Over night at 35˚C¨the dishes were incubated. The antimicrobial area sizes were then evaluated in accordance with CLSI rules 2016 and interpreted.[18]

PCR screening for β-lactamase gene

Isolates indicating resistance to the third-generation cephalosporins have been tested for possible transport of bla_{NDM-1}, $bla_{SHV,}$ and bla_{TEM} mutations, which are the most commonly recorded in the middle east. Pure colony subcultures of Mueller Hinton agar have been utilized for DNA extraction by using the boiling technique. This technique required the inoculums suspended heating, which held in 1000 µl of PCR water for twelve minutes at 95 µl of Eppendorf. Centrifuga separation was then made for 5 minutes at14,000rpm. A PCR model was the supernatant comprising separate DNA. Table (1) β-lactamase gene and fingerprinting of *Pseudomonas aeruginosa* isolates using a (GTG) 5primer to determine their genetic relatedness. The amplified products have been separated with1.5 agarose gel and clusters visualized in gel max UV imaging.

Table 1. P.aeruginosa primers and β-lactamase genes

Gene	Primer sequence (5' → 3')	Annealing (T˚C)	Reference
$(GTG)_5$	GTGGTGGTGGTG	40	[19]
bla_{SHV}	F-TTATCTCCCTGTTAAGCCACC R-GATTTGCTGATTTCGCTCGG	50	[20]
bla_{TEM}	F-GCGGAACCCCTATTTG R-ACCAATGCTTAATCAGTGAG	50	[20]
bla_{NDM-1}	F-GAGATTGCCGAGCGACTTG R-CGAATGTCTGGCAGCACACTT	61	[21]

bla_{SHV}: Sulhydryl β-lactamase variant, bla_{TEM}: Temoneria β-lactamase variant, *bla*: β-lactamase gene, bla_{NDM}: New Delhi β-lactamase, bp: molecular weight in base pair, F: forward primer, R: reverse primer.

RESULTS

A total of 200 specimens was collected in this study, which includes 130(65%)burns webs and 70 (35%) wounds webs, the patient's ages ranging around one year to over 61 years (Table 2).

Table 2. *P. aeurginosa* isolates from swabs of burn and wounds according to the age.

Age group	P.aeruginosa	Other bacteria	No growth	Total
1-10 yr	9	11	3	22
11-20 yr	10	12	2	22
21-30 yr	18	13	3	35
31-40 yr	25	12	3	41
41-50 yr	11	13	3	28
51-60 yr	9	11	4	22

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≥ 61	13	13	2	30

one hundred eighty species of bacteria were isolated from two hundred patients, which include *P.aeruginosa* (n=95,47.5%), followed by *Escherichia coli* (n=27,13.50%), and the lowest percentage were *Klebsiella pneumonia*. (Table 3).

Table 3. Bacterial distribution according to growth.

Types of bacteria	samples	percentage
Negative	20	10.00%
Klebsiella pneumonia	2	1.00%
Pseudomonas putida	8	4.00%
Streptococcus pyogens	11	5.50%
Escherichia coli	27	13.50%
Acinetobacter baumanni	8	4.00%
Pseudomonas aeruginosa	95	47.50%
Staphylococcus aureus	25	12.50%
Staphlococcus epidermids	4	2.00%
Total	200	100%

Antimicrobial susceptibility test

The results of the antibiotic resistance test of *P.aeruginosa* isolates showed different antibiotic resistance profile, (Table 4). In general, 74.5%(n=95) resistant to third-generation ceftazidime (53.68%) of isolates while monobactam resistance, aztreonam (49.47%) were observed. The highest percentage of resistance to gentamicin (92.63%) was reported. Based on the results of the resistance test for fluoroquinolones (85.26%) and (58.94%) of the ciprofloxacin as well as levofloxacin respectively, were resistant.

Table 4. P.aeruginosa susceptibility patterns of different antibiotics.

Antibiotics	**Sensitive (S)**		**Intermediate (R)**		**Resistance (R)**	
	No.of isolate	**percentage**	**No.of isolate**	**percentage**	**No.of isolate**	**percentage**
Imipenem	60	63.15	0	0	35	36.27
Amikacin	9	9.47	4	4.21	82	86.31
cefepime	39	41.05	3	3.15	53	55.78
gentamicin	5	5.26	2	2.10	88	92.63
ciprofloxacin	11	11.57	3	3.15	81	85.26
ceftazidime	39	41.05	5	5.26	51	53.68
Aztreonam	38	40.00	10	10.52	47	49.47
piperacillin	17	17.89	16	16.84	62	65.26
colistin	93	97.89	0	0	2	2.10
Piperacillin/tazobactam	47	49.47	5	5.26	43	45.26

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Ticarcillin/clavulanic acid	49	51.57	3	3.15	43	45.26
Meropenem	60	63.15	0	0	35	36.84
Ticarcillin	38	40.00	5	5.26	52	54.73
Levofloxacin	37	38.94	2	2.10	56	58.94

B-lactamase genes transport

In 9 isolates from the collection of 35, which resistance to Meropenem bla_{NDM-1} was detected. Those isolates were resistant to almost all aminoglycosides and cephalosporins, were also highly resistant to Ciprofloxacin as well as Tazobactam Piperacillin. TEM l-lactamase carriage was the most prevalent for l-lactamase in 35 isolates, and for all b-lactam resistant isolates. Bla_{SHV} carriage was also recorded among Ceftazidime resistant *P.aeruginosa* isolates.

Genetic relatedness of collected *P.aeruginosa* isolates

The strong genetic similarity depending on (GTA) 5 fingerprint was seen in *P.aeruginosa* isolates in various patients who visited Baghdad hospitals (Figure 1). There was also a similarity of about 80% in seven groups depending on isolate banding pattern, antimicrobial agent source,the phenotype of resistance, and genotype. Many isolates of *P.aeruginosa* have been grouped together with similar resistance phenotypes (group 3). Furthermore, the most common isolates factor grouped with each other was the patient sources of the antimicrobial agents, either through hospitals or private pharmacies.

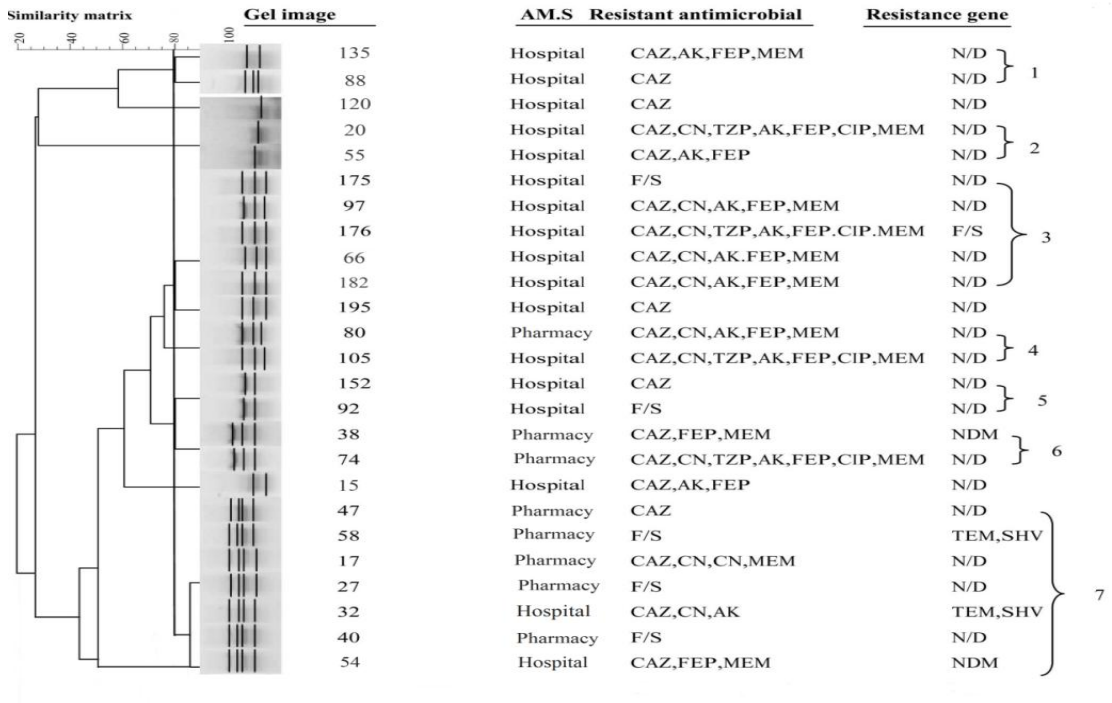

Figure 1.Fingerprint analysis of *P.aeruginosaisolates*, N/D: neither of the target genes has been detected, F/S: Fully susceptible.

DISCUSSION

In this research, 95 *P.aeruginosa* were isolated from burn and wound swabs collected

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from 200 patients touring hospitals of Baghdad. The main age of the participant was 35.6 years. The current research results reported that there is no important relationship between *Pseudomonas infection* and age. This finding was disagree with a study performed by Magliano et al.[22] which documented high rates of *Pseudomonas aeruginosa* infection in the age group (≥60 years). Moreover, the study reported that the predominant isolated bacteria were *P.aeruginosa* (47.5%), followed by *E.Coli* (13.5%) and the lowest was *K.pneumoniae* (1 %). The results are consistent with the study of Gad et al. 2007 Egypt[23]. The research is incompatible with the research by Al-Huraishi in Bagdad[24] which found that *Acinetobacter baumannii* bacteria (31%) was even more predominant than *P.aeruginosa* (12%). The specimen variation and test type used and the diagnosis of the various bacterial species may be attributable to this difference in results.

Further researches have also shown that many people have chosen medicines from community pharmacy, rather than from hospital drugs with a substantial influence on wound infections resistance of *P.aeruginosa*. Self-medication has become a major public health problem and a potential resistance catalyst to antimicrobials in the region. The resistance of *P.aeruginosa* to many antibiotics and antiseptics is common in the environment, which makes it extremely like a patient who is suffering severe wounds or burns and will face this opportunistic organism before the wound is able to heal, Lyczak, et al.[25]and Ulku, et al.[26] confirmed the above. Carbapenems are groups of antibiotics β-lactam of strong antimicrobial activity towards *Pseudomonas aeruginosa.* However, their development, as well as dissemination, have threatened the effectiveness to the therapy efforts and control effort in this organism [27]. Higher resistance incidence of major classes of antibiotics, for example, CAZ (53.68%), Gentamicin (92.36%), and Ciprofloxacin (85.26%), were reported in this study.

The results of the present study have shown that there is no difference between imipenem and meropenem activity towards *P.aeruginosa* (have almost the same percentage of resistance of approximately 36%), which disagrees with Gupta et al. 2006, which showed that the imipenem has a stronger activity than the meropenem [28]. Besides that, study findings have shown that there is high resistance to imipenem as well as meropenem compared to Al-Shara's 2013 study in Najaf [29], which recorded a resistance level of 7.4% and 14.8%, respectively. In the current study, the isolate resistance to Colistin was 2.10%, which did not agree with the research paper in Turkey, which reported that all multi drugs resistants trains are 100% susceptible to Colistin [30]. Colistin resistance is not depending on the metabolic activity of the microbes, and the resistance acquired is rare [31]. In only 12 of the 35 isolates susceptible to Meropenem, the bl_{NDM} carriage was positive. However, only 40% of these figures isolates which carry this gene was positive to bla_{TEM} or bla_{SHV}, suggesting that there is very little proof of co-carriage of that kind of gene with all other bla genes as outlined in a Kanyatta Hospital's latest research on *P. aeruginosa* strains in Kenya [32]. In the research above, 188 *P.aeruginosa* isolated from pus, respiratory tract, blood and, urine samples were also reported 51.9% bla_{NDM}, and 49.6% bla_{VEB}. This can, therefore, lead to potentially transferring an additional β-Lactamase gene to the *P.aeruginosa* isolates[2]. The dissemination of bla_{NDM-1} genes to a large extent because there is no active antibiotic on multidrug resistance bacteria[33].

Certain *P.aeruginosa* isolates appeared high resistance to meropenemhad been negatively tested, and WGS has been presented for other carbapenems. High β-lactam resistance, including carbapenems, has been recorded. It is important that the individuals who did not necessarily treat burns and wounds were used to obtain these isolates. Since there have been some wounds open, these bacteria strains may come from the environment, particularly from the soil. The genetic similarity of the recovered organisms between our

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bacterium isolates was significant. Even more, assessment also shows potential clonal development of pathogen resistance in the hospitals with exactly similar resistance of phenotype as well as elevated genetic similarity. Potent clustering of isolate with distinct strength phenotype also indicates that a comparable range of resistance determinants is independently obtained between isolates with models. Even though we could not determine the feasible transportation of The genetic components, such as plasmids, which may trigger strength characteristics mentioned above, additional genetics analyses and SNP testing relying on WGS information will shine a light on that. Even if (GTG)5 was not an excessive phylogeny method, the NDM strains are likely to be clonal because of their grouping and resistance patterns. It should be noted that perhaps the non-NDM strains were not strongly clustered, and therefore, the clone stability of the high resistance NDM strain could be increased. The distribution for clonal diseases in medical installations was linked to using medical equipment such as catheters and prosthetic[34]. *P.asaeruginosa* strains are capable of survival in the environment, particularly in several areas. Thus, transmission from individual to individual is particularly feasible in contaminated surroundings. Therefore, intentional efforts should be made to decrease transmission and reservoir sources in such environments for resistant strains.

CONCLUSION

The study Conclusion that The development of bla_{NDM-1} in combination with rampant carbapenem abuse is alarming. Accordingly, actions should be launched to institutionalizing the stewardship of antimicrobials such as hospital committees and ongoing tracking of the resistant growth. The risk factor connected with the transport and procurement multidrug resistance species, like self-medication, must be highly avoided. Measures should also be taken to carefully monitor the end of dosing and follow-up after treatment for severe diseases to avoid them.

Conflicts of Interest

No conflict of interest was declared by publishers.

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