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Prevalence of *blaIMP-1* and *blaVIM* genes in carbapenem resistant *Pseudomonas aeruginosa* isolates from patients with thermal injury in Beni-Suef University Hospital

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Abstract

P. aeruginosa is one of the opportunistic pathogens involved in various infections especially in immune-compromised individuals such as those with thermal injuries. There is a world health problem concerning *P. aeruginosa* with multi drug resistance. From a total of 103 consecutive non repetitive clinical samples of burn patients, 32 isolates were confirmed as *P. aeruginosa.* These isolates were studied for MBL production and determination of *bla IMP*, *bla VIM* and *bla NDM* genes. All the 32 were resistant to meropenem. Out of them, 21 were MBL producers by CDT while 17 were MBL producer by Disk Synergy Test (DDST). MBL genes were determined via PCR in 31 out of 32 isolates (96.8% were *bla VIM*, 96.8 % *blaNDM).* None of the strains have *blaIMP* gene. There is an elevated level of PDR to antibiotics by MBL strains. Also, this study showed that CDST is the simplest and cost-efficient test for detecting MBL and it's recommended to be confirmed via PCR in the clinical laboratory setting.

Keywords: *Pseudomonas aeruginosa*, Burns, Meropenem, MBL, PCR.

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1. Introduction

Pseudomonas aeruginosa is actually an infectious threat to burn injury cases. *It exhibits* a primary resistance to wide range of antibiotics due to its outer-membrane barriers, existence of multidrug efflux pumps, in addition to the endogenous inactivation of the antimicrobials [1-2]. Carbapenems (such as imipenem, meropenem, and doripenem) belong to the β-lactam antibiotic class and are frequently utilized for treatment of *P. aeruginosa* infection. In spite of being the highest efficient antibiotic for treatment of MDR *Pseudomonas aeruginosa* infection, development of isolates with high-carbapenem resistance was documented worldwide [3]. There are several mechanisms for carbapenem resistance. These mechanisms include acquiring resistance genes on mobile genetic elements, mutations in genes that change the expression and/or the functions of proteins encoded on the chromosomes [4]. An essential factor that leads to resistance is the production Metallo-β-Lactamases (MBL) that causes hydrolysis of all β lactams that include carbapenems except aztreonam [5].

Documented data revealed that *blaIMP-1*and *blaVIM* genes are the commonest antibiotic resistance marker that

was determined in clinical isolates of imipenem resistant *Pseudomonas aeruginosa* [6].

2. Patients and Methods

This study is a cross sectional study that was carried out at Beni-Suef University Hospital during a period from January 2020 till June 2021. Totally 103 patients with thermal injury were included. Subjects of this study were recruited according to the inclusion including patients with thermal burns caused by fire/flame scald or contact with different degrees of burns patients with chemical burn, electrical injury. One hundred and three samples from septic thermal wounds were taken from inpatients admitted in the plastic surgery unit at Beni-Suef University Hospital. Collection of samples was performed under aseptic precautions and transported immediately to the Microbiology laboratory for processing. Culturing was on nutrient agar, MacConkey agar, Blood agar and Cetrimide agar (Oxiod). The isolated *P. aeruginosa* colonies were identified by conventional methods (Gram stain, cultural characteristics and biochemical tests (catalase and oxidase tests, reduce nitrate to nitrite, Indole, citrate, urea hydrolysis, TSI and Identification was confirmed by VITEK 2 (Biomereux Egypt Distribution). Antimicrobial susceptibility testing was performed via the Kirby-Bauer diffusion method on Mueller–Hinton agar plates (Merck, Darmstadt, Germany), using 9 antibiotics from different classes (ceftazidime, gentamicin, piperacillin, amikacin, aztreonam, cefepime, ciprofloxacin, imipenem and meropenem). Isolates that showed resistance to meropenem were tested for MBL production by two methods: CDT and DDST. Then molecular detection of MBL genes detection by PCR using primer (Table1) (Invitrogen, USA), **(**Fluka, USA) [7].

2.1. Reagents

- **Agarose** a DNase and RNase free, were prepared at 1.75 % concentration.
- **TEA** (Tris EDTA Acetic acid) buffer.
- **Molecular marker (DNA marker):** DNA molecular weight marker (ladder), with molecular size MW 100 bp obtained from (Fischer scientific, USA).

2.2. Statistical Analysis

Collection of data was done followed by coding & analysis via the SPSS version 25 (Statistical package for social science) for windows 10.

3. Results

The current study is a cross sectional study that was carried out at the microbiology department at Beni-Suef University Hospital from January 2020 till June 2021. The minimum required sample size was one hundred patients. And for enhancement of the power of the study, a total of one hundred three cases with thermal injury were enrolled regarding age (Figure 1). About 62.1 % had 2nd-degree burns with a mean burn percentage of 24.7 ± 18.8. About 66.0 % of them had been treated with ceftriaxone and unictam antibiotics. The mean duration of hospital stay was 17.7 ± 8.23 SD and 73.8 % of patients had no chronic diseases (table 4).

4. Discussion

This cross-sectional study included 103 patients with thermal injury. They were recruited and assessed for eligibility from the plastic surgery unit at Beni-Suef University Hospital. As regards the demographic data of the studied patients, the results demonstrated that the largest percentage of the studied patients were≥31 years old (40.8%) followed by patients ≤10 years old (33.0%) (Figure 1). Moreover, more than one-half of them were males (58.3%) and resided in rural areas (63.1%) (table 5). In comparison with a previous study by Radan et al., (2016) that investigated the emergence of CRPA isolates that carry blaIMP among burn cases revealed that the mean age of cases was 25.8 ± 16.4 years, ranging between 3 y and 75 y and the mean BSA burn was 35.85% ± 7.58%. 54% of patients were males (54%) [19]. Regarding the isolated organisms from samples taken in this study, P. aeruginosa was isolated from 31.1% of samples followed by S. aureus (25.2%), Klebsiella (17.5) then Acinetobacter (15.5%) (table 2). Such findings are in harmony with Tchakal-Mesbahi et al., (2021) who revealed that the commonest Gram-negative bacteria in the burn infections isolates was P. aeruginosa (33.91%) [8]. Higher percentages of P. aeruginosa were found in previous studies. A one-year retrospective study by Mohamed, (2016) on the prevalence of critically ill burn injury infection in surgical ICU in Egypt revealed that the commonest organisms were P. aeruginosa (49 percent) followed by Stah. aureus (21 percent) and Klebsiella (15 percent) [9]. Moreover, a previous study by Gupta et al., (2019) revealed that out of 185 wound swabs from burnt patients, P. aeruginosa (43.0%), K. pneumoniae (28.0%), Acinetobacter baumannii (14.83%) and E. coli (6.59%) were the most common isolated organisms [10]. Additionally, Dash et al., (2019) studied the prevalence of burn wound infection and found that the organisms isolated from the burn cases included P. aeruginosa (41.3 percent), then by K. pneumonia (17.3%), Acinetobacter spp (15.3%), Coagulase negative staphylococci (4.7%), Enterococcus spp. (4%), Candida spp. (2.7%), Proteus mirabilis (1.3%) and non-fermenting gram-negative bacilli (1.3percent) approximately, percent P. aeruginosa (96.77%) isolates were from pus samples [11]. Another study by Kabanangi et al., (2021) revealed that out of 103 wound swabs, P. aeruginosa (39 percent), Acinetobacter spp. (28.7 percent)) and Klebsiella spp. (16.2%) were the most frequent Gram-negative microorganisms that cause burn wound infection in hospitalized children [12]. However, lower percentage of P. aeruginosa were found in a previous study by Latifi and Karimi, (2017) that revealed that the most frequent species of burn wound infection was Staph spp. (55.1percent), then Pseudomonas (14.29 percent), Enterococcus (12.24 percent), E. coli (4 percent), Klebsiella & Proteus (2 percent each) [13]. Regarding the risk factors, this study exhibited that the incidence of P. aeruginosa infection was higher among patients ≤ 10 years old (38.2%), males (36.7%), and residents of rural communities, such findings were not statistically significant (P value>0.05) (table 5).

Williams and Lee, (2020) revealed that the documented risk factors for infections in pediatrics with burn injury were the depth of injury, existence of inhalation injury, indwelling device, and total BSA burned. Moreover, the predominant colonization of burn wounds begins with gram-positive microorganisms, that were replaced thereafter by gram-negative microorganisms [14]. Regarding the duration of

hospitalization, significant differences were documented in the P. aeruginosa isolated from patients with short duration of hospital stay (12.1±4.8) compared with those where other organisms were isolated (20.3±8.2) (p-value=0.001) (table 6). This could be due to the early administration of antibiotics on admission that promotes the risk of overgrowth of resistant P. aeruginosa, in addition to the increased mortality among P. aeruginosa positive patients. Contrarily, a previous study by Wanis et al., (2016) revealed that the duration of hospitalization following a burn injury was accompanied by the kinds of bacterial species that were isolated from cases [15]. Within the 1st seven days of hospitalization, P. aeruginosa was rarely detected, representing only eight percent of all Gram-negative isolates. After the 28th day of admission, this percentage increased to fifty five percent. Van Duin et al., (2016) in a single-center study of 5524 burn cases revealed that longer length of hospital stay leads to increased incidence of P. aeruginosa infection [16]. Regarding the resistance of the studied P. aeruginosa isolates to different antibiotics, the present results demonstrated that high percent of the P, aeruginosa was resistant to eight out of the nine antibiotics used, meropenem (75.0%), imipenem (87.5%), amikacin (62.5%), gentamicin (78.12%), piperacillin (53.13%), cefepime (90.6), ceftazidime (71.8%), and ciprofloxacin (68.7%). While 75.0% of the isolates were sensitive to aztreonam (table 7). In contrary with the present finding, Nasirmoghadas et al., (2018) revealed that P. aeruginosa aztreonam resistance was 87% [17]. Similarly, Corehtash et al., (2015) study on P. aeruginosa isolated from burn cases revealed an elevated resistance rate against aztreonam (86.8 percent) [18]. A previous study by Radan et al., (2016) investigated the emergence of CRPA isolates that carry blaIMP gene among all isolates from burn cases and the resistance rate to ciprofloxacin, tobramycin, imipenem, meropenem, amikacin, ceftazidime, as well as cefepime was > 90 percent, whereas the resistance rate to piperacillin/tazobactam was 70.7 percent and aztreonam was 86 percent. Khalil et al., (2021) study on P. aeruginosa from cases suffering burn wounds attending diverse hospitals in Tanta, Egypt revealed that the susceptibility test of 95.7 percent of isolates showed MDR with an elevated incidence of carbenicillin resistance [20]. This study in contrary to a previous one conducted by Safaei et al., (2017) that evaluated the antibiotic resistance pattern of Pseudomonas aeruginosa isolated from burn cases and found that the highest resistance was to amikacin (94.8 percent) and norfloxacin (90.6percent); while only 8.3 percent were resistant to colistin [21]. Nasirmoghadas et al., (2018) study on antimicrobial resistance of P. aeruginosa isolates from burn cases revealed that the organism was resistant to imipenem (ninety percent); levofloxacin (ninety three percent); piperacillin-tazobactam (eighty five percent); tobramycin (ninety two percent); polymyxin b (two percent); and ceftazidime (thirty two percent) [17]. A recent study by Tchakal-Mesbahi et al., (2021) revealed that the frequency of resistant P. aeruginosa isolates from burn wounds included ceftazidime (63.79%), imipenem (70.64%), ciprofloxacin (50%), and amikacin (29.31%) [8]. The present results demonstrated that a high percent of the P. aeruginosa showed pan drug resistance to antibiotics (62.5%). 15.6% were extensive drug-resistant (XDR), 6.3% were multiple drug resistant (MDR) and only 3.1% were sensitive. Antibiotic resistance showed marked increase during COVID-19 pandemic, probably because of the elevated rate of empirical and excessive antimicrobial usage in COVID-19 cases, elevated usage of biocides, and the disrupted optimal healthcare for other cases. A study of El-Shouny et al., (2018) on P. aeruginosa isolated from burn wound infections concluded that 25% of isolates were pan drug resistance and 50% were MDR [22]. Dash et al., (2019) studied P. aeruginosa in burn injuries in addition to the antibiotic resistance of isolated strains and revealed that this pathogen was isolated from 41.3% samples [11]. Out of all the isolates 67.7% showed MDR while 29% showed XDR. And the isolates exhibited the highest resistance to ticarcillin-clavulanic acid (96.77 percent), then cefepime (93.54 percent), levofloxacin (93.54 percent), piperacillin (91.94 percent), netilmicin (91.94 percent), ceftazidime (90.32 percent), doripenem (90.32%), ciprofloxacin (87.1 percent), imipenem (87.1 percent), meropenem (85.49 percent), piperacillin-tazobactam (83.87 percent), gentamicin (58.06 percent), aztreonam (51.61 percent), tobramycin (50 percent) and amikacin (48.4%). Contrarily, Safaei et al. (2017) evaluated the antibiotic resistance pattern of P. aeruginosa isolated from burn cases and revealed that MDR with high rates were determined that include MDR (95.8 percent), XDR (87.5 percent), but no PDR was observed [21]. This study demonstrated that 96.9 percent of the studied P. aeruginosa isolates showed resistance to one or both categories of carbapenems, 68.8% showed resistance to meropenem as well as imipenem, 21.9% showed resistance to imipenem alone, and 6.3% showed resistance to meropenem alone (figure 2). A previous study by Hassuna et al. (2020) who studied carbapenems-resistance of P. aeruginosa clinical isolates obtained from different sources of patients in Upper Egypt and reported that marked resistance to carbapenem (of 64mg/mL or greater) while IRPA isolates were 84.3%, and to meropenem in 96.5% of the isolates [23]. This might be clarified by several mechanisms that include carbapenemase release and efflux-pump over-expression. Rostami et al., (2018) revealed that among all P. aeruginosa isolates obtained from cases suffering from burn injury, 78.5 percent, 46.7 percent, and 15 percent were IRPA, meropenem, and doripenem-resistant, respectively [24]. Moreover, El-Shouny et al. (2018) found that carbapenems resistant P. aeruginosa isolated from burn cases represented 74% [22]. Additionally, Saffari et al. (2016) demonstrated that among the 150 P. aeruginosa isolates from burns, ninety six percent were IRPA on performing the disk diffusion approach [21]. In contrast, McCann et al. (2018) revealed that carbapenem-resistance rates were 14.6 percent and 11.9 percent in P. aeruginosa isolates, respectively [25]. Applying appropriate antibiotic policies in managing CRPA infection may explain the variations in the prevalence rate between various studies. The present results revealed that more than 53.1 % of the examined specimens were MBL producers. By DDST, 65.6% were MBL producers (figure 3).

Furthermore, the prevalence of VIM, and NDM genes were 96.8% and (96.8%) respectively and no blaIMP gene could be detected by PCR (figure 4). In accordance, El Maraghy et al., (2019) studied the metallo-β-lactamases (blaVIM and blaIMP) genes in P. aeruginosa strains in Suez Canal University Hospital in Ismailia, Egypt and found that the blaIMP gene was not expressed in any strain [26]. Additionally, Raouf et al., (2018) studied the incidence of IRPA infections of surgical wounds in particular those caused by MBL production in Minia, Egypt and reported that blaIMP

genes weren't determined the isolates [27]. Moreover, a previous study by Manal et al., (2013) that studied MBL frequency among IMP-resistant as well as susceptible P. aeruginosa isolates via the phenotypic and molecular testing in a medical hospital setting in Cairo, also, revealed absence of blaIMP genes among MBL-producing strains [28]. Moreover, Similarly, a previous study by El-Mahdy and El-Kannishy, (2019) investigated the rate of carbapenemase genes in CRPA accompanying hospital-acquired infection in Mansura Governorate in Egypt and found that 42.5 percent were CRPA [29]. Among CRPA isolates, 61.8 percent were carbapenemase producers and the commonest gene determined was blaVIM. Additionally, Saffari et al., (2016) revealed that among the carbapenem-resistant isolates, all of them were detected as MBL-producing P. aeruginosa isolates by DDST, moreover, eighteen percent of MBL-producing isolates carried bla-VIM-1 gene while 5.5% carried bla-VIM-2 gene [21]. In Saudi Arabia, Shaaban et al., (2017) studied the resistance of P. aeruginosa isolated from diverse clinical samples to carbapenems and demonstrated that eight out of sixteen IRPA strains carry NDM-1 as well as VIM subtypes (VIM 1&2) [30]. Contrarily, several researchers recorded blaIMP gene in their studies. A study of Radan et al. (2016) investigated the emergence of CRPA isolates that carry blaIMP among burn cases revealed that all of the IRPA isolates were MBL positive, and 74.3% of the MBL isolates were positive for the blaIMP gene [19]. A meta-analyses study by Jabalameli et al., (2018), in Iranian burn centers, showed that the prevalence of CRPA, blaIMP, and blaVIM was 76.8% 13.1%, and 21.4%, respectively [31]. Moreover, Rostami et al., (2018) reported that the blaIMP and blaVIM genes were determined in 17.9 percent and 1.2 percent of P. aeruginosa wound burn isolates; respectively [24]. Additionally, Farhan et al., (2019) studied the prevalence of CRPA strains isolated from diverse areas of infections isolated from hospitals in Minia, Egypt and reported that Carbapenemase genes demonstrated include blaIMP (42.8%, 9/ 21), blaVIM (52.3%, 11/ 21), blaGIM (52.3%, 11/ 21), blaSPM (38%, 8/21) [32]. Regarding the blaVIM and blaNDM genes, 70 % and 73.3% of CRPA have the genes respectively which are not statistically significant (P-value, 0.809 and 0.170 respectively). Joji et al. (2019) studied the detection MBL (VIM & NDM-1) genes in carbapenem-resistant P. aeruginosa clinical strains in Bahrain and revealed that 47.5 percent of strains have VIM gene; 2.5 percent strains have the NDM-1 gene, whereas single strain carried both of them. Of note, the imipenem sensitive strains didn't carry any of these the genes [33]. Additionally, lower percentages of NDM-1 were reported in some studies. Joji et al., (2019) found that only one (2.5%) carbapenem-resistant P. aeruginosa strain carry the NDM-1 gene [33]. Zafer et al., (2014) found that the prevalence of the NDM-1 gene was only 4.2 percent [34]. Additionally, Shanthi et al., (2014) reported that only four carbapenem-resistant P. aeruginosa isolates out of 61 carry NDM-1. A previous study by Hashem et al., (2017) study in Suez Canal University Hospital in Ismailia, Egypt revealed that out of 147 Pseudomonas aeruginosa clinical samples recovered from hospitalized and ICU patients with different infections, 26.5 percent of the isolates were CRPA and 64% were positive for MBLs [35]. The frequency of blaVIM and blaIMP-like genes were twenty percent and four percent and the sequences confirmed the isolates to be blaVIM-1, blaVIM-2, blaVIM-4, and blaIMP-1. Mohanam and Menon, (2017) indicated that out of the 213 Pseudomonas aeruginosa isolates, 10% showed resistance to be carbapenem [36]. Among them, 81.8% were found to be MBL producers. Polymerase chain reaction amplification revealed that 91% of isolates have one or more of the MBL genes tested: blaVIM and blaNDM in 32% & 27% isolates, respectively; blaVIM and blaNDM in 14% isolates; blaIMP and blaNDM in 9%; blaVIM and blaIMP in 5% isolates. The blaVIM, blaIMP and blaNDM were proved to be present in single isolate only. The difference in prevalence of genes could be explained as the metallo-β-lactamase determinant are portion of gene cassettes inserted in chromosomal or plasmid-borne integrons located on the nosocomial isolates of *Pseudomonas aeruginosa* that help different recombination and enhance fast transference horizontally [37]. Therefore, variations of MBL among Pseudomonas aeruginosa differs according to the regional areas [38]. Such findings enhance the importance of continuous monitoring and better observance of infection control practices, to avoid more dissemination of such defiant pathogens [39]. Regarding the risk factors for CRPA, the present study demonstrated that a statistically significant increase was determined in cefepime resistance among CRPA patients in comparison with carbapenem-sensitive P. aeroginosa (P-value=0.002) (figure 6, table 7). Such finding is in harmony with a previous study by Radan et al., (2016) that indicated that Carbapenem-resistant Pseudomonas aeruginosa often show resistance to all β-lactam and fluoroquinolone antibiotics. The intrinsic resistance of such organisms causes more limitation to antibiotic selection [19]. El-Shouny et al., (2018) revealed that the elevated rate of resistance to cephalosporin antibiotic might be because of acquired mobile components or transposons between Pseudomonas aeruginosa isolates due to the widespread administration of cephalosporin antibiotic in burn units [22]. Furthermore, massive usage of a particular antibiotic agent enhances selective pressure upon sensitive isolates and induces the occurrence of MDR or PDR isolates. The present results demonstrated that the prevalence of carbapenem-resistant isolates was significantly higher among isolates resistant: amikacin, gentamicin, piperacillin, cefepime, ceftazidime, and ciprofloxacin at P-value equals (0.027, 0.001, 0.001, 0.032, 0.002, and 0.001), respectively. A previous study by Abdelaziz et al., (2021) studied the relationship between the genes responsible for antibiotic resistance in carbapenem-resistant P. aeruginosa and their susceptibility to antibiotics revealed a significant correlation between the existence of the blaSHV gene and the MexA and resistance to fluoroquinolone antibiotic, amikacin, tobramycin, co-trimoxazole and β-lactams and between the aac-6'-Ib gene and resistance to aminoglycoside antibiotic [40].

Mahmoud et al., (2021) indicated that all carbapenem-resistant P. aeruginosa isolates were resistant to ceftazidime, cefepime, ciprofloxacin, gentamicin, levofloxacin, tobramycin, imipenem, and meropenem [41]. Furthermore, high resistance to amikacin, aztreonam, piperacillin and piperacillin- tazobactam was 86.1 percent, 83.3 percent, 94.5 percent and 91.7 percent respectively. The present results demonstrated that the sensitivity, specificity, PPV, and NPV of DDST for detecting MBL were 56.7 percent, one hundred percent, one hundred percent, and 7.1 percent respectively. The k value that denotes the measure of agreement is poor (0.038) (table 8). While the sensitivity, specificity, PPV, and

NPV of CDST for detecting MBL were 66.7%, 0.0%, 95.2%, and 0.0% respectively. The k value that denoted the measure of agreement is poor (-0.03) (table 9, 10). A previous study by Kumar et al., (2018) revealed that regarding the CDST-IMP, the sensitivity, specificity, PPV, NPV and accuracy were 86.21 percent; 30.99 percent; 33.78 percent; 84.62 percent and 47 percent respectively. While regarding the DDST-IMP the sensitivity, specificity, PPV, NPV and accuracy were 82.76%; 35.21%; 34.29%; 83.33% and 49% respectively [42]. The Sensitivity of CDST in a study by Sachdeva et al., (2017) was 97.95 percent while the Specificity was 96.11 percent [43]. Khosravi et al., (2012) demonstrated that the Sensitivity of all the 3 tests (DDST, CDST and E test) was one hundred percent whereas Specificity of DDST was 96.6 percent then E. Test that was 62.1 percent and that of CDST 43.1 percent. It was documented that DDST-IPM was the highest specificity of these 3 tests [44]. Additionally, Lucena et al., (2014) revealed that the DDST assay was the most appropriate approach to assess MBL production in Pseudomonas aeruginosa strains. A previous study by Beig et al., (2021) indicated that the least sensitivity of the CDDT test was because of finding MBL in carbapenemase-producing P. aeruginosa isolates, thus it's not recommended for detecting class B carbapenemase producing isolates. Another study by Sachdeva et al., (2017) revealed that the CDDT was found to be superior to DDST and has the most elevated sensitivity (97.95%) and specificity (96.11%) for detecting MBL product [43].

Table 1: The primers used for MBLs genes detection.

Gene name		Sequence	Melting temp.	Product size
bla*VIM1*	Forward	5- GGTGTTTGGTCGCATATCGCAA-3	58.9	502bp
	Reverse	5- ATTCAGCCAGATCGGCATCGG- 3	59	
bla*VIM2*	Forward	5- GAAGGACTCTCATCGAGCGG-3	60	322 bp
	Reverse	5- AGCGATTTGTGTGCGCTTTT-3	60	
bla*IMP1*	Forward	5-TCGTTTGAAGAAGTTAACG -3	59	568 bp
	Reverse	5-ATGTAAGTTTCAAGAGTGATGC -3	59	
bla*IMP2*	Forward	5- ACGGTCTTGGCTATTTGGGG-3	60	160 bp
	Reverse	5- CCTTTAACAGCCTGCTCCCA-3	60	
Bla NDM	Forward	5- GGTTTGGCGATCTGGTTTTC-3	59	521 bp
	Reverse	6- CGGAATGGCTCATCACGATC-3	59	

Table 2: Distribution of the isolated organisms from the studied samples.

Culture growth		Frequency	Percentage (%)
Gram negative bacilli, (n=66, 64.1%)	*P. aeruginosa*	32	31.1
	Klebsiella	18	17.5
	Acinetobacter	16	15.5
Gram positive cocci, (n=37, 35.9%)	*Staph. aureus*	26	25.2
	Enterococci	11	10.7
Total		103	100.0

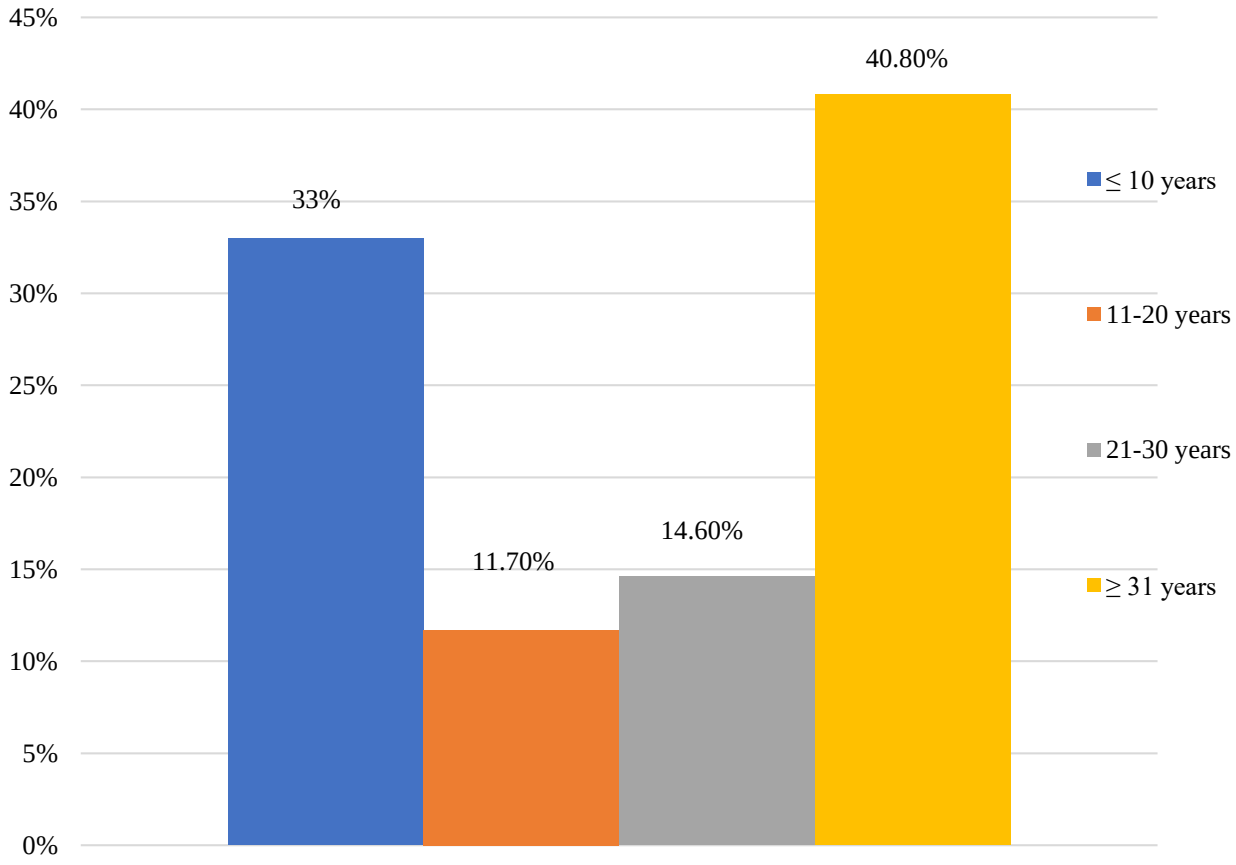

Figure 1: Distribution of the studied patients according to their age; n=103.

Table 3: Antibiotic sensitivity pattern of *P. aeruginosa* isolates (n=32).

		Sensitive	Moderate	Resistance
Meropenem	**N=32 (%)**	8 (25.0)	0 (0.0)	24 (75.0)
Imipenem	**N=32 (%)**	4 (12.5)	0 (0.0)	28 (87.5)
Aztreonam	**N=32 (%)**	24 (75.0)	2 (6.3)	6 (18.8)
Amikacin	**N=32 (%)**	8 (25.0)	4 (12.5)	20 (62.5)
Gentamicin	**N=32 (%)**	6 (18.75)	1 (3.13)	25 (78.12)
Piperacillin	**N=32 (%)**	8 (25.0)	7 (21.87)	17 (53.13)
Cefepime	**N=32 (%)**	3 (9.37)	0 (0.0)	29 (90.63)
Ceftazidime	**N=32 (%)**	9 (28.13)	0 (0.0)	23 (71.87)
Ciprofloxacin	**N=32 (%)**	10 (31.25)	0 (0.0)	22 (68.75)

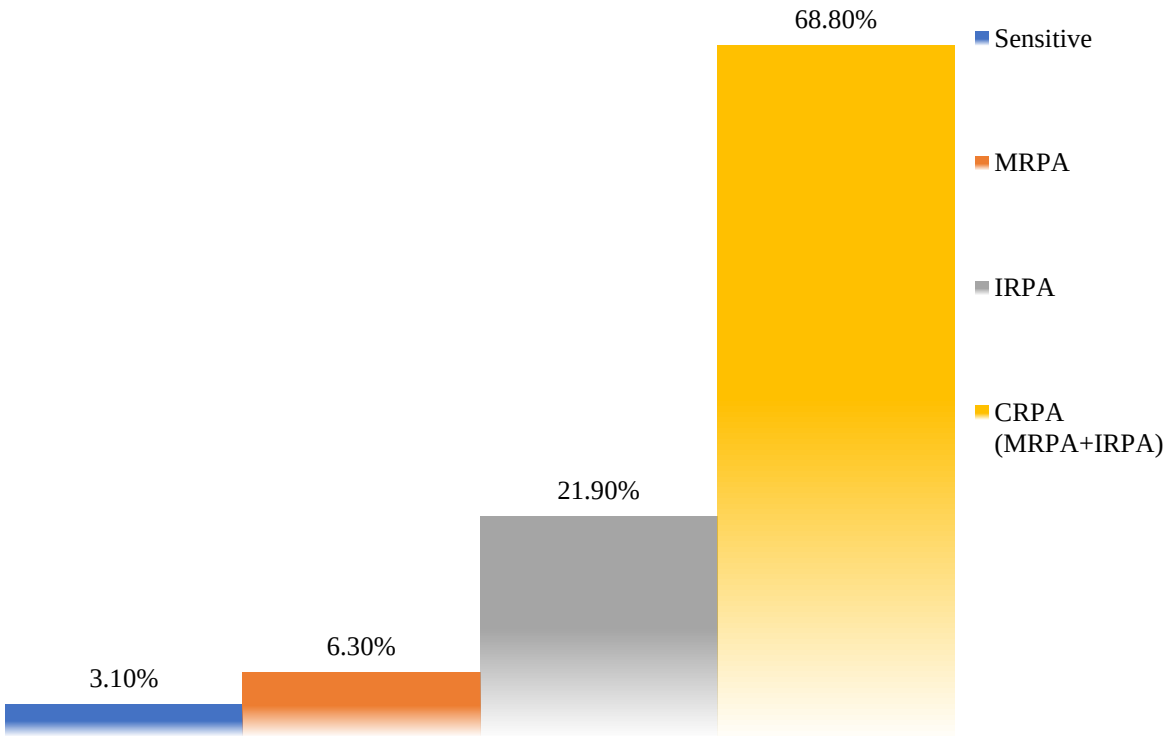

Figure 2: Distribution of the studied *P. aeruginosa* specimens regarding their carbapenem resistance pattern.

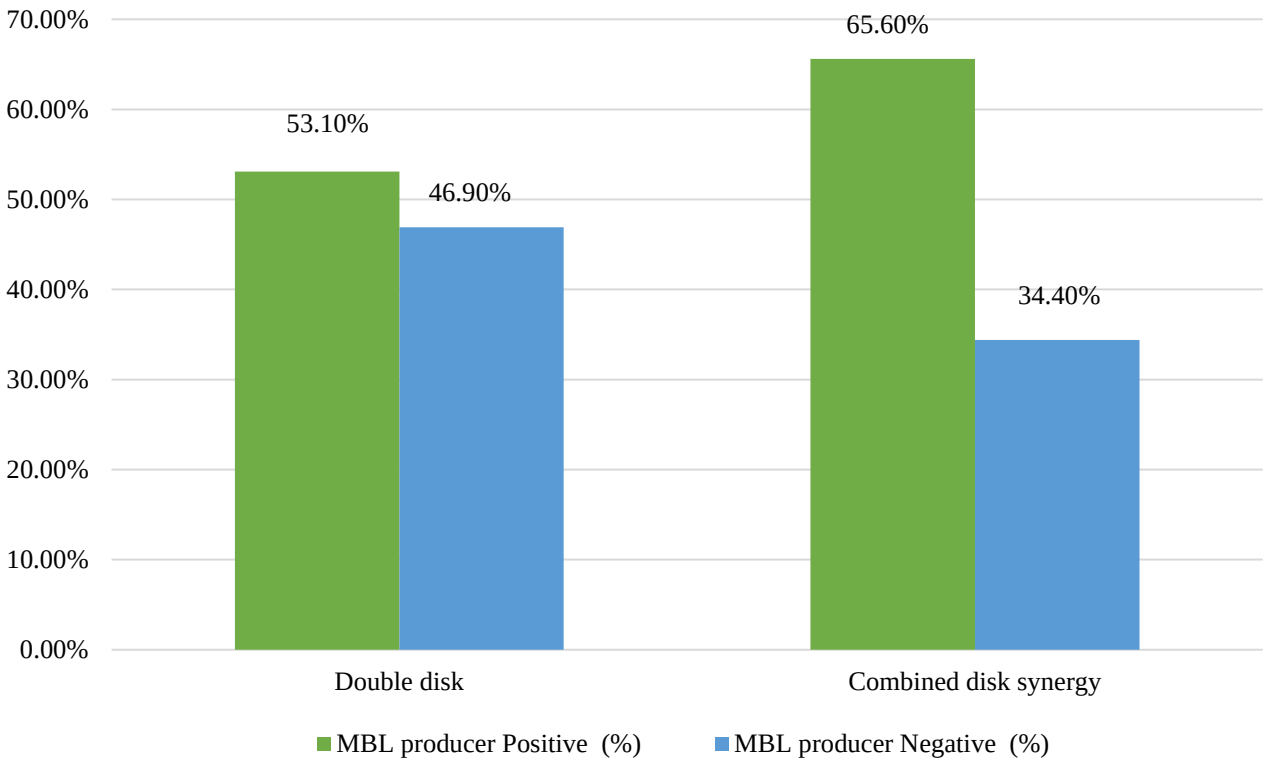

Figure 3: MBL producing *P. aeruginosa* isolates by the screening tests.

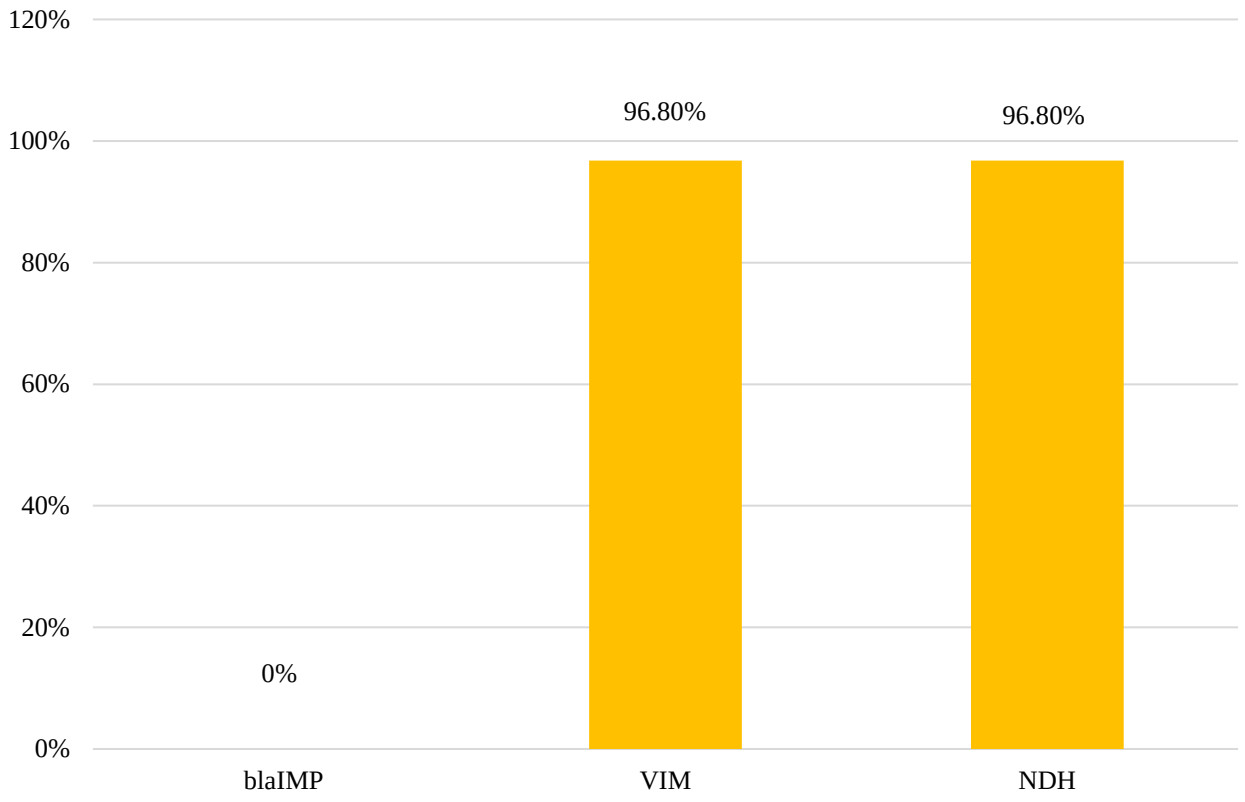

Figure 4: Prevalence of *blaIMP, blaVIM, and NDH* genes in the Carbapenem-resistant isolates.

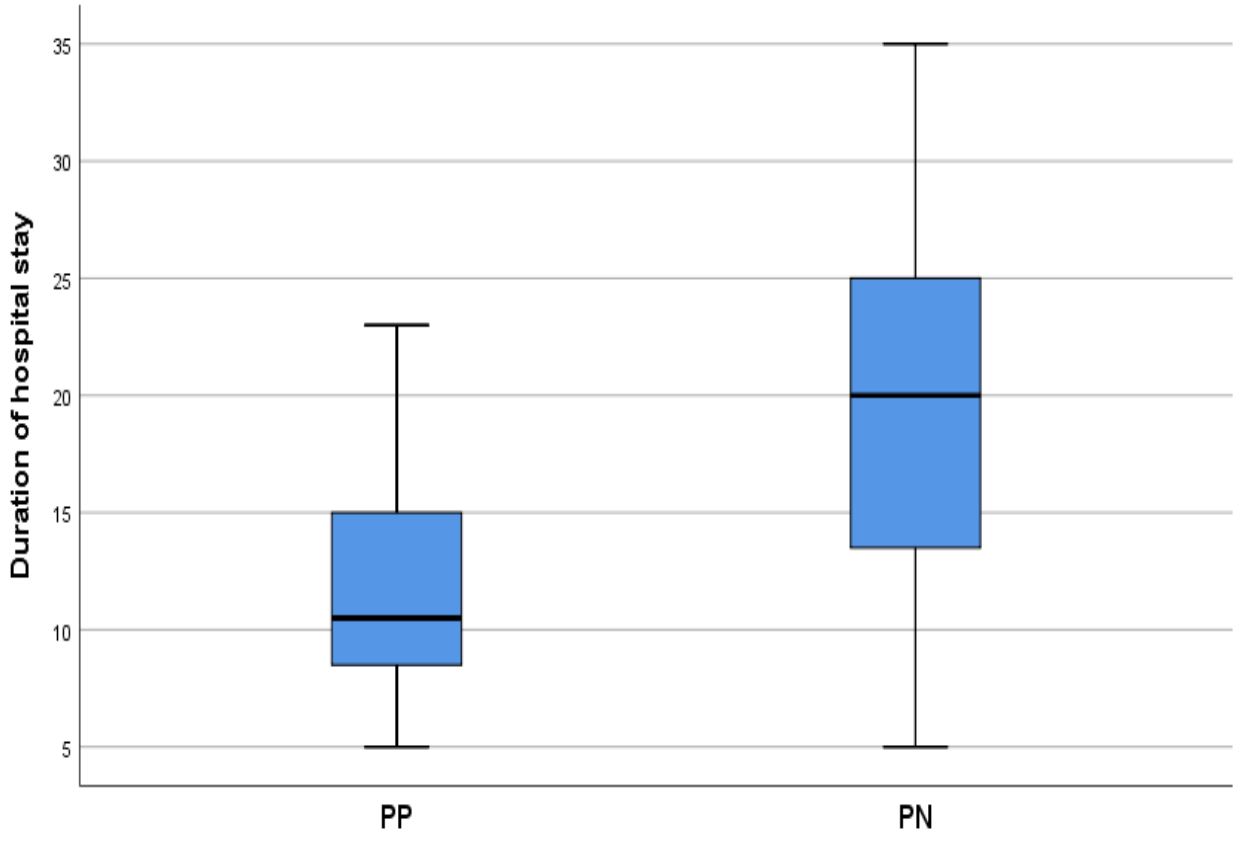

Figure 5: Duration of hospital stay and prevalence of *P. aeruginosa* infection.

Table 4: Distribution of the studied *P. aeruginosa* isolates regarding the clinical data.

Clinical Data		Phytic Results		Total	*P Value*	χ^2/t
		PP	PN			
		N=32 (%)	N=71 (%)	N=103 (%)		
Degree of Burn	1st degree	7 (25.9)	20 (74.1)	27 (100.0)	0.648	0.866
	2nd degree	22 (34.4)	42 (65.6)	64 (100.0)		
	3rd degree	3 (25.0)	9 (75.0)	12 (100.0)		
Percentage of burn	Mean±SD	23.5±13.3	21.03±11.7	21.8±12.2	0.343	0.384
Antibiotic treatment	Ceftriaxone	6 (31.6)	13 (68.4)	19 (100.0)	0.998	0.004
	Ceftriaxone and Unictam	21 (30.9)	47 (69.1)	68 (100.0)		
	Ceftriaxone, Unictam and Ciprofloxacin	5 (31.3)	11 (68.8)	16 (100.0)		
Duration of hospital stay	Mean±SD	12.1± 4.8	20.3±8.2	17.7±8.02	0.001*	11.99
Medical History	No chronic diseases	23 (30.3)	53 (69.7)	76 (100.0)	0.679	2.311
	Diabetes mellitus	4 (28.6)	10 (71.4)	14 (100.0)		
	Hypertension	2 (33.3)	4 (66.7)	6 (100.0)		
	Both diabetic and hypertensive	2 (33.3)	4 (66.7)	6 (100.0)		
	Cardiac	1 (100.0)	0 (0.0)	1 (100.0)		

Statistics were done using the Chi-square test and the student t test / *P-value≤ 0.05 is considered significant.

Table 5: Distribution cabapenem sensitivity pattern of *P. aeruginosa* isolates of the studied patients regarding the sociodemographic data.

		Carbapenem sensitivity		Total	P-value	χ2
		Sensitive	Resistant			
		N=1 (%)	N=31 (%)	N=32 (%)		
Age in years	≤ 10	0 (0.0)	13 (100.0)	13 (100.0)	0.680	1.095
	11-20	0 (0.0)	4 (100.0)	4 (100.0)		
	21-30	0 (0.0)	2 (100.0)	2 (100.0)		
	≥31	1 (7.7)	12 (92.3)	13 (100.0)		
Sex	Male	1 (4.5)	21 (95.5)	22 (100.0)	0.493	4690.
	Female	0 (0.0)	10 (100.0)	10 (100.0)		
Residence	Urban	1 (7.7)	12 (92.3)	13 (100.0)	0.219	1.509
	Rural	0 (0.0)	19 (100.0)	19 (100.0)		

Statistics were done by Chi-square test / *P-value≤ 0.05 is considered significant.

Table 6: Distribution of carbapenem resistant isolates regarding the degree of burn, hospital stay, and medical history of studied patients.

Clinical Data		Carbapenem sensitivity		Total	*P-value*	χ^2/t
		Sensitive	Resistant			
		N=1 (%)	N=31 (%)	N=32 (%)		
Degree of Burn	1st degree	1 (14.3)	6 (85.7)	7 (100.0)	0.158	3.687
	2nd degree	0 (0.0)	22 (100.0)	22 (100.0)		
	3rd degree	0 (0.0)	3 (100.0)	3 (100.0)		
Percentage of burn	Mean±SD	15	23.77±13.39	23.77±13.39	0.524	0.952
Antibiotic exposure	Ceftriaxone	0 (0.0)	6 (100.0)	6 (100.0)	0.541	0.763
	Ceftriaxone and Unictam	1 (4.8)	20 (95.2)	21 (100.0)		
	Ceftriaxone, Unictam and Ciprofloxacin	0 (0.0)	5 (100.0)	5 (100.0)		
Duration of hospital stay	Mean±SD	10	12.13±4.89	12.13±4.89	0.672	-5.256
Medical History	No chronic diseases	1 (4.3)	22 (95.7)	23 (100.0)	0.982	0.404
	Diabetes mellitus	0 (0.0)	4 (100.0)	4 (100.0)		
	Hypertension	0 (0.0)	2 (100.0)	2 (100.0)		
	Both diabetic and hypertensive	0 (0.0)	2 (100.0)	2 (100.0)		
	Cardiac	0 (0.0)	1 (100.0)	1 (100.0)		

Statistics were done using the Chi-square test and the student t-test / *P-value≤ 0.05 is considered significant.

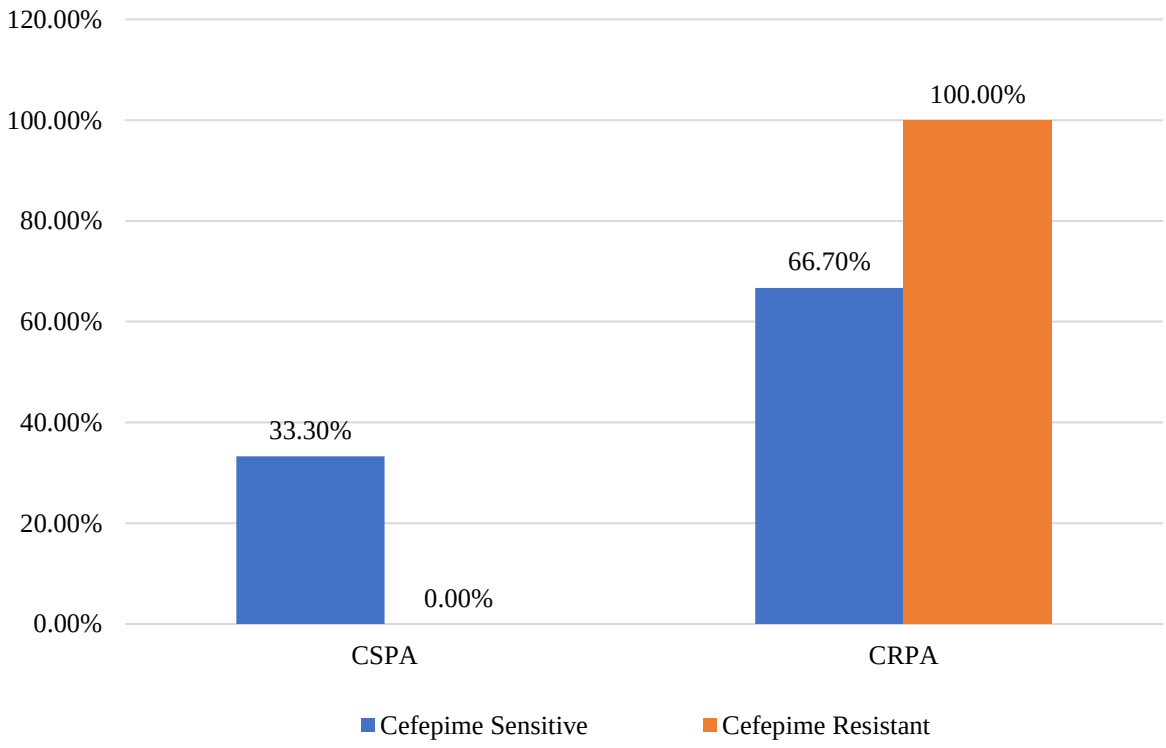

Figure 6: Resistance pattern to Cefepime antibiotic in CRPA and CSPA isolates.

Table 7: Association between carbapenem susceptibility and other antibiotics tested.

		Carbapenem Sensitivity		Total	*P-value*	χ^2
		Sensitive	Resistance			
		N=1 (%)	N=31 (%)	N=32 (%)		
Aztreonam	**Sensitive**	1 (4.2)	23 (95.8)	24 (100.0)	0.482	0.344
	Resistant	0 (0.0)	6 (100.0)	6 (100.0)		
	Moderate	0 (0.0)	2 (100.0)	2 (100.0)		
Amikacin	**Sensitive**	1 (12.5)	7 (87.5)	8 (100.0)	0.213	3.097
	Resistant	0 (0.0)	20 (100.0)	20 (100.0)		
	Moderate	0 (0.0)	4 (100.0)	4 (100.0)		
Gentamicin	**Sensitive**	1 (16.7)	5 (83.3)	6 (100.0)	0.107	4.473
	Resistant	0 (0.0)	25 (100.0)	25 (100.0)		
	Moderate	0 (0.0)	1 (100.0)	1 (100.0)		
Piperacillin	**Sensitive**	1 (12.5)	7 (87.5)	8 (100.0)	0.213	3.097
	Resistant	0 (0.0)	17 (100.0)	17 (100.0)		
	Moderate	0 (0.0)	7 (100.0)	7 (100.0)		
Cefepime	**Sensitive**	1 (33.3)	2 (66.7)	3 (100.0)	0.002*	9.978
	Resistant	0 (0.0)	29 (100.0)	29 (100.0)		
Ceftazidime	**Sensitive**	1 (11.1)	8 (88.9)	9 (100.0)	0.104	2.638
	Resistant	0 (0.0)	23 (100.0)	23 (100.0)		
Ciprofloxacin	**Sensitive**	1 (10.0)	9 (90.0)	10 (100.0)	0.132	2.271
	Resistant	0 (0.0)	22 (100.0)	22 (100.0)		

Statistics were done using the Chi-square test / *P-value≤ 0.05 is considered significant.

Table 8: Comparison of Double disk synergy test and PCR for detecting of MBL.

PCR		Double disk synergy test		*P-value*	χ^2	Sensitivity	Specificity	KAPPA
		Positive MBL producers	Negative MBL producers					
		N=17 (%)	N=14 (%)					
bla IMP	Positive	0 (0.0)	0 (0.0)	NA	--	--	--	--
	Negative	17 (100.0)	14 (100.0)					
blaVIM	Positive	17 (100.0)	13 (92.9)	0.263	1.255	56.7%	100%	0.038
	Negative	0 (0.0)	1 (7.1)					
blaNDH	Positive	16 (94.1)	14 (100.0)	0.356	0.851			
	Negative	1 (5.9)	0 (0.0)					
Total		17 (54.8)	14 (45.2)					

Statistics were done using the Chi-square test / *P-value≤ 0.05 was considered significant/ No association (NA**)**.

Table 9: Comparison of Combined disk synergy test and PCR for detecting MBL.

		Combined disk synergy test		P-value	χ^2	Sensitivity	Specificity	KAPPA
		Positive MBL producer	Negative MBL producer					
		N=21 (%)	N=10 (%)					
bla IMP	**Positive**	0 (0.0)	0 (0.0)	NA	---	--	--	--
	Negative	21 (100.0)	10 (100.0)					
blaVIM	**Positive**	20 (95.2)	10 (100.0)	0.483	0.492	66.7%	0.0%	-0.03
	Negative	1 (4.8)	0 (0.0)					
blaNDH	**Positive**	20 (95.2)	10 (100.0)	0.483	0.492			
	Negative	1 (4.8)	0 (0.0)					

Statistics were done using the Chi-square test / *P-value≤ 0.05 was considered significant/ No association (NA).

Table 10: Relation between PCR results and Carbapenem resistance pattern.

		Carbapenem resistant pattern isolates (n=31)			Total	*P-value*	χ^2
		IRPA only	MRPA only	CRPA			
		N=2 (%)	N=7 (%)	N=22 (%)	N=31 (%)		
bla IMP	Absent	2 (6.5)	7 (22.6)	22 (71.0)	31 (100.0)	NA	---
VIM	Absent	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0.809	0.423
	Present	2 (6.7)	7 (23.3)	22 (70.0)	30 (100.0)		
NDH	Absent	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0.170	3.543
	Present	2 (6.7)	6 (20.0)	22 (73.3)	30 (100.0)		

Statistics were done using the Chi-square test / *P-value≤ 0.05 was considered significant/ No association (NA).

5. Conclusions

In conclusion, *P. aeruginosa* is a serious and the commonest Gram-negative microorganism that cause burn wound infection in hospitalized cases in Beni-Suef University Hospital that showed pan drug resistance with an alarming rate (62.5%), so, firm infection control measures and implementing antimicrobial stewardship programs are highly recommended.

6. Limitation of the study

Follow up of the neonates for colonization at several intervals and at discharge as well as following up the fate of each case could have added more data to the present findings. However, due to lack of fund and materials available, we couldn't extend it any further.

Conflict of interest

None.

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