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Research Article

Detection of virulence determinants and its association with drug resistance in clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* is most commonly noted significant nosocomial pathogen, because of its distribution, of multi drug resistance and expression of various virulence factors. This study was aimed to detect various resistance mechanism and virulence factors of *Pseudomonas aeruginosa* and to determine the significant association between them.
Methods: A total of 203 clinical isolates of *Pseudomonas aeruginosa* were included in this study. All isolates were detected for various virulence factors like Phospholipase, Hemolysin, Gelatinase and DNAse. Screening of β-lactamase like extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase and Metallo β-lactamase (MBL) of *Pseudomonas aeruginosa* were also done.
Results: Of total 203 isolates of *Pseudomonas aeruginosa* studied, 103 were from pus, 50 each from urine and respiratory samples. Virulence factors distribution of *Pseudomonas aeruginosa* showed 80.3% ,70% , 71.4% , 44.8% and 34% were positive for hemolysin, phospholipase, gelatinase, DNAse and biofilm production respectively. Study on prevalence of various β-lactamase in *Pseudomonas aeruginosa* isolated showed 25.6%, 24.1% and 10.3% were ESBL, MBL and AmpC producers respectively.
Conclusions: This study suggests that production of virulence factors may not be significantly associated with antibiotic resistance. However, expression of certain virulence factors, most notably hemolysin and DNAse activity were significantly associated with β-lactamase production. Hence forth, future trends in clinical microbiology laboratories should focus on development of tests for the rapid detection of the most important virulence markers in addition to identification of pathogens and susceptibility pattern.

Keywords: *Pseudomonas aeruginosa*, Virulence factors, Extended spectrum beta-lactamase, AmpC beta-lactamase, Metallo β-lactamase

INTRODUCTION

Pseudomonas aeruginosa, is a gram negative rod shaped bacteria and motile by means of polar flagella. This organism being ubiquitious in nature and one of the most common opportunistic pathogen. In hospitalized individuals, it is the most common cause of nosocomial infection particularly in immunosuppressed, burns and cystic fibrosis patients. This pathogen is significant because of its widespread distribution, of multi drug resistance and multiple arrays of virulence factors produced by it. The objective of this study was to evaluate the in vitro activities of various virulence factors, to study the resistance mechanism of

Pseudomonas aeruginosa and to understand the significant association between them.[1-4]

METHODS

This prospective analytical, single center study was done in a tertiary care teaching hospital in Puducherry. A total of 203, non-repetitive isolates of *Pseudomonas aeruginosa* from all clinical samples were included in this study. Identification was done by conventional biochemical test using standard methods.[1]

Detection of virulence factors by phenotypic methods:

Detection of hemolysin

Sheep blood agar plates inoculated with the colonies, were incubated at 37ºC for 24 h and then checked for zone of hemolysis around them. The results were recorded as α-haemolysis (greenish zones), β-haemolysis (clear zone) or γ-haemolysis (no haemolysis).[2]

Detection of phospholipase

Egg yolk agar was inoculated with colonies from 18-24 hour culture, and incubated at 35ºC for 24-48. Appearance of a milky white opaque halo around the colony was read as positive for phospholipase C production.[3]

Detection of gelatinase

Gelatin productions were determined by inoculating the character was tested by bacterial inoculation tubes containing nutrient gelatin medium. The tubes were incubated for 48 h at 37ºC. Uninoculated tubes were kept as negative control. At the end of incubation period, liquefaction of the culture medium by placing the culture tube at 4°C overnight were observed positive for gelatinase production.[4]

Detection of DNAase activity

Bacterial colonies were spot inoculated in a DNase test agar plates and incubated at 37°C for 24 to 48 hours after which it was flooded 1.0 N HCl. DNase secreted by the bacteria colonies hydrolyse the DNA in the medium, resulting in clearance around the bacterial growth.[5]

Detection of Biofilm production (tube adherence method)

Around 2-3 colonies were inoculated to 5ml of BHI broth in glass tubes. The tubes were incubated at 37°C for 18-20 hrs. After incubation the cultures were aspirated and test tubes were stained with saffranine. Formation of visible stained film on the wall of the tube was interpretated as positive and if the glass remains unstained it is considered as negative for biofilm production.[6]

Routine antimicrobial susceptibility was performed for all isolates of *Pseudomonas aeruginosa* by Kirby-Baeur disk diffusion method according to CLSI guidelines for various antibiotics like Ciprofloxacin (5µg), Norfloxacin antibiotics, namely: Amikacin (30µg), Gentamicin (10µg), Netilmicin (30µg), Tobramycin (30µg), (10µg) Ceftazidime (30µg), Imipenem (10µg), Piperacillin/ tazobactam (100µg/10µg).[7]

Detection of various antibiotic resistance mechanisms like extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase and Metallo β-lactamase (MBL) of Pseudomonas aeruginosa was done by the following methods.

Detection of extended spectrum beta lactamases (ESβLs) by phenotypic confirmatory disc diffusion test (PCDDT)

All isolates were tested for their susceptibility to the 3rd generation cephalosporins (3GCs) ceftazidime (30 µg/disk), cefotaxime (30µg/disk) and ceftriaxone (30 µg/disk) by using the standard disc diffusion method as recommended by CLSI.[7] Isolates which were resistant to at least one of the 3GCs were selected for the study and were processed for ESβLs production. If diameter of zone of inhibition of ≤22 mm for ceftazidime, ≤27 mm for cefotaxime and ≤25 mm for ceftriaxone were recorded, the isolate was considered to be "suspicious for ESβLs production".[7]

Following which Phenotypic confirmatory disc diffusion test (PCDDT) will be used for confirming ESβLs production.

Ceftazidime and cefotaxime discs (30µg) alone and combination with clavulanic acid (30/10µg) were applied to Mueller Hinton agar plates, inoculated with the tested isolates. Diameter of zone of inhibition was measured after overnight incubation at 37ºC. An increase of in diameter of zone of inhibition ≥5 mm for the combined discs compared to cefotaxime or ceftazidime disc alone was considered to be a marker for ESβL sproducing isolate.[7]

Detection of Metallo β-lactamase by disk potentiation test

The carbapenem MDR resistant *Pseudomonas spp*. was further screened for MBL production by disk potentiation test. Two 10 µg imipenem disks were placed on the plate, to one of the disk 10 µl of 50mM zinc sulphate was added after drying, 5µl of 0.5M EDTA solution was then dispensed (930 µg per disc).

The inhibition zones of imipenem and imipenem-EDTA disks were compared after 16 to18 hours of incubation at 35˚C. If there is increase in zone of inhibition ≥7 mm with imipenem and EDTA disk than imipenem disk

alone, the isolate was considered to be the MBL producer.[8]

Detection of AmpC beta-lactamase by disk antagonism test

AmpC β-lactamase production screening was performed by Cefoxitin disk test. Isolates that produced a zone diameter less than 18 mm (screen positive) were further subjected to Disk antagoism test. The disk antagonism test was used for detection of inducible AmpC β-lactamase in all the isolates of *Pseudomonas aeruginosa*. A test isolate (with a turbidity equivalent to that of 0.5 McFarland standards) was spread over a Mueller Hinton agar (Hi-Media) plate. Cefotaxime (30µg) and cefoxitin (30µg) (Hi-Media Mumbai) disks were placed 20 mm apart from center to center.

Isolates showing blunting of the cefotaxime zone of inhibition in adjacent to cefoxitin disk were screened as positive for AmpC β-lactamase. Further confirmation of AmpC production was tested by AmpC disc test.[9]

Statistical analysis

Percentages were calculated for categorical variables. The significant association between drug resistance and virulence factors in different strains of *Pseudomonas aeruginosa* was calculated using Chi-square test/ Fisher's exact test.

RESULTS

Of total 203 isolates of *Pseudomonas aeruginosa* studied, 103 were from pus samples, remaining 50 each from urine and respiratory samples (Table 1).

Table 1: Percentage distribution pattern of *Pseudomonas aeruginosa* in clinical samples.

Sample	Number	Percentage
Pus	103	50.7%
Urine	50	24.6%
Sputum	50	24.6%
Total	203	100%

Table 2: Distribution of virulence factors in clinical isolates of *Pseudomonas aeruginosa*.

Sample	Positive and Negative % of Virulence factors									
	Hemolysin		Phospholipase		Gelatinase		DNAse		Biofilm production	
	Positive%	Negative %	Positive%	Negative %	Positive%	Negative %	Positive%	Negative %	Positive%	Negative %
Pus (103)	77 (74.8%)	26 (25.2%)	68 (66%)	35 (34%)	67 (65%)	36 (35%)	46 (44.7%)	57 (55.3%)	34 (33%)	69 (67%)
Urine (50)	44 (88%)	6 (12%)	38 (76%)	12 (24%)	39 (78%)	11 (22%)	25 (50%)	25 (50%)	20 (40%)	30 (60%)
Sputum (50)	42 (84%)	8 (16%)	36 (72%)	14 (28%)	39 (78%)	11 (22%)	20 (40%)	30 (60%)	15 (30%)	35 (70%)
Total (203)	163 (80.3%)	40 (19.7%)	142 (70%)	61 (30%)	145 (71.4%)	58 (28.6%)	91 (44.8%)	112 (55.2%)	69 (34%)	134 (66%)

Distribution of Virulence factors in clinical isolates of *Pseudomonas aeruginosa* showed 80.3% were positive for hemolysin production, 70% positivity for phospholipase, 71.4% Positivity for gelatinase, 44.8% were positive for DNAse and 34% were biofilm production (Table 2).

The analysis of antibiotic susceptibility pattern of *Pseudomonas aeruginosa* showed maximum sensitivity of 88.2% for drug Imipenem, 80.2% for Piperacillin-Tazobactum, 77.3 % for Tobramycin, 71.9% for Amikacin, 65% for Ciprofloxacin, 64% for Ceftazidime and 52.2% for Gentamicin (Table 3).

Table 3: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=203).

Antibiotics	Sensitive (%)	Interme-diate (%)	Resistant (%)
Amikacin	146 (71.9%)	14 (6.89%)	43 (21.2%)
Gentamicin	106 (52.2%)	25 (12.3%)	72 (35.5%)
Tobramycin	157 (77.3%)	6 (2.95%)	40 (19.7%)
Ciprofloxacin	132 (65%)	10 (4.9%)	61 (30%)
Ceftazidime	130 (64%)	7 (3.4%)	66 (32.5%)
Piperacillin - tazobactum	164 (80.8%)	15 (7.4%)	24 (11.8%)
Imipenem	179 (88.2%)	6 (2.95%)	18 (8.9%)

Study on prevalence of β-lactamase in clinical isolates of *Pseudomonas aeruginosa* showed 25.6% ESBL producers, 24.1% MBL producers and 10.3% were AmpC producers (Table 4).

Analysis of association between Virulence factors and drug resistance in clinical isolates of *Pseudomonas aeruginosa* (Table 5) showed there was a significant association between hemolysin and ESBL producers and DNase and MBL producers ($p<0.05$).

Table 4: Prevalence of ESBL, MBL & AmpC β-lactamase in clinical isolates of *Pseudomonas aeruginosa* (n=203).

Sample	ESBL (%)	MBL (%)	AmpC (%)
Pus (103)	31 (59.6%)	31 (63.3%)	10 (47.6%)
Urine (50)	12 (23.1%)	13 (26.5%)	8 (38.1%)
Sputum (50)	9 (17.3%)	5 (10.2%)	3 (14.3%)
Total (203)	52 (25.6%)	49 (24.1%)	21 (10.3%)

Table 5: Analysis of association between Virulence factors and drug resistance in clinical isolates of *Pseudomonas aeruginosa*.

β-lactamase		Hemolysin		Gelatinase		Phospholipase		DNAse		Biofilm production	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
ESBL	Positive (52)	33	19	37	15	35	17	28	24	19	33
	Negative (151)	130	21	108	43	107	44	63	88	50	101
p value		0.0004		0.95		0.62		0.12		0.65	
MBL	Positive (49)	40	9	32	17	39	10	32	17	16	33
	Negative (154)	123	31	113	41	103	51	59	95	53	101
p value		0.78		0.27		0.09		0.0009		0.82	
AmpC	Positive (21)	19	2	16	5	17	4	11	10	5	16
	Negative (182)	144	38	129	53	125	57	80	102	64	118
p value		0.21		0.60		0.24		0.46		0.29	

DISCUSSION

Pseudomonas aeruginosa as an ubiquitous opportunistic pathogen found in the hospital environment, can cause severe nosocomial infections which involves a broad spectrum of infections including respiratory, gastrointestinal, and urinary tracts as well as wound infections and sepsis.[10-11]

One of the reasons that *P. aeruginosa* is often considered as successful opportunistic pathogen is because of production of multiple array of virulence factors. The genes encoding for these virulence factors are controlled through a mechanism known as quorum sensing (QS). The pathogenesis of *Pseudomonas aeruginosa* is mainly due to production of several cell-associated and secreted extracellular virulence factors which includes elastases, alkaline protease, pyocyanin and rhamnolipids.[12]

In our study on various virulence factors expressed by *Pseudomonas aeruginosa* 80.3% were positive for hemolysin, 70% positivity for phospholipase, 71.4% positivity for gelatinase well in comparable to similar other study which reported 95.2%, 81% and 78% for hemolysin, phospholipase and gelatinase.[13]Another study had reported 87.5% phospholipase activity and 81.25% gelatinase activity concurrent to our study.[14]

Study on DNAse showed 44.8% positivity, in comparison to our findings another study showed 41%, 54% and 64% of isolates from wound swabs, sputum and ear swabs, respectively, showed DNase activity.[15]

In current study, we have reported 34% were biofilm producers. But several studies in fact have reported higher percentage of biofilm producers 76% and 68.7%.[13,14] In our study, we have used tube adherence for routine detection of slime production considering fact of its ease in application and low cost and studies have shown better correlation with PCR s.[16] However there are other study ,stating that tube test correlates well with the TCP test for strong biofilm producing isolates but not between weak and biofilm negative isolates due to the variable results given by different observers.[17] Hence, further studies need to be done to evaluate better method for detection of biofilm in *Pseudomonas aeruginosa*.

The treatment of *Pseudomonas aeruginosa* infection becomes much more complicated, when there is added resistance to multiple antimicrobial drugs which in turn leads to high morbidity and mortality.

In our study more than 80% susceptibility were shown for Imipenem and Piperacillin –Tazobactum. There are several studies showing resistance rate of ceftazidime

above 60%, ciprofloxacin from 48-59%, very much higher than our study.[18-21] Another study by Joseph NM et al, shows that significant reduction in resistant rate of ceftazidime from 50% to 33% and of ciprofloxacin from 49% to 33% much comparable to our study.[22] Several studies showing better anti pseudomonal activity with antibiotics like amikacin and imipenem concurrent to our study.[20-23]

Pseudomonas aeruginosa exhibit varying degrees of innate and acquired resistance mechanism. Acquired resistance mechanism includes extended spectrum β-lactamase, metallo β-lactamase and plasmid mediated AmpC -β-lactamase.[24]

Study on prevalence of β-lactamase in clinical isolates of *Pseudomonas aeruginosa* showed 25.6% isolates were ESBL producers, 24.1% were MBL producers and 10.3% were AmpC producers. Aggarwal et al reported, 20.27% of ESBL production in *Pseudomonas aeruginosa*, in his study.[25] Another study on rates of ESBL, AmpC and MBL production among the ceftazidime resistant *Pseudomonas aeruginosa* showed 19.4 % ESBL Positive, 65.7% MBL Positive and 16.4 % AmpC Positive where they have reported higher percentage of MBL producers than our study.[26] Studies from various other centers on MBL production showed 20.8%, 17.3% and 20.7% well comparable with our study.[27-29]

Although most of the studies focus separately on virulence or resistance, there are only few studies showing relationship and mutual biological impact of one event on the other. In our study, we have done a comparative analysis to study the significant association between antibiotic resistance and virulence factors in clinical isolates of *P. aeruginosa*, which was calculated using Pearson's correlation coefficient. The obtained results showed that there was significant association between Hemolysin and ESBL producers ($p<0.05$) and DNase and MBL producers ($p<0.05$) among various virulence factors and β lactamase studied.

Mansouri et al, studied the possibility of ESBL producing clinical isolates of *P. aeruginosa*, to express several virulence factors under favourable conditions of its growth and survival.[30] Another study of virulence factors among ESβL-producing and nonproducing *Pseudomonas aeruginosa* clinical isolates have shown significant association between ESBL production and various virulence factors.[13]

The ability of the isolates to produce pigment and other virulence factors such as elastase, protease, siderophore and DNAse activity appears to be more significantly associated with multi drug resistance (MDR).[15] Various other studies (Bradbury et al, Deptula and Gospodarek et al) also have shown the association between expression of virulence factors and MDR phenotype.[31,32]

Studies have stated that association between resistance and virulence follows a pattern of Darwinian model, in which those strains that conferred a specific advantage will be selected and become fixed. Those associations which showed a positive effect, i.e., increased resistance plus increased virulence will be selected very rapidly. Those in which selection is apparently negative means increased resistance with diminished virulence will undergo a longer selection process, until a specific virulence advantage is selected and becomes fixed in the population. In another scenario of increased virulence with decreased resistance, compensatory mutations may arise to equilibrate the balance, and finally increased resistance and virulence will proceed together to confer the bacteria with a selective advantage.[33]

CONCLUSION

In conclusion, these results revealed that *Pseudomonas aeruginosa* being a significant opportunistic and nosocomial pathogen is able to accumulate different resistance and virulence factors, making the infections treatment difficult. This study also suggests that production of all virulence factors may not have positive association with antibiotic resistance. However, expression of certain virulence factors, most notably hemolysin and DNAse activity were significantly associated with ESBL and MBL production respectively. Henceforth, future trends in clinical microbiology laboratories should focus not only in identification of pathogens and susceptibility pattern in addition, development of tests for the rapid detection of the most important virulence markers.

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REFERENCES

1. Koneman EW, Allen SD, Janda WM, Schrenkenberger PC, Winn WC. Colour Atlas and Textbook of Diagnostic Microbiology, 5th ed. Lippincott Williams & Wilkins, PA. 1997.
2. Pavlov D, De Wet CME, Grabow WOK, Ehlers MM. Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. Int J Food Microbiol. 2004;92:275-87.
3. Mittal R, Khandwaha RK, Gupta V, Mittal PK, Harjai K. Phenotypic characters of urinary isolates of *Pseudomonas aeruginosa* & their association with mouse renal colonization. Indian J Med Res. 2006;123:67-72.
4. Mac Faddin JF. Biochemical Tests for Identification of Medical Bacteria, 2nd edn. Williams and Wilkins, Baltimor. 1980.
5. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG,

Marmion BP, Simmons A (eds). Mackie and McCartney Practical Medical Microbiology. 14th edn. Edinborough: Churchill Livingstone. 1996:131-52.
6. Prasad SV, Ballal M, Shivananda PG. Slime production a virulence marker in *Pseudomonas aeruginosa* strains isolated from clinical and environmental specimens: A comparative study of two methods. Indian J Pathol Microbiol. 2009;52:191-3.
7. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement ed. CLSI document M100-S20. CLSI: Wayne. PA. 2013.
8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem–EDTA disk method for differentiation of metallo β lactamase-producing clinical isolates of *Pseudomonas spp*. and Acinetobacter spp. J Clin Microbiol. 2002;40:3798-801.
9. Upadhyay S, Sen MR, Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries. 2010;4(4):239-42.
10. Morrison AJ, Wenzel RP: Epidemiology of infections due to *Pseudomonas aeruginosa*. Rev Infect Dis. 1984;6:S627-S642.
11. Pollack M: *Pseudomonas aeruginosa*. In Principles and Practice of infectious diseases Edited by: Mandell GL, Bennett JE, Dolin R. New York: Churchill Livingstone. 1995:1980-2003.
12. Van Delden C, Iglewski BH. Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg Infect Dis. 1998;4:551-60.
13. Khalil MAEF, Sonbol FI, Mohamed AFB, Sameh SA. Comparative study of virulence factors among ESβL-producing and nonproducing *Pseudomonas aeruginosa* clinical isolates. Turk J Med Sci. 2015;45:60-9.
14. Mohammad HH. Phenotypic Investigation for Virulence factors of Pyocine producing *Pseudomonas aeruginosa* Isolated from Burn Wounds, Iraq. International Journal of Scientific & Engineering Research. 2013;4(7):2114-21.
15. Finlayson EA, Brown PD. Comparison of antibiotic resistance and virulence factors in pigmented and non-pigmented *Pseudomonas aeruginosa*. West Indian Med J. 2011;60:24-32.
16. Oliveira A, Maria de Lourdes RS Cunha. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. BMC Research Notes. 2010;3:260.
17. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol. 2006;24(1):25-9.
18. Kaur DC, Wankhede SV. A study of Biofilm formation &Metallo-β-Lactamases in *Pseudomonas aeruginosa* in a tertiary care hospital. International Journal of Scientific and Research Publications. 2013;3(10):1-7.
19. Zafer MM, Al-Agamy MH, El-Mahallawy HA, Amin MA, El-Din Ashour MS. Antimicrobial Resistance Pattern and Their Beta-Lactamase Encoding Genes among *Pseudomonas aeruginosa* Strains Isolated from Cancer Patients. Bio Med Research International. 2014.
20. Chaudhari VL, Gunjal SS, Mehta M. Antibiotic resistance patterns of *pseudomonas aeruginosa* in a tertiary care hospital in Central India. Int J Med Sci Public Health. 2013;2:386-9.
21. Amutha R, Padmakrishnan, Murugan T, Renugadevi MP. Studies on multidrug resistant *Pseudomonas aeruginosa* from pediatric population with special reference to extended spectrum beta lactamase. Indian Journal of Science and Technology. 2009;2(11):11-3.
22. Joseph NM, Devi S, Shashikala P, Kanungo R. Changing Trend in the Antibiotic Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Wound Swabs of Out-Patients and in-Patients of a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. 2013;7(10):2170-2.
23. Senthamarai S, Reddy SKA, Sivasankari S, Anitha C, Somasunder V, Kumudhavathi MS, et al. Resistance Pattern of *Pseudomonas aeruginosa* in a Tertiary Care Hospital of Kanchipuram, Tamilnadu, India. Journal of Clinical and Diagnostic Research. 2014;8(5):30-2.
24. Manchanda V, Singh NP. Occurrence and detection of AmpC b-lactamases among Gram negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. J Antimicrob Chemother. 2008;51:415-8.
25. Aggarwal R, Chaudhary U, Bala K. Detection of extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. Indian J Pathol Microbiol. 2008;51:222-4.
26. Umadevi S, Joseph NM, Kumari K, Easow JM, Kumar S, Stephen S, et al. Detection of Extended Spectrum Beta Lactamases, Ampc Beta Lactamases and Metallobetalactamases In Clinical Isolates Of Ceftazidime Resistant *Pseudomonas aeruginosa*. Brazilian Journal of Microbiology. 2011;42:1284-8.
27. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patient. Indian J Med Res. 2008;127:398-402.
28. Shahid S, Malik A, Agrawal M, Singhal S. Phenotypic detection of extended spectrum and AmpC β-lactamases by a new spot inoculation method and modied three dimensional extract test: Comparison with the conventional three-dimensional extract test. J Antimicrob Chemother. 2004;54:684-7.

29. Shashikala, Kanungo R, Srinivasan S, Devi S. Emerging resistance to carbapenem in hospital acquired *Pseudomonas* infection: A cause of concern. Indian J Pharmacol. 2006;38:287-8.
30. Mansouri S, Norouzi F, Moradi M, Nakhaae N. Comparison of virulence factors among clinical isolates of *Pseudomonas aeruginosa* producing and non-producing extended spectrum beta-lactamases. Curr Res Bacteriol. 2011;4:85-93.
31. Bradbury RS, Roddam LF, Merritt A, Reid DW, Champion AC. Virulence gene distribution in clinical, nosocomial and environmental isolates of *Pseudomonas aeruginosa*. J. Med. Microbiol. 2010;59:881-90.
32. Deptula A, Gospodarek E. Reduced expression of virulence factors in multidrug resistant *Pseudomonas aeruginosa* strains. Arch. Microbiol. 2010;192:79-84.
33. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World? Alejandro Beceiro, María Tomás, Germán Bou. Clin. Microbiol. Rev. 2013;26(2):185.

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