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Prevalence and Antimicrobial Susceptibility Pattern of Pseudomonas Aeruginosa Isolated from Clinical Samples in a Tertiary Care Hospital

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Original Research Article

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Abstract: Pseudomonas aeruginosa is a true opportunistic pathogen and is responsible for causing a variety of infections in clinical settings in both immunocompetent as well as immunocompromised hosts. However, in most cases, *P.aeruginosa* infections occur in patients who have been compromised in some way, e.g. chronic Diabetic Patients. Methods: In this study a total of 280 (10.98%) *P.aeruginosa* isolates were obtained out of 2549 samples collected for a period of 5 years. The isolates were selected on the basis of their growth characteristics on Blood agar, MacConkey agar and Nutrient agar medium. Colonies were subjected to series of biochemical tests to identify the species. Antimicrobial susceptibility testing of all the confirmed *P.aeruginosa* isolates was performed by automated walkaway 96 using NBC 42 and conventionally comparing with Kirby–Bauer disc diffusion method for further confirmation and results were interpreted according to CLSIs guidelines. Results: The prevalence of this pathogen was 10.98% and most of the isolates were mostly isolated from pus/wound swab cultures, followed by urine & sputum cultures, mostly isolated from critical areas such as ICU's. Distribution of Multidrug Resistant *P.aeruginosa* was seen in high percentage from samples of surgical site wound infections followed by ET tip/secretions and urine samples. The susceptibility pattern of *P.aeruginosa* was as follows Colistin 100 %, Amikacin 69.5%, Gentamycin 46.8%, Tobramycin 53.9%, Ceftazidime 51.1%, Cefepime 51.2%, Levofloxacin 49.2%, Ciprofloxacin 32.81%, Pip/Taz 57.42%, Imipenem 77.7 % Multidrug resistant (MDR) strains were totally resistant to Cephalosporins, Aminogycosides, Fluoroquinolones and Carbapenems. Conclusion: High prevalence of *P.aeruginosa* as an opportunistic pathogen has been on the increase with resistance to antimicrobial agents and thus becoming a potential threat as the numbers of susceptible antibiotics are decreasing. This study underlines the importance of culture and sensitivity tests in patients before treatment in order to prevent emergence of drug resistant organisms.

Keywords: Pseudomonas aeruginosa, Pus Samples, Antimicrobial Resistance, Multidrug Resistance [MDR], Colistin & Polymixin B.

INTRODUCTION

P.aeruginosa is a is a Gram negative, bacillary shape, non-spore forming, ubiquitous and versatile human opportunistic pathogen it has been implicated in morbidity, mortality and healthcare costs both in hospitals as well as in the community [1]. *P.aeruginosa* frequently causes life-threatening infections that are difficult to treat as it exhibits intrinsically high resistance to many antimicrobials [2, 3]. It is reported to be the third leading cause of hospital-acquired urinary tract infections, accounting to about 12% of all hospital acquired infections. It generally invades the blood stream from urinary tract and nearly 40% of pseudomonas bacteremia is of UTI origin. Urinary tract catheterization, instrumentation or surgeries are the primary causes of *P.aeruginosa* infections [4]. *P.aeruginosa* intrinsically exhibits high resistance to many antimicrobials and is involved in development of increased multi-drug resistance in health care settings [2, 3]. It develops resistance due to acquisition of resistance genes (like those encoding beta-lactamase and aminoglycoside modifying enzymes) [5, 6], multi-drug resistance efflux pumps, biofilm formation, aminoglycoside modifying enzymes and mutations in different chromosomal genes. Moreover exposures of the organism to board spectrum antibiotics and patient to patient spread have added to rapid increase in isolation of resistant strains [7]. *P. aeruginosa* has a great adaptability to different environmental situations, because of its ability of acquiring resistance to antibiotics and presence of multiple virulence factors that can resist host defenses and allow the development of infections in individuals where defenses are compromised like those on long-term corticosteroid therapy, blood disorders, burn patients, patients with multiple surgeries. Several studies have reported the

influence of previous antibiotic exposure on the susceptibility pattern of *P.aeruginosa* [8-10]. The impact was called collateral damage from antibiotic prescription to refer to ecological adverse effects of antibiotic consumption that is represented by emergence of MDR organisms via mutations [11]. The aim of the present study to determine the prevalence to analyze the susceptibility profile of *P.aeruginosa*, one of the most prevalent bacteria involved in cross-contamination, and the incidence of microbial contamination on surfaces in consulting-rooms, near to and distant from the doctors wash basins, with the purpose of revealing any high risks of infections that could be reduced with effective contamination control procedures.

MATERIALS AND METHODS

A total of 280 clinical isolates of *P.aeruginosa* were prospectively collected between the periods of Jan 2012 to December 2016. The identification of all isolates was performed using conventional methods & fully automated walkAway 96s using NBC 42 panels. Conventionally, all specimens were processed by using standard pre-calibrated loop (Nichrome-SWG 24, 4 mm internal diameter) on Blood agar (BA), Cysteine Lactose Electrolyte Deficient (CLED) medium (for urine) and CHROM agar for preliminary identification of species. Since chromogenic media are now available it can be used for more specific and direct differentiation of bacterial colonies on the primary plate itself. These help in minimizing the requirement for further biochemical tests, and also could help in giving the clinician a preliminary report, as and the need arises, for the early therapy. HiChrom E Agar supported the growth of all routine isolates, and also yeasts. The ability of HiChrom Agar to support the growth of the organisms was concurrent with that of the conventional media. In addition, the advantage of rendering color differentiation to the various isolates for preliminary identification, which were further confirmed tests using MicroScan-walkAway 96s fully automated instrument. The inoculated plates were incubated at 37°C overnight (18-24 hr) and examined the next day morning. The Pseudomonas thus isolated from all cultures were identified by standard conventional methods including the diffusible pigment, panel- negative breakpoint combo 42 and identification by walkaway 96 s fully automated with sensitivity by MIC values. Amikacin 4-32 µg/mL (Ak), Amoxicillin/K Clavulanate 8/4-16/8 µg/mL (Aug), Ampicillin 2-16 µg/mL (Am), Ampicillin/Sulbactam 4/2-16/8 µg/Ml, (A/S), Aztreonam 2-16 µg/Ml, Cefazolin 2-16 µg/Ml, Cefepime 2-16 µg/Ml (Cpe), ESβL Screen - Cefotaxime – 11 µg/mL (CftE), Cefotaxime 2-32 µg/Ml (cft), ESβL Confirmation-Cefotaxime/K Clavulanate (Cft/CA), Cefoxitin - 2-16 µg/Ml, (Cfx), Ceftazidime 2-16 µg/Ml (Caz), ESβL Confirmation - Ceftazidime/K Clavulanate (Caz/CA), Ceftriaxone - 1-2, 8, 32 µg/Ml (Cax), Cefuroxime 4-16 µg/Ml (Crm), Cephalothin 8-16 µg/Ml (Cf), Ciprofloxacin 0.5-4 µg/Ml (Cp), Ertapenem 0.5-4 µg/Ml (Etp), Gentamicin 1-8 µg/Ml (Gm), Imipenem 1-8 µg/Ml (Imp), Levofloxacin 0.5-4 µg/Ml (Lvx), Meropenem 1-8 µg/Ml (Mer), Nitrofurantoin 32-64 µg/mL (Fd), Piperacillin/Tazobactam 8-64 µg/Ml (P/T), Tetracycline 2-8 µg/Ml (Te), Tigecycline 1-4 µg/mL (Tgc), Tobramycin 1-8 µg/mL (To), Trimethoprim/Sulfamethoxazole 0.5/9.5-2/38 (T/S). Keeping a note on the intrinsic resistance of these species, *Pseudomonas aeroginosa* are inherently resistant to Amoxicillin-Clavulanic Acid, Ampicillin-Sulbactam, Cefotaxime, Ceftriaxone, Ertapenem, Tetracyclines, Trimethoprim, Trimethoprim–Sulfamethoxazole, Chloramphenicol, Fosfomycin. Reporting was done as per CLSI guidelines and the antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines.

The isolates were tested for ceftazidime (30µgm) levofloxacin (5µgm) Norfloxacin (10µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Gentamycin (10 µgm), Tobramycin (10 µgm) Piperacillin-Tazobactam (100/10 µg) and Imipenem (10 µg), Meropenem (10µgm), Colistin (10µgm) (Hi-mediA). After adding inoculums of 0.5 McFarland turbidity standards, specified antibiotic discs placed 2 cm apart from each other with sterile forceps and were incubated for 16-18 hours at 37°C aerobically. The degree of sensitivity was determined by measuring zone of growth inhibition around the disc. The growth of bacterium would be inhibited around the discs containing antibiotics to which the bacterium is susceptible, while no inhibitory zone around resistant ones. The results were interpreted as sensitive, intermediately sensitive and resistant to the different drugs. The zone of inhibition was interpreted according to the Kirby-Bauer antibiotic sensitivity chart. Stains, Panels, antibiotic discs and the media QC were assessed weekly using ATCC strains *P.aeruginosa* 27853, *E.coli* 25922, *S.aureus* 29213 and 25923, *E.fecalis* 28212 An isolate was considered as MDR if found resistant to three or more antimicrobials belonging to different classes/groups of antimicrobials. The data regarding the *P. aeruginosa* and sensitivity pattern was obtained from the Microbiology laboratory registers. The patient's details were collected from case sheets in the Medical Records Department and wards.

Resistance to multiple drugs is usually the result of the combination of different machines in a single isolate. There are a variety of mechanisms involved in the resistance of *P. aeruginosa*, among them over expression of efflux pump, acquisition of Extended-Spectrum β-Lactamases (ESBLs) and Metallo-β-Lactamases (MBLs); target site or outer membrane modification are predominant. Production of multiple-β-lactamases by *P.aeruginosa* has led tremendous therapeutic consequences and posed clinical challenges. ESBLs mediate resistance to extended-spectrum Cephalosporins Such As Cefotaxime,

Ceftriaxone, and Ceftazidime. The Carbapenems and β-lactam and β-lactamase inhibitor combination such as Piperacillin plus Tazobactam are the drugs active against cephalosporin resistant *P. aeruginosa*. However, resistance to these drugs has also been increasing worldwide. The production of MBLs, increased expression of the efflux pump, reduced level of drug accumulation are the main factors involved in carbapenem resistance to *P. aeruginosa*. In India, the prevalence of MBLs ranges from 7.5% to 71%. It has been demonstrated that MBLs require divalent cations, usually zinc, as metal co-factor for their enzymatic activity and no therapeutic option is known to be available to control MBLs. Catheter usage is most prevalent in ICU'S, data from NHSN that examined utilization of urinary catheters indicate that 54 to 90 % of all ICU days in Medical and Surgical ICU at a major teaching hospital involved the use of urinary catheter, in the lowest 10 percentile to the 90 percentile respectively of the reporting hospital study in Israel suggested that patients with intermediate duration of cauterization (7 – 30 days) and those catheterized were the indications of obstruction or incontinence are a high risk group that may benefit most from the interventions. Such patients had a higher daily risk (8.6 %) of acquiring infection even during the early period of catheterization.

RESULTS

In the present study there were a total of 2549 samples studies out of which 280 (10.98%) P. *aeruginosa* isolates were obtained. Age wise in males showed between 51-60 yrs showed highest numbers isolated from catheter samples followed by ET secretions, followed by 61 – 70 yrs with advancing age, and more severe underlying illness, the emerging of specialised units for the care of critically ill patients the increased use of multiple invasive devices.

Table-1: Age wise distribution

Age	Males	Females
< 20	4	3
20 -30 yrs	24	7
31- 40 yrs	14	7
41 – 50 yrs	27	9
51 – 60 yrs	33	14
61 – 70 yrs	36	19
71- 80 yrs	25	7
>80 yrs	11	nil

Table-2: Year wise distribution of cases

YEAR	Isolates from Males	Isolates from Females
2012	34	21
2013	30	12
2014	29	8
2015	38	12
2016	43	13

Table -3: Year wise and unit wise distribution of cases involved in the study

	Distribution of cases					Total	percentage
	2012	2013	2014	2015	2016		
3RD Floor	9	7	4	8	3	31	11.69
4TH Floor	6	5	2	9	4	26	9.81
CTOT/CTICU	3	-	2	1	-	6	2.26
5TH Floor	-	-	2	4	1	7	2.64
6TH Floor	-	-	2	6	5	13	4.90
SICU	7	3	1	2	3	16	6.04
EMD	3	1	-	1	1	6	2.26
SDICU	-	2	-	-	1	3	1.13
ICCU	-	-	4	1	-	5	1.89
External	8	-	-	-	-	8	3.02
MICU	9	3	8	7	12	39	14.71
MSICU	2	3	2	7	12	26	9.81
OPD	9	9	-	12	-	30	11.32
RICU	5	5	2	6	8	26	9.81
Gen Ward	2	5	-	-	-	7	2.64
NICU	-	-	1	5	4	10	3.77

Unit wise data shows the prevalence & isolation of *P.aeruginosa* was more from ICU's apart from OPDs which were mostly the post operative the follow up cases, most of them were from the surgical site infections followed by the respiratory infections and UTI. Those isolated from SICU were totally the surgical site infections

Table-4: Showing the samples and numbers of cases showing positive *P.aeruginosa* Total no of samples – 250

Sample type	Total number of cases showing Pseudomonas positive cultures	Percentage
Blood	14	5.6
Bal Fluid	13	5.2
Bed Sore Swab	1	0.4
Bile Fluid	4	1.6
Nasal Sinus Fluid	3	1.2
Central Line	1	0.4
Et Secretions	42	16.8
Jugular Tip	1	0.4
Pus / Wound Swabs	64	25.6
Tissue	4	1.6
Suction Catheter Tip	11	4.4
Sputum	34	13.6
Urine	56	22.4
Throat Swab	1	0.4
Drain Fluid	1	0.4
Total	250	100

Sample wise distribution – shows Pseudomonas isolation predominantly from surgical site infections (25.6 %) followed by UTI (22.4%), includes both asymptomatic & symptomatic catheter associated infections .Those associated with urinary catheters were multidrug resistant strains showing 100% susceptibility to Colistin & Polymixin B, and some strains showed intermediate susceptibility to Carbapenems. A total of total 426 urine positive cultures -205 isolates were *E.coli* 47.97%, 13.57% were Klebsiella pneumonia 0.468% were *Klebsiella oxytoca*, 11.7% were P.*aeruginosa*, 1.40% were Proteus mirabilis, 1.17% were Morganella, 7.25% were Enterococcus *spp*, 3.51% were *Enterobacter spp*, 9.61% were Candida *spp*. Overall antibiotic susceptibility pattern for pseudomonas Amikacin – 69.5%, Gentamycin – 46.8%, Tobramycin- 53.9%, Ceftazidime – 51.1%, Cefepime – 51.2%, Levofloxacin – 49.2%, Ciprofloxacin – 32.81%, Pip/Taz – 57.42%, Imipenem – 77.7 %, Colistin 100 % (figure-1).

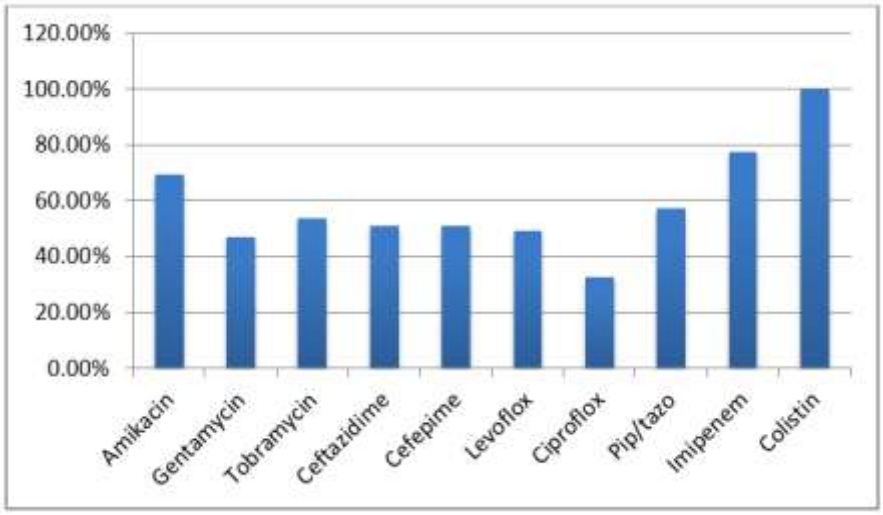

Fig-1: The overall susceptibility pattern of *P.aeruginosa* isolated from all clinical samples

-5: Prevalence of Multidrug Resistant Strains [MDR] in both the sexes with sample wise distribution

Sample	Males	Females	Total
Pus / Swabs	7	5	12
Clt	0	1	1
Tissue	0	2	2
Sputum	1	1	2
Et Tip / Secretions	6	0	6
Urine	2	1	3
Bal Fluid	1	0	1
Total	17	10	27

Table -6: Prevalence of XDR Strains in both the sexes along with sample wise distribution

Sample	Males	Females	Total
Pus / Swabs	2	1	3
Drain fluid	1	0	1
Sputum	1	1	2
Et Tip / Secretions	8	4	12
Urine	22	8	30
Blood	3	2	5
Suction catheter tip	2	0	2
Total	39	16	55

Sex wise distribution shows the prevalence of XDR strains is more in males isolated from catheterised urinary samples , which were mostly asymptomatic CAUTI , followed by ET TIP / secretions, Catheter urine samples showed high percentage of XDR Pseudomonas in both the sexes.

DISCUSSION

A total of 2549 samples studies out of which 280 samples were positive for *P.aeruginosa* the overall prevalence were 10.98%. A study by Vincent J *et al.*, found *P.aeruginosa* was present in 28.7% of samples from ICU in Europe [12]. Pseudomonas isolates and 18.2% of all clinical specimens by Gad GF *et al.*, in Egypt [13]. In the present study the most common samples from which *P.aeruginosa* was isolated were from Pus 25.6%, Urine 22.4% ET secretions 16.8%, and Sputum 13.6%. These findings were similar to other studies done in India [14, 15]. A study by A Samad *et al.*, found 20.05% of prevalence of *P.aeruginosa* in sputum cultures [16]. We in the present study found 13.6% positive for *P.aeruginosa* for sputum cultures. While another study done in Nepal in 2013 by Chander *et al.*, showed 24.10% [17]. In Iran by Anvari *et al.*, in 2014 the isolation rate of Pseudomonas from sputum was 25% [18]. Age wise distribution in this study showed in males between 51-60 yrs have high numbers, isolated from catheter samples followed by ET secretions, followed by 61-70 yrs, with advancing age and more severe underlying illness, the emergence of specialized units for the care of critically ill patients, the increased use of multiple invasive devices, and above all the indwelling catheters provide an environment for such nosocomial infections. In the study we found XDR was found in 22% of cases out of which 15.6% were for male and 6.4% were female cases. The prevalence of XDR strains was more in males isolated from catheterized urinary samples, which were mostly asymptomatic, followed by ET-tip secretions. Catheter urine samples also showed a high percentage of XDR Pseudomonas in both the sexes. In this study we found the presence of MDR in 27 samples out of 250 cases the prevalence was 10.8%. Fauzia Khan *et al.,* reported 30% frequency of MDR *P.aeruginosa* while Gill *et al*., reported 22.7% incidence in Islamabad [16, 19]. Farhatullah *et al*., which reported 29% prevalence of MDR *P.aeruginosa* in burn patients [20]. Resistance of beta-lactam in nosocomial *P.aeruginosa* has become a serious threat particularly against third and fourth generation Cephalosporins, is of major concern. *P.aeruginosa* is notorious and can cause infections at almost all sites, most common being lungs, skin and soft tissues. Most of the infections are encountered in hospitalized patients who get colonized with the organisms either from heavily contaminated hospital environment or from hospital staff (through contaminated hands). *P.aeruginosa* has an ability to grow both under aerobic and anerobic conditions and possesses number of virulence factors that contributes to its pathogenesis [21]. The intrinsic resistance of *P.aerugniosa* is due to an outer membrane barrier and presence of multidrug efflux transporters and endogenous antimicrobial inactivation [22]. Overall antibiotic susceptibility pattern for pseudomonas in the present study Amikacin – 69.5%, Gentamycin–46.8%, Tobramycin-53.9%, Ceftazidime–51.1%, Cefepime–51.2%, Levofloxacin–49.2%, Ciprofloxacin–32.81%, Pip/Taz–57.42%, Imipenem–77.7 %, all these strains showed 100% susceptibility to Colistin & Polymyxin B.K Ahmadi *et al;* in Iran found highest levels of resistance against Ampicillin (93%), Gentamycin (89.5%), Ciprofloxacin (82.5%) and Amikacin (77.3%) [23]. Shahid R *et al*., in Nepal found there was no resistance against Ampicillin, Gentamycin, Norfloxacin

and ofloxacin. The prevalence of resistance against Ceftriaxone, Cephalexin, Ciprofloxacin and Cotrimoxazole were 50% and 100%, respectively [24]. A study by Viren AJ *et al*., in a tertiary care hospital in Gujurat found maximum *P.aeruginosa* infection in urine followed by pus and sputum. Marked reistance against monotherapy was seen in drugs like penicillin, cephalosporin, fluroquinolone, tetracycline and macrolides [25]. In the present study we found 100% sensitivity to Colistin and Polymyxin B.

CONCLUSION

High prevalence of *P.aeruginosa* as an opportunistic pathogen has been on the increase with resistance to antimicrobial agents and thus becoming a potential threat as the numbers of susceptible antibiotics are decreasing. This study underlines the importance of culture and sensitivity tests in patients before treatment in order to prevent emergence of drug resistant organisms.

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REFERENCES

1. Franco, B. E., Martínez, M. A., Rodríguez, M. A. S., & Wertheimer, A. I. (2009). The determinants of the antibiotic resistance process. *Infection and drug resistance*, *2*, 1.
2. Poole, K. (2011). Pseudomonas aeruginosa: resistance to the max. *Frontiers in microbiology*, *2*, 65.
3. Kerr, K. G., & Snelling, A. M. (2009). Pseudomonas aeruginosa: a formidable and ever-present adversary. *Journal of Hospital Infection*, *73*(4), 338-344.
4. Kenneth T. (2005). Online text book of bacteriology. http://textbookofbacteriology.net/ken_todar.html. Accessed 20 March 2018.
5. Zhao, W. H., & Hu, Z. Q. (2010). β-lactamases identified in clinical isolates of Pseudomonas aeruginosa. *Critical reviews in microbiology*, *36*(3), 245-258.
6. Poole, K. (2005). Aminoglycoside resistance in Pseudomonas aeruginosa. *Antimicrobial agents and Chemotherapy*, *49*(2), 479-487.
7. Gill, M. M., Usman, J., Kaleem, F., Hassan, A., Khalid, A., Anjum, R., & Fahim, Q. (2011). Frequency and antibiogram of multi-drug resistant Pseudomonas aeruginosa. *J Coll Physicians Surg Pak*, *21*(9), 531-534.
8. El Amari, E. B., Chamot, E., Auckenthaler, R., Pechere, J. C., & Van Delden, C. (2001). Influence of previous exposure to antibiotic therapy on the susceptibility pattern of Pseudomonas aeruginosa bacteremic isolates. *Clinical infectious diseases*, *33*(11), 1859-1864.
9. Paramythiotou, E., Lucet, J. C., Timsit, J. F., Vanjak, D., Paugam-Burtz, C., Trouillet, J. L., ... & Andremont, A. (2004). Acquisition of multidrug-resistant Pseudomonas aeruginosa in patients in intensive care units: role of antibiotics with antipseudomonal activity. *Clinical infectious diseases*, *38*(5), 670-677.
10. Lepper, P. M., Grusa, E., Reichl, H., Högel, J., & Trautmann, M. (2002). Consumption of imipenem correlates with β-lactam resistance in Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy*, *46*(9), 2920-2925.
11. Paterson, D. L. (2004). "Collateral damage" from cephalosporin or quinolone antibiotic therapy. *Clinical Infectious Diseases*, *38*(Supplement_4), S341-S345.
12. Vincent, J. L., Bihari, D. J., Suter, P. M., Bruining, H. A., White, J., Nicolas-Chanoin, M. H., ... & Hemmer, M. (1995). The prevalence of nosocomial infection in intensive care units in Europe: results of the European Prevalence of Infection in Intensive Care (EPIC) Study. *Jama*, *274*(8), 639-644.
13. Gad, G. F., El-Domany, R. A., Zaki, S., & Ashour, H. M. (2007). Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. *Journal of antimicrobial chemotherapy*, *60*(5), 1010-1017.
14. Mohanasoundaram, K. (2011). The antimicrobial resistance pattern in the clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital; 2008-2010 (A 3 year study). *Clinic Diagnost Res J*, *5*(3), 491-4.
15. Javiya, V. A., Ghatak, S. B., Patel, K. R., & Patel, J. A. (2008). Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*, *40*(5), 230.
16. Khan, F., Khan, A., & Kazmi, S. U. (2014). Prevalence and susceptibility pattern of multi drug resistant clinical isolates of Pseudomonas aeruginosa in Karachi. *Pakistan journal of medical sciences*, *30*(5), 951.
17. Anil, C., & Shahid, R. M. (2013). Antimicrobial susceptibility patterns of Pseudomonas aeruginosa clinical isolates at a tertiary care hospital in Kathmandu, Nepal. *Asian J Pharm Clin Res*, *6*(3), 235-238.
18. Anvari, M. S., Naderan, M., Boroumand, M. A., Shoar, S., Bakhshi, R., & Naderan, M. (2014). Microbiologic spectrum and antibiotic susceptibility pattern among patients with urinary and respiratory tract infection. *International journal of microbiology*, *2014*.
19. Gill, M. M., Usman, J., Kaleem, F., Hassan, A., Khalid, A., Anjum, R., & Fahim, Q. (2011).

Frequency and antibiogram of multi-drug resistant Pseudomonas aeruginosa. *J Coll Physicians Surg Pak*, *21*(9), 531-534.

20. Ullah, F., Malik, S. A., & Ahmed, J. (2009). Antimicrobial susceptibility and ESBL prevalence in Pseudomonas aeruginosa isolated from burn patients in the North West of Pakistan. *Burns*, *35*(7), 1020-1025.
21. Schurek, K. N., Breidenstein, E. B., & Hancock, R. E. (2012). Pseudomonas aeruginosa: a persistent pathogen in cystic fibrosis and hospital-associated infections. In *Antibiotic Discovery and Development* (pp. 679-715). Springer, Boston, MA.
22. Poole, K. (2011). Pseudomonas aeruginosa: resistance to the max. *Frontiers in microbiology*, *2*, 65.
23. Ahmadi, K., Hashemian, A. M., Pouryaghobi, S. M., Akhavan, R., Rozmina, S., & Bolvardi, E. (2016). Antibiotic Resistance Properties of Pseudomonas aeruginosa Isolated From Cases of Superficial Infections at the Emergency Unit. *Jundishapur journal of microbiology*, *9*(1).
24. Raza, M. S., Chander, A., & Ranabhat, A. (2013). Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. *Open Journal of Medical Microbiology*, *3*(3), 159.
25. Javiya, V. A., Ghatak, S. B., Patel, K. R., & Patel, J. A. (2008). Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*, *40*(5), 230.