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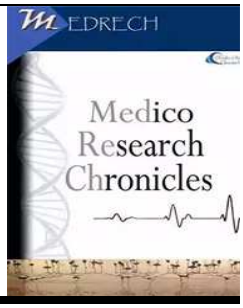

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES IN JASHORE MEDICAL COLLEGE HOSPITAL.

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Background: Pseudomonas aeruginosa is one of the leading causes of hospital-acquired infections. Increased resistance in this organism continues to pose a significant threat to patient care because of limited therapeutic options. The main objective of this study was to find out the prevalence and current antimicrobial susceptibility pattern of P. aeruginosa isolates obtained from various clinical samples at a tertiary care hospital.

Methods: The study was conducted in the Bacteriology laboratory of the Department of Microbiology, Jashore Medical College Hospital, Jashore, Bangladesh. All clinical samples received from various departments from January 2021 to December 2021. The colonies which were grown on culture media were identified by different standard biochemical tests. Antimicrobial susceptibility testing was done using Kirby–the Bauer disc diffusion method and the results were interpreted according to the CLSI guidelines. Quality control of the test was done by standards ATCC strain of P. aeruginosa 27853.

Result: A total of 167 cultured organisms were recorded and analyzed in this study. Among 167 cultured organisms, there were 37 isolates identified as P. aeruginosa. Among the antibiotic sensitivity patterns of P. aeruginosa, we found that the most sensitive drug was colistin 35 (94.59%), followed by levofloxacin 31(83.78%), cefuroxime axetil 29(78.38%), gentamicin 26(70.27%), and each cefperazone+sulbactum

& netilmicin has a percentage of 24(64.86%) On the other hand, we found P.aeruginosa showed resistance towards Ofloxacin 24(64.86%), Piperacillin 23(62.16%), Ceftazidime 21(56.76%), Cefoprazone 20(54.05%), Cefipime 20(54.05%), Aztreonam 19(51.35%), Cefaprazone + sulbactum 16(43.24%) and Gentamycin 17(45.00%).
Conclusion: Most of the P. aeruginosa strains were isolated from sputum, urine, respiratory secretions and pus samples and were found to be MDR. Piperacillin-tazobactam was the most sensitive chemotherapeutic agent followed by Colistin and levofloxacin.

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INTRODUCTION

The genus Pseudomonas, being one of the most complex bacterial genera, contains more than 140 species, among which most of them are saprophytic. Around 25 species are associated with humans to cause opportunistic infections. Some of the important clinically relevant species are P. aeruginosa, P. fluorescens, P. putida, P. stutzeri, P. mallei, P. pseudomallei, P. maltophilaand P. putrefaciens.[1, 2]Pseudomonas aeruginosa is the most commonly identified one from the clinical specimens of hospital-admitted patients.[3] Even though it is a commensal of microflora in healthy humans, it is a commonly encountered causative agent of infections seen in hospitalized patients, particularly in burns, respiratory diseases, and catheterized and immunocompromised patients. It is one of the commonest gram-negative bacteria that take advantage of an individual's weakened immune status to cause infection by its tissue-damaging toxins. Infections caused by Pseudomonas aeruginosa can infect any anatomical site; hence it can be isolated from various body fluids such as wounds, urine, blood, eye or ear swabs, and sputum. It is also a grave concern to cancer and burns patients. It can cause infections such as Urinary Tract Infections (UTI), respiratory infections, particularly Ventilator-Associated Pneumonia (VAP) in debilitated patients, bone and joint infections, dermatitis, otitis media, bacteremia and other numerous varieties of systemic infections. [4, 5] It is ubiquitous in nature. It can thrive and colonize anywhere, being widely distributed in the environment-soil, vegetation, water bodies, sewage, hospitals and even on the moist sites of the skin of healthy individuals. And its ability to resist antibacterial and antiseptic agents makes it more complex. This organism is hard to treat because of its acquired and intrinsic resistance. [1, 6] The defense mechanism of Pseudomonas aeruginosa makes it immune to many antibiotics by different means such as chromosomally encoded genes, restricted outer membrane permeability, production of antibiotic inactivating enzymes or the efflux system that pumps antibiotics out of the cell. [7, 8] Keeping in view the knowledge about the ability of Pseudomonas spp. to thrive in a myriad of habitats, its etiology as well as the pathology and its wide range of mechanisms of resistance to antibiotics**.** Independent risk factors have been identified for multi drug-resistant (MDR) or pan-resistant P. aeruginosa infection like prior to use of antibiotics, history of P. aeruginosa infection or colonization within the previous year, length of hospital stay, being admitted as in-patient or in the intensive care unit (ICU), mechanical ventilation, malignant disease and history of chronic obstructive pulmonary disease.[9,10,11] The antibiotic resistance mechanisms include the acquisition of extended-spectrum β-lactamases, carbapenemases, aminoglycoside modifying enzymes, and 16S ribosomal ribonucleic acid methylases. Mutational changes causing the up-regulation of multidrug efflux pumps, depression of ampC, modification of antimicrobial targets and

changes in the outer membrane permeability barrier are also described.[12] Development of antimicrobial resistance limits the therapeutic options that lead to high mortality and morbidity.[13] Emergence of antibiotic resistance in P. aeruginosa has been an increasing trend. There is a diversity of definitions to describe MDR isolates of P. aeruginosa. According to different studies, the term MDR P. aeruginosa has been described as resistance to at least three antibiotics from a variety of antibiotic classes, mainly Aminoglycosides, Penicillins, Carbapenems, Cephalosporins and Quinolones.[14] Hidron et al, considered MDR P. aeruginosa when resistant to only a single important anti -P. aeruginosa agent.[15] Current study followed the definition of MDR P. aeruginosa as stated by European Center for Disease Prevention and Control (ECDC) and Centre for Disease Control and Prevention (CDC), where MDR P. aeruginosa was defined as the one that has acquired non-susceptibility to atleast one agent in three or more categories of antimicrobials.[16] Therefore, knowledge of the current drug resistance pattern of the common pathogenic bacteria in a particular region is useful in clinical practice. Hence, the present study was conducted to find out the prevalence and the antimicrobial susceptibility pattern of P. aeruginosa isolates obtained from various clinical samples at the Microbiology department in Jashore medical college hospital.

METHODOLOGY & MATERIALS

The present study was conducted in the Bacteriology laboratory of the Department of Microbiology, Jashore Medical College Hospital, Jashore, Bangladesh. All clinical samples received from various departments from January 2021 to December 2021 were processed for isolation and identification of P. aeruginosa was made according to the Standard microbiological techniques. Blood agar, MacConkey agar and Nutrient agar were used as growth media for the culturing of samples [7]. The plates were then incubated at 37°C for 24 hours to get the growth and were then processed further for identification using standard procedures. P. aeruginosa was identified by -Gram staining, motility test and biochemical tests like the oxidase test, O/F test, and growth at 420 C[8]

Antibiotic sensitivity pattern of P. aeruginosa isolates to Ceftazidime (30mcg), Cefipime(30mcg), Piperacillin+Tazobactum (5mcg), Cefperazone+sulbactum (75/30mcg), Azetreonam (5mcg), Imipenem (10mcg), Meropenem (10mcg), Gentamicin (10mcg), Amikacin (30mcg), Netilmicin (5mcg), Colistin (10mcg), Ciprofloxacin (5mcg), Ofloxacin (30mcg), Levofloxacin(5mcg), Cefuroxime+Clavulanic (30 mcg), Amoxicillin+Clavulanic acid (30 mcg), Cefuroxime axetil (30 mcg) was investigated by Kirby-Bauer method on Mueller Hinton Agar (MHA). The final bacterium inoculation concentration was approx 108 cfu/ml which was equal to 0.5 McFarland. MHA plates were incubated overnight at 370, and the diameter of each inhibition zone was measured with a special scale supplied by Himedia Mumbai.8. Statistical analysis was done by descriptive statistics using simple ratios and percentages. Microsoft Office 2010 was used to generate Tables.

RESULT

A total of 167 cultured organisms were recorded and analyzed in this study. Among 167 cultured organisms, there were 37 isolates identified as P. aeruginosa. Out of 37 isolated P. aeruginosa, the most common specimens with positive growth were wound swab 3(42.86%), sputum 17(28.81%), urine 7(17.95%), respiratory secretions 6(23.08%), pus 3(11.11%), blood 1(11.11%) as shown in Table 1. Among 37 P. aeruginosa, 23(62.39%) cases were isolated from male patients, and 14(37.61%) were from female patients, as shown in Figure 1. The highest number of P. aeruginosa was recovered from patients who were more than 40 up to 60 years of age

15(40.54%), followed by those who were more than 60 years of age 9(24.32%), as shown in Table 2. Figure 2 shows that most isolates (81.20%) were recovered from patients attending inpatient departments than from outpatient departments (18.80%). Table-3 shows antibiotic sensitivity patterns of P. aeruginosa. We found that the most sensitive drug was Colistin 35(94.59%), followed by Levofloxacin 31 (83.78%), Amoxicillin+Clavulanic acid 30 (81.08%), Cefuroxime axetil 29 (78.38%), Cefuroxime+Clavulanic 28 (75.68%), Imipenem 23 (62.16%), Ciprofloxacin 22(59.46%), Amikacin 21 (56.76%), Cefperazone+sulbactum 21 (56.76%), Azetreonam 20(54.05%), Netilmicin 19 (51.35%), Gentamicin 18 (48.65%), Meropenem 17 (45.95%), Cefipime 17 (45.95%), Ceftazidime 19 (43.24%), Piperacillin+Tazobactum 13 (35.14%) and Ofloxacin 13(35.14%). On the other hand, we found P.aeruginosa showed resistance towards Ofloxacin 24 (64.86%), Piperacillin+Tazobactum 24 (64.86%), Ceftazidime 21 (56.76%), Meropenem 20(54.05%), Cefipime 20 (54.05%), Gentamicin 19 (51.35%), Netilmicin 18 (48.65%), Azetreonam 17 (45.95%), Amikacin 16(43.24%), Cefperazone+sulbactum 16(43.24%), Ciprofloxacin 15 (40.54%), Imipenem 14 (37.84%), Cefuroxime+Clavulanic acid 9 (24.32%), Cefuroxime axetil 8 (21.62%), Amoxicillin+Clavulanic acid 7 (18.32%), Levofloxacin 6 (16.22%), Colistin 2 (5.41%). Table 4 shows the distribution of MDR P.aeruginosa isolates among clinical specimens showed out of 37 sample 18(48.65%) were MDR P.aeruginosa where in relation to individual source, wound swab 2(66.67%), sputum 9(52.94%), urine 2(28.00%), respiratory secretions 4(99.67%), pus 1(50.00%) were MDR strains but there were no MDR P.aeruginosa isolates from blood samples.

Table-1: Distribution of total isolates of P. aeruginosa strains from different samples (N=37)

Clinical samples	Total No of Sample	Total isolates of P. aeruginosa strains	Percentage (%)
Sputum	59	17	28.81
Urine	39	7	17.95
Pus	27	3	11.11
Wound swab	7	3	42.86
Respiratory secretions (E.T. tip/ bronchial wash etc.)	26	6	23.08
Blood	9	1	11.11
Total	**167**	**37**	22.16

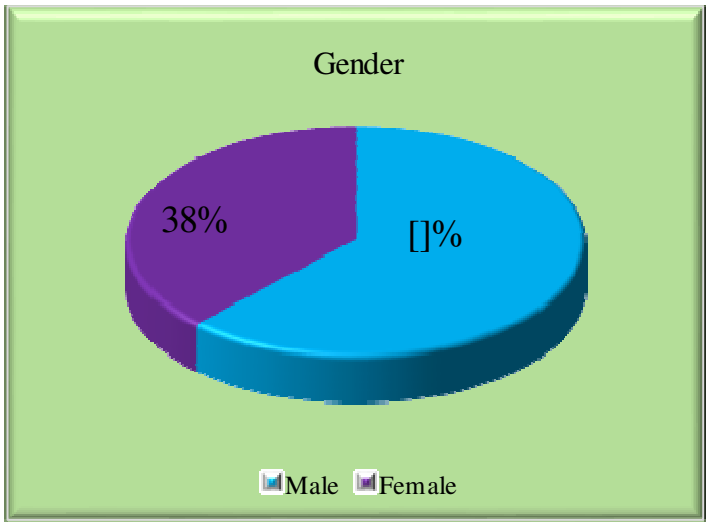

Figure-1: Gender distribution of P. aeruginosa strains(N=37).

Table-2: Age-wise distribution of isolates of P. aeruginosa (N=37)

Age in years	Total samples	Percentage (%)
0-15	2	5.41
16-30	4	10.81
31-45	7	18.92
46-60	15	40.54
>60	9	24.32
Total	**37**	**100.00**

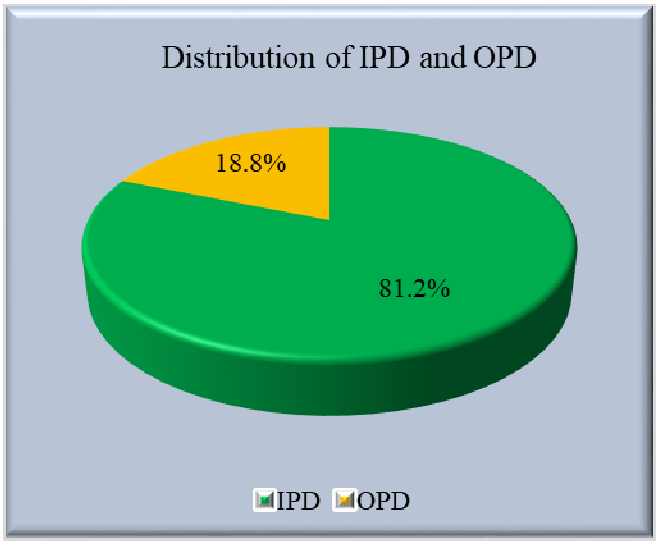

Figure-2: Distribution of P. aeruginosa strains among outpatient department (OPD) and inpatient department (IPD) (N=37)

Table-3: Antimicrobial susceptibility of P. aeruginosa isolates (n = 117) to various antibiotics(N=37).

S. No	Name of antibiotics	Sensitivity number of samples	Sensitivity %	Resistant number of samples	Resistance %
1	Ceftazidime	16	43.24	21	56.76
2	Cefipime	17	45.95	20	54.05
3	Piperacillin+ Tazobactum	13	35.14	24	64.86
4	Cefperazone+ sulbactum	21	56.76	16	43.24
5	Azetreonam	20	54.05	17	45.95
6	Imipenem	23	62.16	14	37.84
7	Meropenem	17	45.95	20	54.05
8	Gentamicin	18	48.65	19	51.35
9	Amikacin	21	56.76	16	43.24
10	Netilmicin	19	51.35	18	48.65
11	Colistin	35	94.59	2	5.41
12	Ciprofloxacin	22	59.46	15	40.54
13	Ofloxacin	13	35.14	24	64.86
14	Levofloxacin	31	83.78	6	16.22
15	Cefuroxime+ Clavulanic acid	28	75.68	9	24.32
16	Amoxicillin+ Clavulanic acid	30	81.08	7	18.92
17	Cefuroxime axetil	29	78.38	8	21.62

Table-4: Distribution of MDR *P. aeruginosa* isolates among clinical specimens (N=37)

S. No.	Clinical samples	Total number of samples	Number of MDR strains	Percentage (%) of MDR isolation
1	Sputum	17	9	52.94
2	Urine	7	2	25.00
3	Pus	3	1	50.00
4	Wound swab	3	2	66.67
5	Respiratory secretions	6	4	66.67
6	Blood	1	0	0.00
Total		**37**	**18**	48.65

DISCUSSION

P. aeruginosa presents a serious therapeutic challenge for the treatment of both communities acquired and nosocomial infections. Infections caused by P. aeruginosa are notoriously difficult to treat due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance. Our study measures the rate of isolation of P. aeruginosa (22.15%) which is quite similar to previous studies by Tadvi et al.[17] (22.67%), Viren et al[18] (26.79%), and

Ruhil et al[19] (27.70%). The occurrence of P. aeruginosa is found to be higher in males, in age group >60 & 46-60 years and in inpatients & surgery department, which is same as reported by Viren et. Al[18], Ali Hussein et al[20], Shampa et al[21] and Rakesh et al.[22] Most of isolates showed that the most sensitive drug was colistin 35(94.59%), followed by Levofloxacin 31 (83.78%), Amoxicillin+Clavulanic acid 30 (81.08%), Cefuroxime axetil 29 (78.38%), Cefuroxime+Clavulanic 28 (75.68%), Imipenem 23(62.16%), Ciprofloxacin 22(59.46%), Amikacin 21 (56.76%), Cefperazone+sulbactum 21 (56.76%), Azetreonam 20 (54.05%), Netilmicin 19(51.35%), Gentamicin 18 (48.65%), Meropenem 17 (45.95%), Cefipime 17(45.95%), Ceftazidime 19 (43.24%), Piperacillin+Tazobactum 13 (35.14%), Ofloxacin 13 (35.14%), Sensitivity pattern of P. aeruginosa nearly coincides with that of Viren et al., Tadvi et al.[17] , Ruhil et al.[18], and Aggarwal et al.[22] P. aeruginosa showed resistance towards Ofloxacin 24 (64.86%), Piperacillin+Tazobactum 24 (64.86%), Ceftazidime 21 (56.76%), Meropenem 20 (54.05%), Cefipime 20 (54.05%), Gentamicin 19 (51.35%), Netilmicin 18 (48.65%), Azetreonam 17 (45.95%), Amikacin 16 (43.24%), Cefperazone+sulbactum 16 (43.24%), Ciprofloxacin 15 (40.54%), Imipenem 14 (37.84%), Cefuroxime+Clavulanic acid 9 (24.32%), Cefuroxime axetil 8 (21.62%), Amoxicillin+Clavulanic acid 7 (18.32%), Levofloxacin 6(16.22%), Colistin 2 (5.41%), which was comparable with previous studies done in India as by Arora et al.[24], Jamshaid et al[23] and Bhatt et al.[25] In present study prevalence of MDR P.aeruginosa was 24.15%,which is very much close to the study by Chander et al[26] (20.69%) and Shampa et al.[21] (18.00%).

LIMITATIONS OF THE STUDY:

The study was conducted in a single hospital with small sample size. So, the results may not represent the whole community**.**

CONCLUSION AND RECOMMENDATIONS

Inevitably, the antibiotic susceptibility pattern of bacterial pathogens like P. aeruginosa in specialized clinical units should be continuously monitored to minimize the resistance to the use of routine antibiotics. Judicious usage of antibiotics and creating a standard antibiotic policy that supports the clinician to choose the appropriate drug of choice helps in preventing drug resistance, whereas proper infection control measures and timely identification of resistant pathogens can help in reducing the spread of multidrug-resistant strains. An increase in antibacterial resistance in P. aeruginosa is a cause of concern. Regular antimicrobial susceptibility monitoring is essential for local, regional, and national level isolates. This would help prescribe the right combination of chemotherapeutic agents and prevent the emergence of MDR strains of P. aeruginosa.

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

REFERENCES

1. Palleroni NJ. Introduction to the family Pseudomonadaceae. In The prokaryotes 1981 (pp. 655-665). Springer, Berlin, Heidelberg.
2. Mohanty S, Baliyarsingh B, Nayak SK. Antimicrobial Resistance in Pseudomonas aeruginosa: A concise review. Antimicrobial Resistance-A One Health Perspective. 2020 Feb 6.
3. Rahman MA, Nair PO. Prevalence And Antibiotic Susceptibility Pattern Of Pseudomonas Species Isolated From Clinical Samples In A Tertiarycare hospital. International Journal of Current Pharmaceutical Research. 2021; 13(1):50-3.

4. Bekele T, Tesfaye A, Sewunet T, Waktola HD. Pseudomonas aeruginosa isolates and their antimicrobial susceptibility pattern among catheterized patients at Jimma University Teaching Hospital, Jimma, Ethiopia. BMC research notes. 2015 Dec; 8(1):1-4.
5. Deshmukh KA, Manthale D. Prevalence and antibiotic susceptibility of Pseudomonas aeruginosa isolated from chronic suppurative otitis media. International Journal of Otorhinolaryngology and Head and Neck Surgery. 2017 Jan; 3(1):56-60.
6. Yayan J, Ghebremedhin B, Rasche K. Antibiotic resistance of Pseudomonas aeruginosa in pneumonia at a single university hospital center in Germany over a 10-year period. Plos one. 2015 Oct 2; 10(10):e0139836.
7. Alnour TM, Ahmed-Abakur EH. Multidrug resistant Pseudomonas (P) aeruginosa: medical impact, pathogenicity, resistance mechanisms and epidemiology. JSM Microbiol. 2017; 5(3):1046.
8. Juayang AC, Lim JP, Bonifacio AF, Lambot AV, Millan SM, Sevilla VZ, Sy JK, Villanueva PJ, Grajales CP, Gallega CT. Five-year antimicrobial susceptibility of Pseudomonas aeruginosa from a local tertiary hospital in Bacolod City, Philippines. Tropical medicine and infectious disease. 2017 Jul 12; 2(3):28.
9. Ohmagari N, Hanna H, Graviss L, Hackett B, Perego C, Gonzalez V, et al. Risk factors for infections with multidrug-resistant Pseudomonas aeruginosa in patients with cancer. Cancer 2005; 104:205-12.
10. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, CarmeMultidrug-resistantistant Pseudomonas aeruginosa: Risk factors and clinical impact. Antimicrobial Agents Chemotherapy 2006; 50:43-8.
11. Arruda EA, Marinho IS, Boulos M, Sinto SI, Caiaffa HH, Mendes CM, et al. Nosocomial infections caused by multiresistant Pseudomonas aeruginosa. Infect Control Hosp Epidemiology 1999; 20:620-3.
12. Dash M, Padhi S, Narasimham MV, Pattnaik S. Antimicrobial resistance pattern of Pseudomonas aeruginosa isolated from various clinical samples in a tertiary care hospital, South Odisha, India. Saudi J Health Sci 2014; 3:15-9
13. Zavascki AP, Carvalhaes GC, Picão RC, Gales AC. Multidrugresistant Pseudomonas aeruginosa and Acinetobacter baumannii: resistance mechanisms and implications for therapy. Expert Rev Anti-Infect Ther. 2010;8(1):71-93. doi: 10.1586/eri.09.108
14. Falagas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multi-drug resistant (MDR) and pandrug-resistant (PDR) Acinetobacter baumannii and Pseudomonas aeruginosa. J Med Microbiol. 2006;55:1619-1629. doi 10.1099/jmm.0.46747-0
15. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol. 2008;29:996–1011. doi: 10.1086/591861.
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert

proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281. doi: 10.1111/j.1469-0691.2011.03570.x
17. Tadvi J, Javadekar TB, Bhavsar R, Garala N. Prevalence & antibiogram of Pseudomonas aeruginosa at SSG hospital, Baroda, Gujarat, India. J Res Med Den Sci. 2015 Jan; 3(3):204-7.
18. Javiya VA, Ghatak SB, Patel KR, Patel JA. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian journal of pharmacology. 2008 Oct; 40(5):230.
19. Kiran R, Bharti A, Himanshu A. Pseudomonas aeruginosa isolation of post-operative wound in a referral hospital in Haryana, India. Journal of Infectious Diseases and Antimicrobial Agents. 2009; 26(2):43-8.
20. Singh B, Purohit M, Mutha A. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital of central India.
21. Shampa A, Bhattacharjee A, Garg A, Sen MR. Antimicrobial susceptibility of P. aeruginosa isolated from wound infection. Ind J Dermatol. 2006; 51(4):286-8.
22. Ninama GL, Mistry K, Parmar R, Patel K, Vegad MM. Antibiotic resistance pattern in Pseudomonas aeruginosa species isolated at a tertiary care hospital, Ahmadabad. National journal of medical research. 2012 Jun 30; 2(02):156-9.
23. Agarwal G, Kapil A, Kabra SK, Das BK, Dwivedi SN. Characterization of Pseudomonas aeruginosa isolated from chronically infected children with cystic fibrosis in India. BMC microbiology. 2005 Dec; 5(1):1-1.
24. Arora D, Jindal N, Kumar R, Romit M. Emerging antibiotic resistance in Pseudomonas-A challenge. Int J Pharm Pharm Sci. 2011; 3(2):82-4.
25. Bhatt P, Rathi KR, Hazra S, Sharma A, Shete V. Prevalence of multidrug resistant Pseudomonas aeruginosa infection in burn patients at a tertiary care centre. Indian Journal of Burns. 2015 Jan 1; 23(1):56.
26. Singh B, Purohit M, Mutha A. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital of central India.