ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 7, Issue – 2, April – 2022 , Page No. : 306 - 312

Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in a tertiary care centre in South Tamilnadu

[1]Dr. Bharathi Krishnasamy, Assistant Professor, Institute of Microbiology, Madurai Medical College, Madurai

[2]Dr. Mangala Adisesh, Director (Retd., Institute of Microbiology, Madurai Medical College, Madurai

[3]Dr. Susitha T, Associate Professor, Nagapattinam Govt. Medical College, Paliyur

[4]Dr. M.R. Vasanthapriyan, Assistant Professor, Institute of Microbiology, Madurai Medical College, Madurai

Corresponding Author: Dr. Bharathi Krishnasamy, Assistant Professor, Institute of Microbiology, Madurai Medical College, Madurai.

Citation this Article: Dr. Bharathi Krishnasamy, Mangala Adisesh, Dr. Susitha T, Dr. M.R. Vasanthapriyan, "Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in a tertiary care centre in South Tamilnadu", IJMSIR- April - 2022, Vol – 7, Issue - 2, P. No. 306 – 312.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Objective: To evaluate and determine the level of resistance to the routinely used antibiotics for psedumonas aeruginosa from the clinicalisolates.

Methods: The microbiology laboratory statistical data of all clinical isolates of P. aeruginosa at the Institute of Microbiology, Madurai Medical College and Government Rajaji Hospital, Madurai, Tamilnadu from August 2018 to January 2019 was reviewed. The antimicrobial susceptibility patterns were analysed by a standardized method.

Results: One Thousand Four Hundred and Fifty isolates of P. aeruginosa were tested in this current study. These strains were commonly isolated from patients admitted in surgery wards followed by intensive care units. Among the samples respiratory tract was the most common source of infection. The antibiotic susceptibility rates were as follows: ciprofloxacin 92.2%, meropenem 91.6%, imipenem 90.2%, amikacin 85.8%, ceftazidime 81.8%piperacillin/tazobactam 81.3% and gentamicin 77.7%. Among 1450 strains 6.4% were designated as being multidrug resistant. These were commonly isolated from respiratory tract specimens of patients in intensive care units.

Conclusion: The clinical significance of these findings is important in the selection of appropriate and effective treatment for P. aeruginosa infections. This study also emphasizes the importance of a conservative approach to antibiotic therapy and continued antimicrobial susceptibility testing surveillance programs to curtail the problem of antibiotic resistance. Based on results institutional antibiotic policy also framed.

Pseudomonas aeruginosa is a leading cause of nosocomial infections, ranking second among the gram-negative pathogens reported by National Nosocomial Infectious Surveillance (NNIS) system. Pseudomonas aeruginosa was the third most common pathogen among bloodstream isolates. In the European Prevalence of

Infection in intensive care (EPIC) study, P. aeruginosa was the predominant gram-negative species isolated from bronchopulmonary infection. Pseudo monas aeruginosa commonly causes bronchopulmonary infections and less frequently urinary tract infections, infections of surgical wounds and bacteremia.4 Particularly at the risk are extreme age groups, premature babies, ventilated patients, those with severe burns or wound injuries.3,5

Infections caused by P. aeruginosa are frequently life threatening and often difficult to treat due to its intrinsic resistance to a large number of routinely used antimicrobial drugs.8-11Resistance to antipseudomonal antibiotics is an increasing threat, and emergence of antibiotic resistance during therapy occurs with high frequency leads to therapeutic challenges.11,12 The trend of changing and easy acquisition of resistance in P. aeruginosa requires rapid and effective surveillance procedures to identify and represent the whole reality of the current situation.13

Table 1: Types of patients with Pseudomonas aeruginosa infection

Location	n	(%)
Surgical wards	448	30.8
Intensive care units	430	29.6
General medical wards	392	27.03
Outpatient	75	5.17
Burn	48	3.31
Renal transplant	13	0.89
Oncology	33	2.27
Hematologic malignancies	11	0.75

Table 2: Sources of Pseudomonas aeruginosa infection.

Sources	n	(%)
Respiratory specimens (N=440)		
Sputum	359	24.7
Bronchoalveolar lavage	81	5.58
Wound (N=410)		
Surgical site infection	342	23.58
Others (chronic wounds and so forth)	68	4.68
Urine specimens (N=265)		
Catheter urine specimens	217	14.90
Midstream urine specimens	48	3.31
Surveillance swabs	140	9.65
Blood culture	130	8.96
Intravascular catheter tips	43	2.96
Sterile body fluids	22	1.51

Methods

A retrospective study was conducted at Institute of Microbiology, Madurai Medical College & Government Rajaji Hospital, Madurai, Tamilnadu. The Microbiology laboratory statistical data was used to identify all clinical cultures from patients that were positive for P. aeruginosa during a 6 month period between August 2018 to January 2019 without duplicationof strains from the same patient and sample. Pseudomonas aeruginosa was identified according to a test panel consisting of Gram stain, colony morphology in solid media, Pigment production, Oxidase reaction and ability of the Pathogen grown at $42^{O}C$. The promyogenic P aeruginosa strains and those isolated from sterile sites were confirmed by standard culture methods and Bio chemical properties as per CLSI standards . Anti-Microbial susceptibility testing was performed by disc diffusion method (Kirby bauer method) as described by CLSI Clinical Laboratory Standards Institute (CLSI).[14] Briefly all inocula were prepared from a pure agar plate culture, with isolates that were 18-24 hour old. Organisms were prepared in 0.9% saline and adjusted to match 0.5 McFarland standard with a spectro photometer. All organisms were

tested for Anti-microbial susceptibility pattern on Muller-Hinton Agar (HI media Laboratories). The antibiotics discs (obtained from Hi media) were used: Ceftazidime(30ug),Piperacillin/Tazobactam (30ug/10ug), Imipenem (10ug), Meropenem (10ug),gentamicin (10ug), netilmicin (10ug), amikacin (30ug), ciprofloxacin (5ug), aztreonam (30ug), and polymyxin B (300ug).

Interpretation of zone diameter was based upon CLSI guidelines.[15] Escherichia coli ATCC 25922 and P. aeruginosa ATCC 27853 were included as quality control strains following the protocol as described by CLSI guidelines.

Table 3: In vitro susceptibility of 1450 isolates of Pseudomonas aeruginosa to commonly used antimicrobial agents (expressed as percentage)

Antimicrobial agents	Resistant	
	n	(%)
Amikacin	202	13.93
Aztreonam	353	24.34
Ceftazidime	251	17.31
Ciprofloxacin	112	7.72
Colistin	0	0
Gentamicin	319	22
Imipenem	136	4.37
Meropenem	121	8.34
Netilmicin	234	16.13
Piperacillin/tazobactam	271	18.68

Results

A total of 1450 P. aeruginosa isolates were tested. Eight Seventy four patients were males (60.27%) and Five Seventy Six were females (39.72%). The age range was 14 -85 years. Majorities of P. aeruginosa strains were isolated from surgicalwards followed by ICUs (Table 1). The most common source of specimens was the respiratory tract (Table 2). The majority of respiratory specimens were from patients in ICUs followed by medical patients with chronic obstructive airway disease. The most common specimens from the surgical wards were wound specimens from surgical site infections followed by catheter urine specimens (CSU) and sputum. Respiratory specimens mostly Sputum and Broncho Alveolar Lavage were the most common specimens from ICUs followed by wound specimens from surgical site infections, blood cultures and CSU. The most common specimens from other patients were as follows, sputum from outpatients, wound specimens from burn and renal transplant, and blood culture form oncology or haematology patients. Data on the in vitro susceptibility of P. aeruginosa isolates to antimicrobial drugs are presented in Table 3. All isolates were susceptible to polymyxin B. The susceptibility rate for ciprofloxacin was 92.2% followed by meropenem 91.6%, imipenem 90.2%, amikacin 85.8%, ceftazidime 81.8%, piperacillin/tazobactam 81.3%, and gentamicin 77.7%. Table 4 summarizes the cross resistance of P. aeruginosa isolates to antimicrobial agents. Of the carbapenem-resistant isolates 136 (9.37%) were resistant to imipenem and 121 (8.34%) to meropenem. Cross resistance between the 2 carbapenems was observed in 76 (5.24%) of the isolates, 39 (28.67%) of imipenem-resistant isolates were susceptible to meropenem and the reverse was observed in 19 (15.70%). These carbapenem-resistant P. aeruginosa strains were commonly isolated from respiratory specimens from ICUs. These isolates were frequently cross resistant to ceftazidime, piperacillin/tazobactam, and gentamicin and to a lesser extent ciprofloxacin. Approximately 21.9% of the ceftazidime resistant isolates were susceptible to piperacillin/tazobactam and 70% were susceptible to carbapenems and ciprofloxacin. Amikacin

resistant isolates (10%) were susceptible to gentamicin. Approximately one half of gentamicin resistant isolates was susceptible to amikacin. More than one half of ciprofloxacin resistant isolates was susceptible to carba penems and piperacillin/ tazobactam. Among the piperacillin/ tazobactam resistant isolates cross resistant with the 2 carbapenems was observed in approximately 28% of isolates. Forty-five (6.4%) of isolates were multiresistant. Nineteen (42.2%) were resistant to both ciprofloxacin and aminoglycosides with variable beta-lactam susceptibility. Twelve (26.7%) were resistant to both beta-lactam and aminoglycoside. Two (2.2%) were resistant to beta-lactams and ciprofloxacin.[12] Of these strains 26.7% were demonstrated in vitro activity to polymyxin B only. These multiresistant strains were commonly isolated from respiratory specimens from ICUs followed by general wards.

Table 4: Cross-resistance of Pseudomonas aeruginosa isolates.

Antimicrobial agents	Percentage of strain resistant to:										
	N of strains	AK	AZT	CAZ	CIP	COL	GN	IMP	MER	NET	P/T
Amikacin	202	-	68	58	38	0	90	34	36	87	57
Aztreonam	353	38	-	59	22	0	49	27	26	41	59
Ceftazidime	251	43	86	-	25	0	54	29	27	42	78
Ciprofloxacin	112	67	69	60	-	0	75	33	35	64	55
Colistin	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	319	55	56	44	25	0	0	29	29	62	61
Imipenem	136	52	68	57	30	0	65	0	74	55	52
Meropenem	121	41	75	63	31	0	78	86	0	63	59
Netilmicin	234	96	63	52	32	0	88	31	33	0	54
Piperacillin/tazobactam	271	54	83	80	23	0	59	28	27	48	0

AK - amikacin, AZT - aztreonam, CAZ - ceftazidime, CIP - ciprofloxacin, COL - colistin, GN - gentamicin, IMP - imipenem, MER - meropenem, NET - netilmicin, P/T - piperacillin/tazobactam

Discussion

The data of the present study showed that the isolates from ICUs were more resistant than those from the outpatient and other none ICU settings as shown by other studies.[3,13,16,17] Although ciprofloxacin is in particular jeopardy in Europe, USA and Latin America where the rates of susceptibility are between 60-75%, this agent is associated with the highest susceptibility rate (92%) after polymyxin B in our institution.[3,18,19] This can be attributed to the implementation of antibiotic policy, which restricts its use to special cases. Carbapenems were the most potent among beta-lactam antibiotics but resistance to these agents is a emerging problem.[21,22]. In our study, the resistance rate to meropenem (8.4%) are less than those reported by Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study (1999-2000) in Middle East and Asia (10%). Also our imipenem resistance rate (9.8%) is much lower than was reported by MYSTIC study (42%), which can be explained by low selective pressure.[23] Cross resistance

between the 2 carbapenems exists.[24-26] It isimportant to note that a higher proportion of imipenem-resistant isolate were susceptible to meropenem, which may be due to its superior intrinsic antipseudomonal activity.[21] Carbapenem resistance does not necessarily mean blanket resistant to all available drugs. However, it appears that it is associated with higher resistance rate to ceftazidime, piperacillin/tazobactam and gentamicin but to a lesser extent to ciprofloxacin. The resistance to piperacillin/tazobactam among P. aeruginosa strains is an emerging problem and piperacillin/tazobactam exposure was a strong risk factor.[25,26] Our results showed a resistance rate of 18%, which is higher than those reported from USA and Middle East by MYSTIC study which may be due to its frequent usage in our ICUs and certain clinical settings according to hospital antibiotic policy.[17,19] Ceftazidime still retain a good activity despite its use for long periods.[27,28] The greater potency of ceftazidime has been reported previously by Fluit et al.[29] Aztreonam was the least active among beta-lactams despite its uncommon use. Amikacin was the most potent drug tested among aminoglycosides whereas gentamicin was the least active. Gentamicin resistance rate (22%) is much lower than those reported by MYSTIC study in Europe (46%).

Multidrug resistant (MDR) P. aeruginosa is increasingly being isolated and against some isolates; the only therapeutic option is polymyxin B.[29,30,31] Six percent of our P. aeruginosa were MDR and the majority was isolated from non-cystic fibrosis patients, which is alarming.[29,32,33] A high proportion of our MDR isolates were resistant to both ciprofloxacin and aminoglycoside with variable susceptibility to other beta-lactams, which reflect the multifactorial nature of beta-lactamresistance in this organism.[34]

The risk of emergence of antibiotic resistance in P. aeruginosa may vary with different antibiotic treatments. Judious use of antibiotics, infection control measures and periodic surveillance studies provide a useful mean in controlling this serious problem. Framing institutional antibiotic policy will help to preserve some drugs with high susceptible range for future.

Acknowledgment: We are grateful to all Microbiology Staff who made this work possible.

References

1. Centre for Disease Control and Prevention. National Nosocomial Infectious Surveillance (NNIS) system report: data summary from October 1986 to April 1998, issued June 1998. Am J Infect Control 1998; 26: 522-533.

2. Diekma DJ, Pfaller MA, Jones RN, Doern GV, Win Okur PL, Gales AC et al. Survey of blood-stream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada and Latin America for the SENTRY Antimicrobial Surveillance Program. Clin Infect Dis 1999; 29: 595-607.

3. Vincent JL, Bihari DL, Suter PM, Bruining HA, White J, Nicolas-chanion M et al. The prevalence of nosocomial infection in intensive care units in Europe: results of the European Prevalence of Infection in Intensive Care (EPIC) study. JAMA 1995; 74; 639-644.

4. Pollack M. Pseudomonas aeruginosa. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice ofinfectious diseases. Vol 2. Philadelphia (PA): Churchill Livingstone; 2000. p. 2310-2335.

5. Grundman H, Kropec A, Hartung D, Berner R, Daschner F. Pseudomonas aeruginosa in a neonatal

intensive care unit: reservoirs and ecology of nosocomial pathogen. J Infect Dis 1993; 168: 943-947.

6. Moss RB. Cystic Fibrosis: pathogenesis, pulmonary infection and treatment. Clin Infect Dis 1995; 21: 839-845.

7. Rolston KV, Tarrand JJ. Pseudomonas aeruginosa - still a frequent pathogen in patients with cancer: 11-year experience at a comprehensive cancer centre. Clin Infect Dis 1999; 29: 463-464.

8. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa and other non-fermentative gram-negative bacteria. Clin Infect Dis 1998; 27 (Suppl 1): S93-S99.

9. Livermore MD. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 2002; 34: 634-640.

10. Giamarellou H. Prescribing guidelines for severe Pseudomonas infections. J Antimicrob Che mother 2002; 49: 229-233.

11. Carmeli Y, Troillet N, Karchmer AW, Sam ore MH. Health and economic outcomes of antibiotic resistance in Pseudomonas aeruginosa. Arch Intern Med 1999; 159: 1127-1132.

12. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother 1999; 43: 1379-1382.

13. Bouza E, Garcia-Garrote F, Cercenado E, Marin M, Diaz MS. Pseudomonas aeruginosa: a survey of resistance in

136 hospitals in Spain. Antimicrob Agents Che mother 1999; 43: 81-82.

14. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk susceptibility testing. Document M2-A7. Wayne (PA): NCCLS; 2000.

15. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial susceptibility testing. Document M100-S11. Wayne (PA): NCCLS; 2001.

16. Saiman L, Schidlow D, Smith A, editors. Concepts in care: microbiology and infectious disease in cystic fibrosis. Vol V. Bethesda (MD): Cystic Fibrosis Foundation; 1994.

17. Archibald L, Phillips L, Monnet D, McGowan JE Jr, Tenover F, Gaynes R. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis 1997; 24: 211-215.

18. Intensive Care Antimicrobial Resistance Epidemiology (ICARE). Surveillance report data summary from January 1996 through December 1997. A report from the National Nosocomial Infections Surveillance (NNIS) system. Am J

Infect Control 1999; 27: 279-284.

19. Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of Pseudomonas aeruginosa isolates: occurrence rates antimicrobial susceptibility patterns, and molecular typing in the Global SENTRY antimicrobial surveillance program 1997-1999. Clin Infect Dis 2001; 32 (Suppl 2): S146-S155.

20. Turner PJ. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection): a global overview. J Antimicrob Che mother 2000; 46: 4-23.

21. Naka T, Nakajima A, Ono T, Saito K, Yoneyama H. Resistance to beta-lactam antibiotics in Pseudomonas aeruginosa due to interplay between the Mex AB-Oprm efflux pump and beta-lactamase. Antimicrob Agents

Che mother 1999; 43:1301-1303.

22. Livermore DM. Of pseudomonas, porins, pumps and carbapenems. J Antimicrob Che mother 2001; 47: 247-250.

23. Pai H, Kim JW, Kim J, Lee JH, Choc Kw, Gotoh N. Carbapenem resistance mechanisms in Pseudomonas aeruginosa clinical isolates. Antimicrob Agent Che mother 2001; 45: 480-484.

24. Troillet N, Samore MH, Carmeli Y. Imipenem-resistant Pseudomonas aeruginosa: risk factors and antibiotic susceptibility patterns. Clin Infect Dis 1997; 25:1094-1098.

25. Iaconis J, Pitkin DH, Sheikh W, Nadler HL. Comparison of antibacterial activities of meropenem and six other antimicrobials against Pseudomonas aeruginosa isolates from North American studies and clinical trials. Clin Infect

Dis 1997; 24 (Suppl 2): 191-196.

26. Harris AD, Perencevich E, Rough Mann MC, Morris G, Kaye KS, Johnson JA. Risk factors for piperacillin/tazobactam resistant Pseudomonas aeurginosa among hospitalized patients. Antimicrob Agents Che mother 2002; 46: 854-858.

27. Mokaddas EM, Sanyal SC. Resistance patterns of Pseudomonas aeruginosa to carbapenems and piperacillin/tazobactam. J Che mother 1999; 11: 97-102.

28. Lee SC, Fung CP, Liu Pr, Wang TC, See L, Lee N et al. Nosocomial infections with ceftazidime resistant Pseudomonas aeruginosa risk factors and outcome. Infect Control Hosp Epidemiol 1999; 20: 205-207.

29. Fluit AC, Verhoef J, Schmitz FJ, The European SENTRY Participants. Antimicrobial resistance in European isolates of Pseudomonas aeruginosa. Eur J Clin Microbial Infect Dis 2000; 19: 370-374.

30. Harris A, Torres-Viera C, Venkataraman L, Degirolami P, Samose M, Carmeli Y. Epidemiology and clinical outcomes of patients with multi-resistant Pseudomonas aeruginosa. Clin Infect Dis 1999; 28: 1128-1133.

31. Saiman L, Mehar F, Niu WW, Neu HC, Shaw KJ, Miller G et al. Antibiotic susceptibility of multiply resistant Pseudomonas aeruginosa isolated from patients with cystic Fibrosis, including candidates for transplantation. Clin Infect Dis 1996; 23: 532-537.

32. Levin AS, Barone AA, Peng J, Santos MV, Marinho IS, Arruda E et al. Intravenous colistin therapy for nosocomial infections caused by multidrug-resistant Pseudomonas and Acinetobacter Baumannii. Clin Infect Dis 1999; 28:1008-1011.

33. Arruda EA, Marinho IS, Boulos M, Sinto SI, Caiaffa H, Mendes CM et al. Nosocomial infections casued by multiresistant Pseudomonas aeruginosa. Infect Control Hosp Epidemiol 1999; 20: 620-623.

34. Panzig B, Schroder G, Pitten FA, Grundling MA. Large outbreak of multiresistant Pseudomonas aeruginosa strains in North-eastern Germany. J Antimicrob Che mother 1999; 43: 415-418.

35. Srikumar R, Tsang E, Pole K. Contribution of Mex AB Opr M multidrug efflux system to the beta-lactam resistance of penicillin binding protein and beta-lactamase-derepressed mutants of Pseudomonas aeruginosa. J Ani m rob Che mother 1999; 44: 537-540.