Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa

Asma M. Al-Jasser, *KSF (Path), Jordanian Board (Micro),* ***Noura A. Elkhizzi,*** *KSF (Path), FRCPath (Micro).*

ABSTRACT

Objective: To determine the level of resistance to the widely used antipseudomonal antibiotics in clinical isolates of *Pseudomonas aeruginosa* (*P. aeruginosa*).

Methods: The microbiology database of all clinical isolates of *P. aeruginosa* at the Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia, from January 1999 to December 1999 was reviewed. The antimicrobial susceptibilities were determined by a standardized method.

Results: Seven hundred and four *P. aeruginosa* isolates were tested. These strains were commonly isolated from surgical patients followed by intensive care units. Respiratory tract was the most common source of isolation. 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 704 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 empiric treatment of serious *P. aeruginosa* infections. It emphasizes the importance of a conservative approach to antibiotic therapy and continued antimicrobial susceptibility testing surveillance programs to curtail the problem of antibiotic resistance.

Saudi Med J 2004; Vol. 25 (6): 780-784

Pseudomonas aeruginosa is a leading cause of nosocomial infections, ranking second among the gram-negative pathogens reported to National Nosocomial Infectious Surveillance (NNIS) system from January 1995 to March 1996.[1] *Pseudomonas aeruginosa* was the third most common pathogen among bloodstream isolates in the most recent SENTRY study.[2] In the European Prevalence of Infection in intensive care (EPIC) study, *P. aeruginosa* was the predominant gram-negative species isolated from bronchopulmonary infection sites of patient hospitalized in 1417 intensive care units (ICUS) of 17 Western-European countries.[3] *Pseudomonas aeruginosa* commonly causes bronchopulmonary infections and less frequently urinary tract infections, infections of surgical wounds and bactremia.[4] Particularly at risk are the aged, premature babies, tracheostomized patients; those with severe burns or wound injuries; cystic fibrosis and immune deficiencies.[3,5-7] Infections caused by *P. aeruginosa* are frequently life threatening and often difficult to treat due to its intrinsic susceptibility to a limited number of antimicrobial agents.[8-11] Resistance to antipseudomonal antibiotic is an increasing problem, and emergence of antibiotic resistance during therapy occurs with relatively high frequency.[11,12] The changing and easy acquisition of

From the Department of Microbiology, Armed Forces Hospital, Riyadh, *Kingdom of Saudi Arabia.*

Received 20th October 2003. Accepted for publication in final form 9th February 2004.

Address correspondence and reprint request to: Dr. Asma M. Al-Jasser, Consultant Microbiologist, Department of Microbiology, Armed Forces Hospital, PO Box 7897, Riyadh 11159, *Kingdom of Saudi Arabia.* Tel. +966 (1) 4791000 Ext. 4096. Fax. +966 (1) 4783033. E-mail: asjass2002@yahoo.com

resistance in *P. aeruginosa* requires rapid surveillance procedures to represent the whole reality of the situation.[13]

Methods. A retrospective study was conducted at Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia, which is a tertiary health care center with a 1200 beds capacity. The microbiology laboratory database was used to identify all clinical cultures from patients that were positive for *P. aeruginosa* during a one year period between January 1999 to December 1999 without duplication of strains from the same patient and sample. *Pseudomonas aeruginosa* was identified according to a test panel consisting of gram stain, color appearance, pigment production, oxidase reaction and growth at 42°C. The a pyocyanogenic *P. aeruginosa* strains and those isolated from sterile sites were confirmed by Analytical Profile Index, Biomeriuex, France (API) system. Susceptibility testing was performed by disc diffusion method as described by National Committee for Clinical Laboratory Standards (NCCLS).[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 photometer. All organisms were tested on Muller-Hinton Agar (Becton, Dickinson, United States of America [USA]). The following discs (obtained from Oxoid, UK) 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 NCCLS guidelines.[15] *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were included as quality control strains following the protocol described above.

Results. A total of 704 non-repetitive *P. aeruginosa* isolates were tested. Four hundred and thirteen patients were males and 291 were females. The age range was 1-97 years. Majorities of *P. aeruginosa* strains were isolated from surgical wards 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. Respiratory specimens from 5 patients with cystic fibrosis were received. 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 tracheal aspirates

Table 1 - Types of patients with *Pseudomonas aeruginosa* infection.

Location	n	(%)
Surgical wards	216	(30.7)
Intensive care units	211	(30)
General medical wards	190	(27)
Outpatient	30	(4.3)
Burn	25	(3.6)
Renal transplant	13	(1.8)
Oncology	11	(1.6)
Hematologic malignancies	8	(1.4)

Table 2 - Sources of *Pseudomonas aeruginosa* infection.

Sources	n	(%)
Respiratory specimens (N=205)		
Sputum	103	(14.6)
Tracheal aspirates	91	(12.9)
Bronchoalveolar lavage	11	(1.6)
Wound (N=197)		
Surgical site infection	111	(15.8)
Bed sores	41	(5.8)
Others (chronic wounds and so forth)	45	(6.4)
Urine specimens (N=131)		
Catheter urine specimens	106	(15.1)
Midstream urine specimens	25	(3.6)
Surveillance swabs	77	(10.9)
Blood culture	66	(9.4)
Intravascular catheter tips	20	(2.8)
Sterile body fluids	8	(1.1)

Table 3 - In vitro susceptibility of 704 isolates of *Pseudomonas aeruginosa* to commonly used antimicrobial agents (expressed as percentage)

Antimicrobial agents	Resistant n	(%)
Amikacin	100	(14.2)
Aztreonam	175	(24.9)
Ceftazidime	128	(18.2)
Ciprofloxacin	55	(7.8)
Colistin	0	-
Gentamicin	157	(22.3)
Imipenem	69	(9.8)
Meropenem	59	(8.4)
Netilmicin	114	(16.2)
Piperacillin/tazobactam	132	(18.2)

were the most common specimens from ICUs followed by wound specimens from surgical site infections, blood cultures and CSU. From the medical wards: wound specimens, sputum and CSU were the most common specimens. 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 hematology patients. Six percent of *P. aeruginosa* isolates were mucoid in phenotype. The mucoid isolates were more susceptible than non-mucoid ones. Data on the in vitro susceptibility of *P. aeruginosa* isolates to antimicrobial agents 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 69(9.8%) were resistant to imipenem and 59 (8.4%) to meropenem. Cross resistance between the 2 carbapenems was observed in 51 (7.2%) of the isolates, 18 (26.1%) of imipenem-resistant isolates were susceptible to meropenem and the reverse was observed in 8 (13.6%). 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 carbapenems and piperacillin/tazobactam. Among the piperacillin/tazobactam resistant isolates cross resistant with the 2 carbapenems was observed in approximately 28% of isolates. The definition for multiple resistance in this study was resistance to ≥2 classes of antipseudomonal agents; beta-lactam antibiotics including the carbapenems and monobactams, aminoglycosides or fluoroquinolone (ciprofloxacin). This definition was developed at microbiology and infectious diseases consensus conference sponsored by cystic fibrosis foundation in 1994.[14] 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 medical wards.

Table 4 - Cross-resistance of *Pseudomonas aeruginosa* isolates.

Antimicrobial agents	N of strains	Percentage of strain resistant to: AK	AZT	CAZ	CIP	COL	GN	IMP	MER	NET	P/T
Amikacin	100	-	68	58	38	0	90	34	36	87	57
Aztreonam	175	38	-	59	22	0	49	27	26	41	59
Ceftazidime	128	43	86	-	25	0	54	29	27	42	78
Ciprofloxacin	55	67	69	60	-	0	75	33	35	64	55
Colistin	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	157	55	56	44	25	0	0	29	29	62	61
Imipenem	69	52	68	57	30	0	65	0	74	55	52
Meropenem	59	41	75	63	31	0	78	86	0	63	59
Netilmicin	114	96	63	52	32	0	88	31	33	0	54
Piperacillin/tazobactam	128	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. The beta-lactams exhibited a wide range of potency.[20] Carbapenems were the most potent among beta-lactam antibiotics but resistance to these agents is a growing 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 is important 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, which may be a therapeutic option in these cases. 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-lactam resistance 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.

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, Winokur 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 of infectious diseases. Vol 2. Philadelphia (PA): Churchill Livingstone; 2000. p. 2310-2335.
5. Grundmann 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 Chemother*** 2002; 49: 229-233.
11. Carmeli Y, Troillet N, Karchmer AW, Samore 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 Chemother*** 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 Jannuary 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 Chemother*** 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 Chemother*** 1999; 43:1301-1303.
22. Livermore DM. Of pseudomonas, porins, pumps and carbapenems. ***J Antimicrob Chemother*** 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 Chemother*** 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, Roughmann MC, Morris G, Kaye KS, Johnson JA. Risk factors for piperacillin/tazobactam resistant *Pseudomonas aeurginosa* among hospitalized patients. ***Antimicrob Agents Chemother*** 2002; 46: 854-858.
27. Mokaddas EM, Sanyal SC. Resistance patterns of *Pseudomonas aeruginosa* to carbapenems and piperacillin/tazobactam. ***J Chemother*** 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 Northeastern Germany. ***J Antimicrob Chemother*** 1999; 43: 415-418.
35. Srikumar R, Tsang E, Pole K. Contribution of MexAB Opr M multidrug efflux system to the beta-lactam resistance of penicillin binding protein and beta-lactamase-derepressed mutants of *Pseudomonas aeruginosa*. ***J Animrob Chemother*** 1999; 44: 537-540.