Antimicrobial Susceptibility Survey of *Pseudomonas Aeruginosa* Strains Isolated from Clinical Sources

Fitzroy A. Orrett, MD
Champs Fleurs, Trinidad and Tobago

A two-year prospective study of 554 *Pseudomonas aeruginosa* isolates was recovered from various clinical sources throughout Trinidad, and their resistance patterns to antipseudomonal antimicrobial agents were determined. Of the 554 *P. aeruginosa* isolates, 20.6% (114/554) were community isolates, 17.3% (96/554) from the intensive care unit (ICU), 10.1% (56/554) from the nursery, and the remaining 52% (288/554) were from other hospital inpatient services. Respiratory tract infections were the predominant source of *P. aeruginosa* isolates from the ICU—46.9% (45/96)—and nursery—21.4% (12/56), whereas wounds were the principal source of *P. aeruginosa* from the surgical services—77.0% (141/183). Community isolates of *P. aeruginosa* were predominantly from ear—100% (51/51)—and urinary tract infections—35.5%, (33/93). The overall prevalence of resistance was low for both hospital isolates (13.9%) and community isolates (3.8%). All community isolates were fully sensitive to four of the nine antimicrobials tested. Resistance rates among community strains ranged from 2.6% (ciprofloxacin and ceftazidime) to 12.3% for piperacillin. All isolates from hospital were fully sensitive to imipenem, but resistance rates for the other drugs ranged between 2.5% and 27.3%. The study showed that the overall resistance pattern of *P. aeruginosa* was relatively low. This is an encouraging observation but invites caution since resistance to the newly introduced drug, cefepime, has now emerged within the hospital environment and may present serious therapeutic problems within the near future. Policies governing the use of antimicrobials in many institutions are lacking. Such policies must be instituted in order to limit the spread of resistance and also to reduce the emergence of resistance to newly commissioned drugs within the country.

Key words: *Pseudomonas aeruginosa* ■ antimicrobial resistance ■ nosocomial infections ■ community-acquired infections ■ antipseudomonal drugs

 From Department of Paraclinical Sciences, Unit of Pathology and Microbiology, University of the West Indies and the Microbiology Laboratory, Diagnostic Laboratories, Eric Williams Medical Sciences Complex, Champs Fleurs, Trinidad and Tobago. Send correspondence and reprint requests for *J Natl Med Assoc.* 2004;96:1065–1069 to: F.A. Orrett, P.O. Box 371, Curepe Post Office, Curepe, Trinidad and Tobago, West Indies; e-mail: drfao4301@yahoo.com and drfao@ tstt.net.tt

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous organism frequently isolated from clinical specimens. Because these organisms are usually inherently resistant to many antimicrobial agents, treatment of pseudomonal infections is usually difficult, and mortality is usually high.[1-3] This intrinsic resistance is mainly a result of the diffusion barrier of the bacterial outer membrane; amino-acid substitution in the target molecules, such as Gyr A and/or Par C, via point mutation in each genetic determinant; and antimicrobial inactivating enzymes.[4] In most hospital environments, this inherent resistance is further complicated by mutations mediated via chromosomes and the acquisition of resistant genes from plasmids and transposons.[4] One type of mutation simultaneously comprises penicillins, cephalosporins, and the fluoroquinolones, and enhances resistance to chloramphenicol and tetracyclines by accelerating multidrug efflux.[5] Other mutations involve loss of the D2 porins that mediate carbapenem resistance,[6,7] reduce uptake of aminoglycosides across the outer or cytoplasmic membrane,[8] and inactivation by aminoglycosides-modifying enzymes.[9] Recently, strains of *P. aeruginosa* resistant to all antimicrobials except polymyxin B were reported.[10] Genetic analyses of these strains identified two unique extended-spectrum beta-lactamase genes. One—bla (VIM-7)—encoded a metallo-beta-lactamase, and the other—bla (OXA-45)—encoded a class-D extended-spectrum beta-lactamase.

P. aeruginosa resistance is not restricted to the hospital environment but has also been seen in community-acquired infections, such as otitis externa,[11] folliculitis,[12] osteomyelitis,[13] outpatients with chronic indwelling urethral catheters,[14] and endocarditis in intravenous drug users.[15] The prevalence of resistance among *P. aeruginosa* strains has been extensively reported, especially with the expansion in the number of β-lactam agents with improved antipseudomonal activity. Resistance may not be as widespread as reported, and there may be consider-

able geographic variation. Recently, several deaths due to septicemia with multiresistant *P. aeruginosa* were reported at a neonatal intensive-care unit (ICU) in a southern hospital in Trinidad.[16] No data on the susceptibility patterns among *P. aeruginosa* strains have been documented in Trinidad.

The purpose of this study, therefore, was to evaluate the current level of resistance among *P. aeruginosa* isolates from various centers throughout Trinidad to the currently available antipseudomonal antimicrobial agents.

MATERIALS AND METHODS

Background and Collection of Isolates

From January 1, 2001 to December 31, 2002, *P. aeruginosa* isolates were recovered from patients seen at various centers in Trinidad. Isolates were collected from community sources, (privately operated microbiology laboratories, general practitioners offices, outpatients clinics) and hospitals (specimens from the ICUs, surgical services, nursery, adult and pediatric medical wards, and the obstetric and gynecology wards) [*See Acknowledgements*], and then submitted to the Eric Williams Medical Sciences Complex Microbiology Laboratory (EWMSC). All isolates from community sources and other hospitals were received on Mueller-Hinton agar plates. Data obtained for each isolate were the patient's age, sex, the source of the isolate, whether the patient was nonhospitalized or hospitalized in an ICU, surgical ward, or on a neonatal unit. Specimens with incomplete data were few and therefore were excluded from the study. The EWMSC is a fee-for-service, 560-bed tertiary hospital complex located in the northwestern part of Trinidad. Trinidad is about 4,828 km^2 in area and is the larger of the twin-island republic, Trinidad and Tobago, located about 11 km off the northern coast of Venezuela in South America. The population of the republic is about 1.25 million people.[17]

Bacteriologic Methods

P. aeruginosa were identified by colonial morphology, a positive oxidase reaction, pyocyanin production on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD), motility, growth at 42°C on cetrimide agar and biochemical tests. O serotyping was not done. Antimicrobial susceptibility testing was done on Mueller-Hinton agar following recommendations of the National Committee for Clinical Laboratory Standards,[18] using: ciprofloxacin 5 μg, amikacin 30 μg, ceftazidime 30 μg (Oxoid, UK), imipenem 10 μg, gentamicin 10 μg, tobramycin 10 μg, aztreonam 30 μg, piperacillin 100 μg, and cefepime 30 μg (Becton Dickinson, USA. Mueller-Hinton broth was used as the growth medium. The final bacterial inoculum concentration was approximately 1.5 x 10^8 colony-forming units (CFU)/ml. Before the antibiotic discs were placed, the Mueller-Hinton plates were inoculated with swabs that were submerged in the final inoculum concentration and streaked over the entire surface of the plates. Plates were incubated aerobically at 35–37°C for 18–24 hours.

The control organism was *P. aeruginosa* ATCC strain 27853, obtained from the Caribbean Epidemiology Center, a local branch of the Pan American Health Organization/World Health Organization. Information on the amount and cost of antimicrobials consumed by each service in the hospital was obtained from the pharmacy department and the Ministry of Health.

Table 1. Proportion (%)* of *Pseudomonas aeruginosa* Strains Recovered from Various Sources in Relation to Patient Category

	Patient Category From					
Source	**ICU[1]**	**Surgical Services**	**Other Services[2]**	**Nursery**	**Community**	**TOTAL**
Wounds	20 (7.9)	141 (55.7)	53 (20.9)	11 (4.3)	28 (11.2)	253
Respiratory tract	45 (57.0)	3 (3.8)	17 (21.5)	12 (15.2)	2 (2.5)	79
Urinary tract	16 (17.2)	21 (22.6)	16 (17.2)	7 (7.5)	33 (35.5)	93
Ear	0	0	0	0	51 (100.0)	51
Blood	6 (16.2)	10 (27.0)	8 (21.6)	13 (35.2)	0	37
Eye	4 (30.8)	3 (23.0)	6 (46.2)	0	0	13
CSF[a]	0	0	0	8 (100.0)	0	8
Miscellaneous[b]	5 (25.0)	5 (25.)0	5 (25.0)	5 (25.0)	0	20
TOTAL	96	183	105	56	114	554

[1] Intensive care unit; [2] Other services: adult and pediatric medical, obstetric/gynecological; [a] CSF: cerebrospinal fluid; [b] Miscellaneous: umbilical swab, stool, peritoneal dialysate fluid; (%)*: percentage.

RESULTS

From 650 cultures that were received from participating centers, 554 (85.2%) were recovered on subculture and subsequently confirmed as *P. aeruginosa*. All of the 554 isolates that were confirmed as *P. aeruginosa* produced pyocyanin. Ninety-six of 554 (17.3%) of the isolates were recovered from the ICU, and 10.1% (56/554) from the nursery. Other inpatients services accounted for 288 isolates (52.0%%) and community isolates of *P. aeruginosa* accounted for only 20.6% (114/554) of all isolates (Table 1). Respiratory tract isolates were the predominant source of *P. aeruginosa* from the ICU and the nursery, whereas wounds were the principal source from other inpatients. More than 98% (112/114) of the community isolates of *P. aeruginosa* were recovered from ear and urinary tract infections.

Table 2 shows the frequency of resistance among isolates from hospital and community sources. Community source isolates were fully sensitive to four of the nine antimicrobials tested but showed very low levels of resistance to ciprofloxacin and ceftazidime (each 2.6%) and gentamicin (7.9%). Imipenem was the only drug to which all hospital and community source isolates were fully sensitive. Although relatively low, hospital isolates showed varying degrees of resistance to the other antimicrobials.

Table 2. Prevalence of Resistance to Antimicrobial Agents among *Pseudomonas Aeruginosa* Strains isolated from Hospital and Community Sources

	Number (%)* of isolates resistant from:	
Antimicrobial	**Hospital N=440**	**Community N=114**
Piperacillin	120 (27.3)*	14 (12.3)
Gentamicin	112 (25.5)	9 (7.9)
Tobramycin	95 (21.6)	0
Ceftazidime	88 (20.0)	3 (2.6)
Ciprofloxacin	52 (11.8)	3 (2.6)
Aztreonam	43 (9.8)	10 (8.8)
Cefepime[a]	30 (6.8)	0
Amikacin	11 (2.5)	0
Imipenem	0	0

[a] Cefepime: Introduced into the country in January 2000; (%)*: percentage.

Except for the major hospitals (SFGH, POSGH, and the EWMSC), the varied sources of each isolate was not always available. However, from among known hospital sources, the most resistant *P. aeruginosa* isolates were from the respiratory tracts and infected wounds. The drugs to which these strains were most resistant were piperacillin, ceftazidime, and the aminoglycosides, gentamicin, and tobramycin.

DISCUSSION

The prevalence of resistance of *P. aeruginosa* strains to the nine antimicrobials tested was relatively low among hospital strains. Strains from community sources were fully sensitive to four of the nine drugs. No local or regional data could be found in the literature to which resistance rates among *P. aeruginosa* could be compared. However, increasing resistance to the different antipseudomonal drugs particularly among hospital strains has been reported worldwide,[19-21] and this presents a serious therapeutic problem in the management of diseases due to these organisms. Reports from Turkey[22] have shown *P. aeruginosa* resistance rates of 73.6% and 68.0% to piperacillin and gentamicin, respectively. In Russia, 79% and 75% of isolates were found to be resistant to piperacillin and gentamicin,[23] respectively, while from Bangladesh,[24] rates of resistance of 51.0% to tobramycin and 21.0% to ciprofloxacin have been recorded.

Eight of the nine antipseudomonal drugs can only be given parenterally, and resistance to these parenteral agents is slowly increasing in our hospital.[16,25] The only oral antipseudomonal agent tested in this study, ciprofloxacin, recently became available in the hospital but widely available at local pharmacies. However, although recently available in the hospital formulary, ciprofloxacin was still used, because relatives of some inpatients were able to purchase the drug at local pharmacies. Resistance in the hospital environment may be affected by the frequent or infrequent availability of antimicrobials as well as the prudent use of these drugs. Although the resistance rate of *P. aeruginosa* is relatively low, the overall resistance rate among other gram-negative organisms at this institution and at a sister institution about 28 miles away has been increasing.[16,25-28] This may be related to the yearly demand and increase in antimicrobial consumption. For example, the total cost for antimicrobials alone at this institution for 1998 was approximately TT$2.2 million [TT$6 = US$1]. In 1999, the cost rose to approximately TT$3.6 million, an increase of about 39%. (Data from the hospital pharmacy records and the Ministry of Health). If the rate of consumption continues, figures for 2000–2002 may show a greatly increased cost for antimicrobials alone.

The community isolates of *P. aeruginosa* strains causing urinary tract infections were recovered from male patients with chronic in-dwelling urethral catheters. These patients are treated with antipseudomonal antimicrobials in the outpatient clinics. These men have prostatic diseases and are either

unfit for surgery, awaiting surgery, or have refused surgery.[14] Catheters are changed once every six weeks in most cases, or earlier if complaints of fever gross hematuria or acute blockage are made. Excessive prescribing of a single drug often leads to resistance and a decrease in the usefulness of the antimicrobial. Antimicrobial administration and the presence of in-dwelling urethral catheters correlate with the development of bacterial resistance.[29]

One significant observation in this study was the increasing resistance to cefepime. This drug was recently introduced into the country but was not in the hospital formulary during the study period, and 7.5% of *P. aeruginosa* strains have been found to be resistant. Cefepime was supplied as samples by drug representatives to selected clinicians in the hospitals in an effort to promote the use of the drug. Although communication between the hospital laboratories and the clinicians have been good, the absence of an antibiotic policy at most hospitals in Trinidad and the aggressive sales pitch by most drug representatives have contributed to poor compliance with respect to restrictive use of this drug and others. Cefepime is a fourth-generation cephalosporin, which has enhanced stability in the presence of Bush-Jacob-Medeiros group-1 β-lactamases. These β-lactamases will inactivate all other currently available cephalosporins.[30] Therefore, cefepime is active against stably derepressed class-1 β-lactamases of *P. aeruginosa* and members of the *Enterobacteriaceae* family. Cefepime has also been shown to be active against most strains of ceftazidime-resistant *P. aeruginosa*.[30]

The study showed that the overall resistance pattern of *P. aeruginosa* to the antimicrobial agents was relatively low. It was also evident from the results that resistance to the relatively new drug, cefepime, may present a serious problem in the near future and that policies governing antimicrobial use in the country be formulated. In the meantime, physicians in both hospital and community practices must exercise adequate care in the prescribing of antimicrobials, especially in cases of *P. aeruginosa* infections, to prevent emergence of resistant strains.

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