CLINICAL CASE REPORT

Infective endocarditis of a native aortic valve due to *Pseudomonas aeruginosa* complicated by progressive multi-drug resistance

Khuloud Aldhaheri MD[1], Nisha Andany MD, MPH[1,2], Alireza Eshaghi PhD[3], Andrew E Simor MD[1,2], Lesley Palmay BSc, BScPhm, MSc[4], Samir N Patel PhD[3], Philip W Lam BScPhm, MD, MSc[1,2]

BACKGROUND: Treatment of infective endocarditis secondary to *Pseudomonas aeruginosa* can be challenging because of this organism's ability to acquire antimicrobial resistance over time. **METHODS:** We describe a patient with native aortic valve infective endocarditis due to *P. aeruginosa* who developed progressive multi-drug resistance while on therapy. The resistance mechanisms were characterized using whole-genome sequencing. **RESULTS:** We identified two mutations in subsequent isolates (*dacB* and *OprD*) that conferred resistance to anti-pseudomonal penicillins, cephalosporins, and carbapenems. The patient was treated with combination high-dose continuous infusion meropenem and ciprofloxacin therapy, in addition to bioprosthetic aortic valve replacement and repair of ventricular septal wall defect. Antibiotics were continued for 6 weeks post–cardiac surgery and the patient remains infection free 18 months post-completion of antibiotic therapy. **CONCLUSION:** Clinicians should be aware of the ability of *P. aeruginosa* to acquire resistance mechanisms in response to selective antibiotic pressures in high-inoculum infections such as infective endocarditis. The mutations identified in this case report correlated well with the evolving antimicrobial resistance profile observed.

KEYWORDS: infective endocarditis, Pseudomonas aeruginosa

HISTORIQUE : Il peut être difficile de traiter une endocardite infectieuse causée par un *Pseudomonas aeruginosa* en raison de la capacité de cet organisme à acquérir une résistance aux antimicrobiens. **MÉTHODOLOGIE :** Les chercheurs décrivent un patient atteint d'une endocardite infectieuse de la valve aortique d'origine, attribuable à un *P. aeruginosa*, qui a acquis une multirésistance progressive pendant son traitement. Le mécanisme de résistance était caractérisé par le séquençage du génome entier. **RÉSULTATS :** Les auteurs ont dépisté deux mutations dans les isolats subséquents (*dacB* et *OprD*), responsables d'une résistance aux pénicillines, aux céphalosporines et aux carbapénèmes antipseudomonaux. Le patient a reçu une polythérapie de perfusion continue de méropénem à forte dose et de ciprofloxacine, en plus du remplacement d'une valve aortique bioprothétique et de la réparation d'une communication interventriculaire. L'antibiothérapie s'est poursuivie six semaines après l'opération, et le patient n'avait pas d'infection 18 mois après la fin de l'antibiothérapie. **CONCLUSION :** Les cliniciens devraient savoir que le *P. aeruginosa* peut acquérir des mécanismes de résistance en réponse aux pressions antibiotiques sélectives en cas d'infections comportant un titre élevé d'inoculum comme une endocardite infectieuse. Les mutations constatées dans le présent rapport de cas étaient bien corrélées avec l'évolution du profil de résistance antimicrobienne observé.

MOTS-CLÉS : endocardite infectieuse, Pseudomonas aeruginosa

[1]*Department of Medicine, University of Toronto, Toronto, Ontario, Canada;* [2]*Division of Infectious Diseases, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada;* [3]*Public Health Ontario Laboratory, Public Health Ontario, Toronto, Ontario, Canada;* [4]*Department of Pharmacy, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada*

Correspondence: Philip W Lam, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue E209, Toronto, Ontario M4N 3M5 Canada. Telephone: 416-480-6100 ext 7249. E-mail: philip.lam@sunnybrook.ca

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CASE PRESENTATION

A 64-year-old woman presented to hospital with a 2-day history of left leg erythema, pain, and fever. Her medical history was significant for recurrent lower extremity cellulitis secondary to chronic lymphedema and severe aortic stenosis (aortic valve area 0.68 cm^2). Blood cultures drawn on admission grew *Pseudomonas aeruginosa* susceptible to all first-line agents (Table 1). She was treated empirically with piperacillin–tazobactam 4.5 g intravenously (IV) every 6 hours. Her clinical status improved, and as a result, blood cultures were not repeated to assess for clearance. She was subsequently transitioned to oral cephalexin and ciprofloxacin to complete a 14-day course of therapy for bacteremia and cellulitis.

Four days after completion of antibiotic therapy, she re-presented to hospital with fever, rigors, and night sweats. On physical examination, she was hypotensive and febrile (39°C) with a previously identified systolic ejection murmur at the right upper sternal border. There were no signs of cellulitis or new skin lesions during this second hospital presentation. She was re-started empirically on piperacillin–tazobactam 4.5 g IV every 6 hours. Admission blood cultures grew *P. aeruginosa* with the same susceptibility profile as the prior isolate (Table 1).

Despite appropriate antimicrobial therapy with piperacillin–tazobactam, she remained persistently febrile and bacteremic. Subsequent isolates of *P. aeruginosa* from blood cultures demonstrated progressive resistance to multiple antimicrobial agents (Table 1), prompting a change in therapy to meropenem and tobramycin on day 8 of admission.

On day 9 of admission, the patient developed vertigo and nystagmus. MRI of the brain demonstrated multifocal cerebral and cerebellar infarcts with a left temporal lobe ring-enhancing lesion suspicious for abscess (Figure 1A). Computed tomography (CT) of the abdomen demonstrated multiple new splenic infarcts (Figure 1B). Given the suspicion of a cardioembolic source, a work-up for infective endocarditis was initiated. Transthoracic and transesophageal imaging did not demonstrate evidence of vegetation. CT angiogram of the head and chest did not identify any evidence of aneurysm.

Table 1: Antimicrobial susceptibility results for sequential *Pseudomonas aeruginosa* isolates

Antibiotics	Minimum inhibitory concentration (mg/L) and interpretation*				
	Blood, first admission	Blood, day 0[†]	Blood, day 4	Blood, day 8[‡]	Aortic valve, day 13
Amikacin	≤4 (S)	≤4 (S)	≤4 (S)	≤4 (S)	8 (S)
Aztreonam	8 (S)	8 (S)	>16 (R)	4 (S)	16 (I)
Cefepime	≤2 (S)	≤2 (S)	16 (I)	≤2 (S)	16 (I)
Ceftazidime	4 (S)	4 (S)	>16 (R)	2 (S)	>16 (R)
Ceftolozane–tazobactam	NC	≤4/4 (S)	≤4/4 (S)	NC	≤4/4 (S)
Ciprofloxacin	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Colistin	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)
Doripenem	0.25 (S)	0.25 (S)	0.5 (S)	0.5 (S)	>2 (R)
Gentamicin	2 (S)	2 (S)	2 (S)	2 (S)	4 (S)
Imipenem	2 (S)	2 (S)	2 (S)	2 (S)	>8 (R)
Levofloxacin	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)
Meropenem	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)	4 (I)
Piperacillin–tazobactam	8/4 (S)	8/4 (S)	32/4 (I)	8/4 (S)	≥64/4 (R)
Polymyxin B	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)
Ticarcillin–clavulanic acid	32 (I)	32 (I)	128 (R)	32 (I)	128 (R)
Tobramycin	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)	2 (S)

* Interpretation of minimum inhibitory concentrations based on Clinical & Laboratory Standards Institute M100 Performance Standards
† Day number is relative to second hospitalization
‡ Two different biotypes were isolated from day 8 blood cultures. Only one biotype (susceptibility results shown) was sequenced;
the second biotype (not shown) demonstrated an identical susceptibility profile to the day 4 blood culture isolate
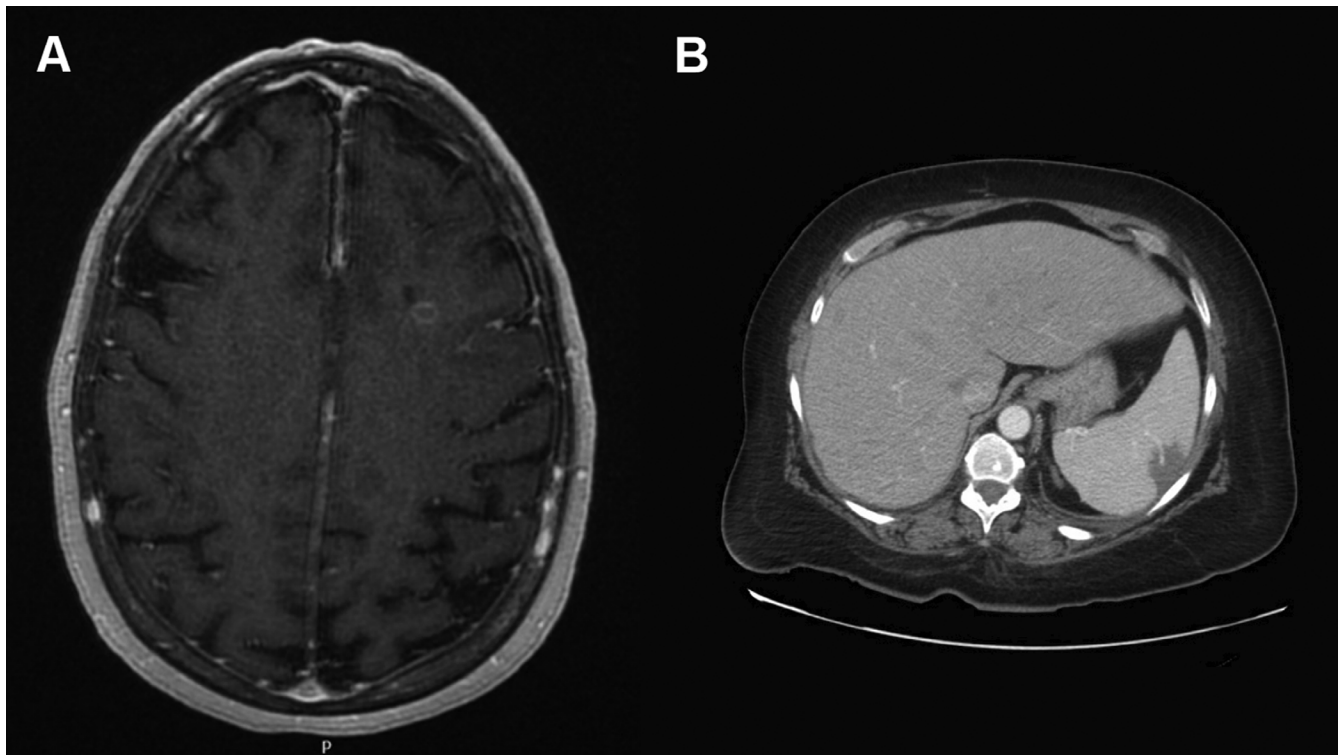*S = Susceptible, I = Intermediate, R = Resistant, NC = Not completed*

Figure 1: Brain MRI showing parietotemporal infarct and leptomeningeal enhancement (A) and abdomen CT demonstrating splenic infarcts (B)

CT = Computed tomography

DIAGNOSIS

On day 11 of admission, the patient developed cardiogenic shock with evidence of cardiac tamponade. She was taken emergently to the operating room for exploration. Intra-operatively, the aortic valve was noted to be bicuspid with no clear evidence of vegetation, but resection of the valve revealed a deep aortic root abscess invading the septum. Evacuation of pericardial effusion, bioprosthetic aortic valve replacement, and repair of ventricular septal wall defect were performed. Bacterial cultures of the aortic valve and ventricular septum grew *P. aeruginosa* (Table 1).

All blood and operative culture isolates (including those from the first admission) were sent to the Public Health Ontario Laboratory for whole-genome sequencing. All isolates were found to be identical to each other (sequence type 252) with one to two single nucleotide polymorphism differences. Bacterial isolates from day 4 and 13 demonstrated a 12-nucleotide deletion in the *dacB* gene. In addition, insertion of a cytosine nucleotide causing a frameshift mutation in the *OprD* gene was noted from the day 13 (aortic valve) culture isolate.

Given the concerns about multi-drug resistance and significant toxicities associated with prolonged aminoglycoside use, the patient was transitioned to 1 g meropenem IV every 4 hours via continuous infusion (given the intermediate susceptibility result from the aortic valve culture) and 400 mg ciprofloxacin IV every 8 hours for 6 additional weeks postoperatively. Ceftolozane–tazobactam was avoided because of the findings of brain abscess and the lack of data supporting adequate central nervous system penetration of this antibiotic. The patient made a complete recovery and remains well 18 months after completion of antibiotic therapy.

DISCUSSION

P. aeruginosa is an aerobic, non-spore-forming, gram-negative bacillus that is commonly found in freshwater environments. Infections resulting from *P. aeruginosa* typically occur among patients with underlying immunocompromise; however, infections among immunocompetent patients also occur, particularly in the nosocomial setting (1). Common clinical syndromes associated with *P. aeruginosa* include bacteremia, pneumonia, urinary tract infections, head and neck infections (particularly of otologic origin), and skin and soft tissue infections. Injection drug use is an important risk factor for *P. aeruginosa* bacteremia, specifically when water contaminated with this organism is used as a diluent (2).

In the absence of injection drug use, endocarditis due to *P. aeruginosa* is uncommon; approximately 3% of endocarditis

cases overall are due to this organism (3). The predominant risk factors for pseudomonal endocarditis unrelated to injection drug use include presence of a prosthetic heart valve, intra-cardiac device, and central venous catheter, as well as underlying diabetes mellitus or receipt of immunosuppressive therapy (4,5). Among persons with nosocomial endocarditis, the proportion of cases due to *P. aeruginosa* may be higher (6). Mortality due to left-sided endocarditis caused by *P. aeruginosa* is high even with appropriate antimicrobial therapy (7). For this reason, clinical practice guidelines suggest surgical intervention in combination with antimicrobial therapy (8).

P. aeruginosa has the potential for multi-drug and high-level antimicrobial resistance as a result of various mechanisms. These mechanisms of resistance may be intrinsic or acquired and include enzymatic inactivation of antimicrobial agents, alterations in penicillin-binding proteins, porins, and efflux pumps. Initial case reports in the 1980s described the failure of piperacillin–tazobactam therapy among patients with *P. aeruginosa* endocarditis, with isolates demonstrating significant increases in piperacillin minimum inhibitory concentrations and beta-lactamase production post-therapy (9).

The availability of whole-genome sequencing has allowed for further characterization of these resistance mechanisms. Domitrovic and colleagues described the development of cephalosporin resistance in five sequential *P. aeruginosa* blood culture isolates over a 7-month period in a patient with relapsed prosthetic aortic valve endocarditis treated with beta-lactam therapy (10). All isolates also possessed a premature stop codon in *OprD* as well as mutations in *gyrA*, *parC,* and *nalC,* thereby conferring resistance to carbapenems and quinolones, respectively (10). Gürtler and colleagues reported a case of *P. aeruginosa* prosthetic aortic valve endocarditis and persistent bacteremia in which the responsible organism sequentially developed resistance to piperacillin–tazobactam, ceftazidime, and meropenem (11). Genetic sequencing demonstrated mutations in *ampR*, *ampD,* and *OprD* (11).

The findings of a mutation in *dacB* and *OprD* genes in our case correlate with the susceptibility profile observed. Resistance to anti-pseudomonal penicillins and cephalosporins from the day 4 and 13 isolates coincide with the mutation in the *dacB* gene. The inactivation of *dacB* (which encodes for penicillin-binding-protein 4) in experimental knockout strains of *P. aeruginosa* has been shown to significantly increase *ampC* expression, which likely explains the observed resistance pattern (12). To our knowledge, this is only the second case report of resistance development through this gene mutation during therapy (13). The finding of selective imipenem resistance in the aortic valve culture isolate is consistent with loss of *OprD* function as the underlying mechanism (14). Two different biotypes were isolated from day 8 blood cultures, one demonstrating an identical susceptibility to the day 0 isolate, whereas the second isolate possessed the same susceptibility pattern as the day 4 isolate, suggesting the presence of hetero-resistant subpopulations.

Guidelines from the Infectious Diseases Society of America suggest combination antimicrobial therapy for cases of endocarditis due to non-HACEK (*Haemophilus* spp, *Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens,* and *Kingella kingae*) gram-negative aerobic bacilli, particularly *P. aeruginosa* (8). However, studies examining the optimal approach to the medical management of pseudomonal endocarditis in humans are limited, and no randomized controlled trials have been performed with this population. In the largest prospective cohort study of 58 patients hospitalized with endocarditis due to non-HACEK gram-negative bacteria (of which approximately one-fifth of cases were due to *P. aeruginosa*), infection due to a multi-drug-resistant organism was independently associated with increased mortality (5). Almost all patients (86%) were treated with combination antibiotic therapy (5). Mortality was similar between those treated medically and those who received a combined medical–surgical approach; however, the subset of patients who developed complications (such as abscess or valve perforation) had higher mortality rates when treated with medical therapy alone (5).

Despite the lack of echocardiographic evidence of endocarditis in our case, clinical suspicion of endocarditis was high. The patient possessed multiple indications for early surgical intervention, including persistent bacteremia and recurrent embolic phenomena despite appropriate combination antimicrobial therapy. Ultimately, the diagnosis of infective endocarditis was made intra-operatively. Although there is growing evidence to support the use of positron emission tomography and CT in diagnosing select cases equivocal for infective endocarditis (15), this imaging modality may not be readily accessible at many centres and in this case would not have changed management given the high suspicion for endovascular infection and progressive deterioration necessitating surgical intervention.

We suspect that chronic skin breakdown from lymphedema and chronic dermatomycosis served as a portal of entry for *P. aeruginosa* to contribute to an initial skin and soft tissue infection, with subsequent bacteremia and seeding of a vulnerable bicuspid aortic valve. A high inoculum of bacteria within the vegetation (before cardiac surgery) likely allowed for evolution of resistance in response to selective antimicrobial pressure. The ability of *P. aeruginosa* to quickly develop resistance highlights the importance of repeating susceptibility testing on serial isolates when persistent bacteremia is encountered.

In conclusion, this case highlights the ability of *P. aeruginosa* to acquire antimicrobial resistance during

therapy, particularly in high-inoculum infections such as infective endocarditis. Using whole-genome sequencing, we were able to identify mutations in *OprD* and *dacB* that corresponded to the development of progressive antibiotic resistance during the patient's treatment course. Although the optimal approach to management of pseudomonal infective endocarditis remains unclear, combination antimicrobial therapy in addition to surgical intervention may be needed.

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