Possible Prosthetic Valve Endocarditis by *Pandora* *pnomenusa* and Specific Virulence Mechanisms

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Abstract: Bacteremia by *Pandora* spp. has rarely been described before. We report the first case of a *P. pnomenusa* possible prosthetic valve endocarditis, according to the modified Duke criteria, in a 37-year old male injecting drug user suffering from recurrent endocarditis. Furthermore, we demonstrate biofilm formation by the *P. pnomenusa* isolates of this patient and investigate antibiotic resistance.

Keywords: prosthetic valve endocarditis, central venous catheter, bacteremia, *Pandora* *pnomenusa*, biofilm formation, antibiotic resistance

Introduction

The genus *Pandora* was first described in 2000¹ and is mostly isolated from individuals with cystic fibrosis (CF). The genus currently contains 28 validly published taxa,^{2,3} with more being added continuously.⁴ Although the true prevalence is unknown, it seems to be a rare organism.⁵ Current evidence suggests a pathogenic role of *Pandora* spp. in chronic lung infection with accelerated decline of lung function.⁵ Biofilm formation is a key virulence factor associated with colonization and infection in CF lungs. In addition, biofilm formation can lead to antibiotic resistance and persistence of various pathogens in infected lungs.⁵⁻⁸

Currently, biofilm formation is insufficiently studied in *Pandora* spp., and little is known about the virulence factors of *Pandora* *pnomenusa*.^{9,10} Several cases of bloodstream infections (BSI) with *Pandora* spp. have been described¹¹⁻¹⁵ and thus far, only two cases of *P. pnomenusa* bacteremia have been reported.^{12,13} We aim at reporting the possible case of *P. pnomenusa* prosthetic valve endocarditis (PVE) in a patient suffering from recurrent endocarditis. We also investigated biofilm formation and antibiotic resistance determinants.

Case Presentation

A 37-year old male injecting drug user (IDU) with recurrent biological PVE resided in a cardiac rehabilitation clinic after valve re-replacement surgery for an episode of *Enterococcus faecalis* and *Candida dubliniensis* endocarditis. Two days after completion of his six-week antibiotic treatment with intravenous amoxicillin/clavulanic acid and whilst still under isavuconazole treatment, he developed fever accompanied by elevated inflammatory markers. Subsequently, two blood culture bottles yielded growth of *P. pnomenusa* drawn simultaneously

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from his peripherally inserted central venous catheter (PICC) and from his elbow vein. The patient was started on empirical treatment with tigecycline. Upon suspicion of a central venous catheter-associated bloodstream infection (CLABSI), the patient was readmitted to the University Hospital of Zurich on day 6 of the current episode. Blood cultures drawn upon admission showed a marked difference in time to positivity of 9h between the PICC (14 hours) and the peripherally drawn blood cultures (23 hours), indicative of CLABSI. The central line was removed immediately without showing any growth microbiologically. Initial testing demonstrated susceptibility towards piperacillin/tazobactam but resistance to tigecycline when interpreted according to *Pseudomonas* clinical breakpoints. Accordingly, therapy was switched to piperacillin/tazobactam on day 8 of the current episode. Three days after initiation of targeted therapy, a new PICC was inserted until novel blood cultures on day 22 yielded growth of *P. pnomenusa* again, which led to another change of the PICC. At the same time, transesophageal echocardiography (TOE) demonstrated filiform, floating structures on the central catheter without valve vegetation, whereas the positron emission computed tomography (PET/CT) did neither show a metabolic activity of the prosthetic valve nor of the catheter tip. A small echo-dense structure at the anterior pocket of the annulus had already been described in earlier investigations. Based on suggestive clinical and microbiological criteria (fever, persistent positive blood cultures not meeting major criteria, predisposing heart condition and IDU¹⁶) a treatment duration of six weeks for possible PVE was determined. After a three-week course of piperacillin/tazobactam, therapy was switched to trimethoprim/sulfamethoxazole. To date, *P. pnomenusa* was not isolated in the patient again.

Methods

The BacT/ALERT Virtuo blood culture system was used for incubation (bioMérieux (Suisse) SA, 1213 Petit Lancy).

Antimicrobial susceptibility testing was performed according to EUCAST.¹⁷ Minimal inhibitory concentrations (MIC) were determined by ETEST (bioMérieux (Suisse) SA, 1213 Petit Lancy, Switzerland) or MIC strip test (Liofilchem srl, Roseto degli Abruzzi, Italy). Additionally, the Rapidec Carba NP test was performed to confirm carbapenemase activity (bioMérieux

(Suisse) SA, 1213 Petit Lancy, Switzerland). *Klebsiella pneumoniae* ATCC BAA-2146 was used as positive control strain.

We evaluated quantitative biofilm formation according to O'Toole.¹⁸ Colonies of *Pandoraea* spp. were suspended in LB Broth and grown overnight at 37°C, crystal violet was solubilized with 96% ethanol and optical density (OD) was measured at 620nm. In parallel, the bacterial suspensions were seeded in imaging chambers (ibidi) and grown to form biofilms at 37°C, as described previously.¹⁹ After fixation with 4% paraformaldehyde (Electron Microscopy Sciences), biofilms were stained with FM[®] 4-64FX (Molecular Probes) and analyzed by confocal microscopy (excitation 561 nm, emission 600–620 nm). Image processing was performed with ImageJ software (<https://imagej.net/Fiji>).

For whole-genome sequencing (WGS) DNA libraries were prepared following the QIAseq FX DNA Library Kit (QIAGEN AG, Hombrechtikon, Switzerland). Quality control of the library was performed using capillary electrophoresis (Fragment Analyzer Automated CE System, Advanced Analytical). Sequencing was done on the MiSeq Platform (Illumina). The Comprehensive Antibiotic Resistance Database (CARD) was used to identify resistance genes.²⁰

Microbiological Results

Initial 16S sequencing did not yield reliable results. Thus, species identification was done by whole genome sequencing and the isolate was identified as *P. pnomenusa*.³, ([Supplementary Figure 1](#)). We identified an OXA-151 carbapenemase with two amino acid replacements V180L and S271A.²¹ The isolates were highly resistant to meropenem but susceptible to imipenem. The Rapidec Carba NP test yielded no perceivable hydrolysis of imipenem. The susceptibility of piperacillin/tazobactam was surprising ([Table 1](#)).

We assessed the ability of the clinical *P. pnomenusa* isolates to produce static biofilms on abiotic surfaces. As controls, we included the bacterial species *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. 18 h biofilms were stained with FM[®] 4-64FX to label the bacteria and immediately examined by confocal microscopy. Analysis revealed that all *P. pnomenusa* isolates, as well as the control species, formed three-dimensional structures in a rich medium with irregular and fluffy aggregates ([Figure 1](#)). These aggregates formed in some areas of the surface, while in other areas single

Table I Susceptibility Testing of the *P. pnomenusa* Strains

Antibiotic	Strain 1 [mm]	Strain 1 [mg/L]	Interpretation	Strain 2 [mm]	Strain 2 [mg/L]	Interpretation
Piperacillin		24	R		8	R
Piperacillin/tazobactam	23	0.19	S	24	0.047	S
Ceftazidime	6	24	R	6	16	R
Ceftazidime/avibactam		24	R		24	R
Cefepime	26		S	27		S
Imipenem	29	1.5	S	27	1.5	S
Meropenem	6	≥32	R	6	≥32	R
Meropenem/vaborbactam		32.0	R		24	R
Ciprofloxacin	28	0.5	S	28	0.5	S
Levofloxacin	28		S	30		S
Trimethoprim/sulfamethoxazole	36	0.64	S	33	0.64	S
Amikacin	14		R	9		R
Gentamicin	6		R	6		R
Tobramycin	11		R	6		R
Ceftolozane/tazobactam		≤0.016	S			
Tetracyclin		3.0				
Minocycline		0.38				
Tigecycline		1.5	R			

Note: For disc diffusion testing EUCAST breakpoints for *P. aeruginosa* were applied.

cells attached to the surface. Biofilm formation on abiotic surfaces can be quantified by crystal violet staining of the biomass attached to the surfaces of 96-well plates. Under the conditions tested, absorbance at 620nm of *P. pnomenusa* isolates 1 and 2 were 0.42 and 0.60, respectively (Figure 2). Compared to the control strains, *P. pnomenusa* produced comparable amounts of biofilm-associated biomass.

Discussion

We report the case of a possible PVE due to the uncommon biofilm-producing pathogen *P. pnomenusa*.

Pandora spp. is an emerging pathogen in CF patients.⁵ Rarely BSI are reported in patients with other comorbidities.^{11–15} A fatal case of *P. pnomenusa* BSI was reported in a 30-year old patient suffering from end-stage pulmonary sarcoidosis with secondary nocardiosis and mycetomas.¹² The second published case of *P. pnomenusa* BSI occurred in 10-month old child with acute lymphoblastic leukemia that was successfully treated with imipenem and removal of the port-a-cath system.¹³ BSI with *Pandora* spp. other than *P. pnomenusa* were reported in immunocompromised patients after lung,¹⁴ liver transplantation¹¹ and cystic fibrosis.¹⁵

To date a possible PVE due to *Pandora* spp. has not been reported. Certain elements of this case suggest a possible endocarditis,¹⁶ whereby neither the TOE nor the PET/CT could ultimately confirm the diagnosis. The patient was treated with piperacillin/tazobactam although piperacillin/tazobactam was reported to be resistant in *Pandora* spp thus far.²² *Pandora* spp. are known to harbor OXA family carbapenemases, and OXA-62-like carbapenemases were described in *P. pnomenusa*.²² However, we identified an OXA-151 carbapenemase with two amino acid replacements V180L and S271A. Interestingly, these AA “replacements” are found in OXA-62, while L18P, A33T and T245A are discordant from OXA-62 but found in OXA-151.²² Nonetheless, the strain was highly resistant to meropenem but susceptible to imipenem.

We demonstrated that *P. pnomenusa* is capable of forming biofilms. Thus far, this had been demonstrated solely for *Pandora pulmonicola*.⁹ For many pathogens, biofilm formation contributes to virulence in CF lungs.⁸ Arguably, biofilm formation also facilitates the colonization of intravenous catheters and promotes endocarditis.^{8,16} With this case report, we hope to expand the current understanding of *Pandora* spp. but further research is warranted, especially to prove a causal link

Figure 1 *Pandora pneumoniae* forms biofilm on abiotic surfaces. Overnight fully aerated planktonic culture of *P. pneumoniae* patient isolates (strains #1 and #2) as well as the biofilm-producing species *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 (ie, positive controls) were diluted 1:100 in fresh LB medium and grown at 37°C, under agitation, to exponential phase. Cultures were then inoculated into tissue culture treated imaging chambers and let form biofilms at 37°C. At given time-points, biofilms were washed using PBS to remove non-sessile bacteria and, when indicated, fixed with 4% paraformaldehyde. Bacterial membranes were subsequently stained with the fluorescent probes FM 4-64FX to visualise the biofilm biomass by confocal laser scanning microscopy. 3D reconstructions of the Z optical section stacks ($x = 260 \mu\text{m}$, $y = 260 \mu\text{m}$, $z = 30 \mu\text{m}$) indicates the formation of dense and rough biofilms of distinct morphotypes. *K. pneumoniae* produced small, uniformly distributed aggregates. *P. aeruginosa* and *P. pneumoniae* developed large aggregates, distributed across the whole well.

Figure 2 Quantitative biofilm assay. Blank wells containing Müller-Hinton-broth were used to determine background optical density (OD). Each bar represents the arithmetic mean of independent triplicates with at least four measurements each. Standard deviation is depicted as whiskers. Represented above are the Bonferroni adjusted p-values obtained by t-test.

between biofilm formation by *Pandoraea* spp. and virulence in CF patients, CLABSI and endocarditis.

Sequence Information

Sequences are available at the European nucleotide archive.

Strain 1ERS5743847

Strain 2ERS5743848

Ethical Approval

Ethical approval was not required to publish the case details.

Informed Consent

The patient has given written permission to publish this case report.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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