Infection and Drug Resistance

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CASE REPORT

Sepsis Due to *Pandoraea sputorum* Infection After Multiple Trauma in a Non-Cystic Fibrosis Patient: A Case Report from Southeast China

Zhiyi Ma[1,*], Xin Zou[1,*], Jinhao Lin[2,*], Chunchun Zhang[3], Shuhua Xiao[4]

[1]Department of Pulmonary and Critical Care Medicine, Longyan First Affiliated Hospital of Fujian Medical University, Longyan, People's Republic of China; [2]Department of Pulmonary and Critical Care Medicine, Longyan People's Hospital, Longyan, People's Republic of China; [3]Department of Laboratory Medicine, Longyan People's Hospital, Longyan, People's Republic of China; [4]Department of Intensive Care Unit, Longyan People's Hospital, Longyan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jinhao Lin, Department of Pulmonary and Critical Care Medicine, Longyan People's Hospital, No. 31 Denggaoxi Road, Longyan, People's Republic of China, Tel +865973102968, Email playplayone2659@163.com

Abstract: *Pandoraea sputorum* (*P. sputorum*) infection is of great concern as these gram-negative bacillus species are multidrug-resistant and usually isolated from the patients' respiratory tract suffering from cystic fibrosis (CF). A few cases of infection have also been reported in non-CF patients due to its rare pathogenic nature with unclear and overlapping clinical, biochemical, and microbiological characteristics with other species. Here, we report an unusual case of a 46-year-old non-CF female, who presented with multiple pelvic fractures, acute traumatic brain injury, multiple rib fractures, and multiple burns (18% of the total body surface area, II°) by the collapse of a brick kiln, suffered from *P. sputorum* sepsis due to wound infection. Pandoraea species were isolated both from her blood and wound secretion. Antibiotic susceptibility testing indicated susceptibility to imipenem, tetracyclines, sulfamethoxazole, and ampicillin/sulbactam but resistance to meropenem, quinolones, aminoglycosides, and other beta-lactams. 16S ribosomal RNA (rRNA) PCR assays and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were used to confirm the bacteria as *P. sputorum*. After effective anti-infection of intravenous antibiotics (imipenem 1.0 Q8H with tigecycline 50 mg Q12H for 14 days), wound care, and other comprehensive treatment for two months, the patient improved and was discharged from the hospital eventually. After reviewing the literature, we observed that the susceptibility results of Pandoraea species were often multidrug-resistant and had a unique pattern of being resistant to meropenem but sensitive to imipenem. Biofilm formation, carbapenemase production, and unique gene procession differed from the environmental isolates could help explain its resistance. This case report highlights the potential virulence of *Pandoraea species* as a pathogen in patients with no underlying disease. Although they are often multi-resistant, imipenem can be a preferred treatment for Pandoraea species in the earliest identification steps.

Keywords: *Pandoraea sputorum*, sepsis, multidrug-resistant, non-cystic fibrosis, multiple trauma

Introduction

Initially, Coenye et al[1] described the characteristic features of *Pandoraea* species as aerobic, non-spore-forming, non-nitrate-reducing, non-lactose-fermenting, Gram-negative rods with a single polar flagellum that are mostly isolated from cystic fibrosis (CF) patients. To date, 7 named Pandoraea species and 4 unnamed genomospecies have been identified.[1–3] The source of Pandoraea species' isolation is vast; it can be isolated even from environmental samples such as soil, water, and powdered milk, as well as from patients' clinical samples like wound sites, blood, lung tissue, and urine.[4–6] Six clinically relevant *Pandoraea* Species (*Pandoraea apista, Pandoraea norimbergensis, Pandoraea pulmonicola, Pandoraea pnomenusa, Pandoraea sputorum*, and *Pandoraea fibrosis*) have been isolated from human specimens, which were predominantly from the respiratory tract of cystic fibrosis (CF) patients in European and North America.[4–8] Their potential harm is still unclear and under-investigated, they have been demonstrated to cause decreased lung function and cross-infection activity in patients with CF.[3,4] In addition to lung

Received: 9 September 2022
Accepted: 17 November 2022
Published: 2 December 2022

colonization, a few cases of invasive infections in different sites were also reported worldwide, mostly in non-CF patients with underlying diseases.[5] Above all, the *Pandoraea* species has grabbed the attention of clinicians due to its high resistance to multiple drugs and and invasive fatality if patients have not received appropriate antibiotics.[9,13,19] Here, we investigated a case of a 46-year-old female patient without underlying disease infected with sepsis by *Pandoraea sputorum* (*P. sputorum*) after multiple pelvic fractures and burns. The *Pandoraea* species were isolated from both her blood samples and wound secretion. Further detection using the 16S ribosomal RNA (rRNA) gene sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) confirmed the bacteria as *P. sputorum*. Informed consent was obtained from the patient to publish this case report and accompanying images, and institutional review board (IRB) approval was granted.

Case Presentation

On August 12th, 2018, a 46-year-old female patient was admitted to the emergency ward of the People's Hospital (Longyan, China) due to severe trauma caused by the collapse of a brick kiln. She had no history of any chronic or infectious diseases earlier. An initial physical examination found that her vital signs were stable and within normal range. She was still conscious but with limited activity. Further examination revealed that her whole body was burned with blisters (2% of the abdomen, 3% of the right waist, 4% of the back, 7% of the legs, and 2% of the arms). Her right eyelid was turned and exploited, while her left forehead skin and labium were exuded with blood because of the contusion. The back side of her left hand was injured, with exposure of bones and tendons. Her iliac region was also swollen with skin bruises and congestion, she felt obvious pain, and her pelvic extrusion and separation tests were positive. An emergency computer tomography (CT) of her brain, chest, and abdomen was recommended. Chest CT showed mild dilation in the right middle lobe, the right lower lobe, and the left lower lobe (Figure 1). Blood analysis showed a white blood cell (WBC) count of 8.13×10^9/L, hemoglobin (HGB) of 130g/L, and platelet (PLT) count of 174 $\times10^9$/L. After clinical examination and imaging studies, she was diagnosed to have multiple fractures (right sacral promontory, bilateral superior pubic ramus, right inferior pubic ramus, right 1–5th rib), multiple lacerations (scalp, right canthus, right eyelid), acute traumatic craniocerebral injury, superficial skin contusion (right zygomatic, left frontal), and multiple burns (II°, 18% of the total body surface area [TBSA]). A comprehensive treatment of debridement, analgesics, anti-infection (levofloxacin 0.4 QD+ cefazolin 1.0 Q12H, I.V.), hemostasis, tetanus prevention, wound exposure, and rehydration was carried out on the first day. On hospital day 2, her blood pressure dropped to 85/50mmHg, and HGB reached a nadir of 104g/L. We suspected the patient was in a state of hemorrhagic shock due to intra-abdominal hemorrhage and pelvic fracture and transferred her to the intensive care unit (ICU) immediately. She received enhanced dilatation and rehydration treatment, anti-infection (ceftazidime 1.0 Q8H + levofloxacin 0.4 QD, I.V. for 11 days), analgesia, nutritional support, single room isolation, appropriate braking, wound disinfection, external application of burn cream, and exposure treatments. Her situation improved gradually, with stable blood pressure. But she had an intermittent moderate fever (38.2°C of the highest level) without any symptoms of respiratory or urinary tract infection. There was still much exudation from the wound on her back and upper right leg. Bilateral blood samples were collected to culture both aerobic and anaerobic bacteria on the 7th day. Her blood analysis still showed a normal WBC count of 3.75×10^9/L, an elevated CRP of 145 mg/L, and a mildly elevated PCT of 0.81 ng/mL. Four days later, her first blood culture reports revealed the presence of bacterial *Pandoraea* species (Figure 2). In vitro susceptibility testing with the Vitek2 compact system

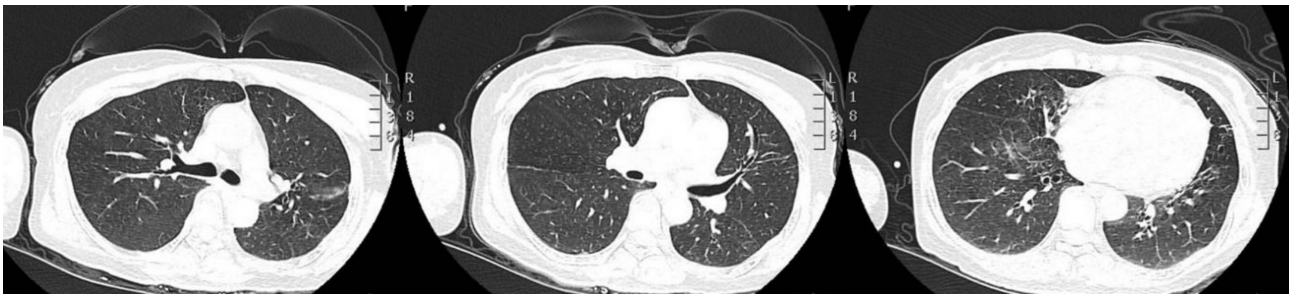

Figure 1 The first chest CT scan of the patient when she was sent to the emergency.

Figure 2 Bacteria colony of Pandoraea species cultured on Columbia blood plate for 48 hours.

(bioMerieux, Marcy-I'Etoile, France): interpreted according to the 2018 Clinical and Laboratory Standards Institute (CLSI) criteria for non-fermentative isolates: showed the isolate to be only sensitive to sulfamethoxazole, imipenem, and ampicillin/sulbactam (Table 1). On hospital day 12, her body temperature rose to a higher level of 38.9°C. The second bilateral blood sample for bacterial culture was collected again. Repeat blood analysis still showed a normal white blood cell count of 4.21×10^9/L, an elevated C-reactive protein (CRP) of 93.7 mg/L, and mildly elevated procalcitonin (PCT) of 0.74 ng/mL. A repeat CT scan of the chest and abdomen showed no new pulmonary shadows but still obvious soft tissue edema and subcutaneous swelling in the right abdominal wall and the right iliac region. The bacterial *Pandoraea* species presented in her blood was considered the responsible pathogen for her infection. On hospital day 13, we changed her anti-infection treatment to imipenem 1.0 Q8H + tigecycline 50 mg Q12H I.V. Four days later, her second blood culture reports again confirmed the presence of *Pandoraea* species, which were sensitive to the three antibiotics mentioned above and minocycline (Table 1). The source of her sepsis was presumed to be wound-associated, as no respiratory or urinary infection happened during the hospital day. On day 18, bacteria culture and the high-throughput gene detection of pathogenic microorganisms were performed using the samples from her wound secretion. Although the results of high-throughput gene sequencing of pathogenic microbial DNA only showed DNA sequences of Staphylococcus epidermidis and Candida parapsilosis, *Pandoraea* species was again cultured six days after sample collection (Table 1). 16S ribosomal RNA (rRNA) gene sequencing of bacteria from her first blood culture sample confirmed it as *Pandoraea* species, which has the first-highest similarity to *Pandoraea sputorum* (99.89%), the second-highest similarity to *Pandoraea vervacti* (99.66%), and the third-highest similarity to *Pandoraea* oxalalivorans (99.66%). The MALDI-TOF MS method was further applied to confirm it as *Pandoraea sputorum*. No bacteria were isolated from her blood sample collected on the hospital days 17th and 23rd. After 14 days of treatment with appropriate anti-infection and wound care, the patient's condition improved with a normal body temperature, a normal CRP of 6.0mg/L, a normal PCT of 0.11ng/mL, and scanty wound exudation (Figure 3). Her situation was stable enough to shift her to the Department of Burn and Plastic Surgery to continue her wound reparation. Two months later, she was discharged from our hospital, her wounds and fractures healed, and she could move freely. The clinical manifestations, significant examination results, and related antibiotic treatments have been presented as a timeline (Figure 4).

Table 1 Antibiotic Susceptibility of Pandoraea Species from Blood and Wound Secretion Samples in Our Case

Samples	Blood (8.19)		Blood (8.23)		Wound Secertion (8.24)	
Antibiotic/Suspecibility	**MIC(mg/l)/ KB(mm)**	**S/R**	**MIC(mg/l)/ KB(mm)**	**S/R**	**MIC(mg/l)/ KB(mm)**	**S/R**
Trimethoprim/Sulfamethoxazole	<=20	S	<=20	S	<=20	S
Piperacillin	≥128	R	≥128	R	–	–
Piperacillin/tazobactam	≥128	R	–	–	–	–
Cefazolin	≥64	R	≥64	R	≥64	R
Cefuroxime sodium	≥64	R	32	R	32	R
Ampicillin	≥32	R	≥32	R	≥32	R
Cefuroxime axetil	≥64	R	32	R	32	R
Cefotetan	≥64	R	≥64	R	≥64	R
Ceftazidime	≥64	R	≥64	R	≥64	R
Ceftriaxone	32	R	16	I	16	I
Cefepime	≥64	R	32	R	32	R
Aztreonam	≥64	R	≥64	R	≥64	R
Imipenem	21mm	S	<=1	S	2	S
Meropenem	≥16	R	≥16	R	≥16	R
Amikacin	≥64	R	≥64	R	–	–
Gentamicin	≥16	R	≥16	R	≥16	R
Tobramycin	≥16	R	≥16	R	≥16	R
Ciprofloxacin	≥4	R	≥4	R	≥4	R
Levofloxacin	≥8	R	4	I	4	I
Ampicillin/sulbactam	8	S	4	S	4	S
Tetracycline	–	–	14mm	I	–	–
Minocycline	–	–	25mm	S	–	–

Notes: Susceptibility testing with the Vitek2 compact system (bioMerieux, Marcy-l'Etoile, France): interpreted according to the 2018 Clinical and Laboratory Standards Institute (CLSI) criteria for non fermentative isolates.
Abbreviations: KB, Kirby-Bauer disk diffusion method; MIC, minimal inhibitory concentration; S, susceptible; R, resistant; I, intermediate.

Discussion

The *Pandoraea* species was first isolated by Coenye et al[1] from a mixed population of other well-known CF pathogens. This *Pandoraea* species belongs to the β-subclass of Proteobacteria, including Pseudomonas. It is closely related to two Gram-negative rods of the genera Burkholderia and Ralstonia species that closely resemble their characteristic features. The source of isolation of *Pandoraea* species is vast; it can be isolated even from environmental samples such as soil, water, and powdered milk, as well as from patients' clinical samples like wound sites, blood, lung tissue, and urine.[5,6] In most of the epidemiology cases of CF, *Pandoraea* species was isolated from respiratory samples in Australia, Europe, and America,[4,7,8] and very scanty reports are available from Asia, which may consist with the epidemiology of CF and imply that the detection of *Pandoraea* spp. might be underestimated owing to the difficulty of routine diagnostic tests. *Pandoraea* species has been considered a significant pathogen because it has the infectious nature of causing cross-infection among CF patients, like other well-known bacteria such as *Burkholderia cepacia* complex, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*.[3,4]

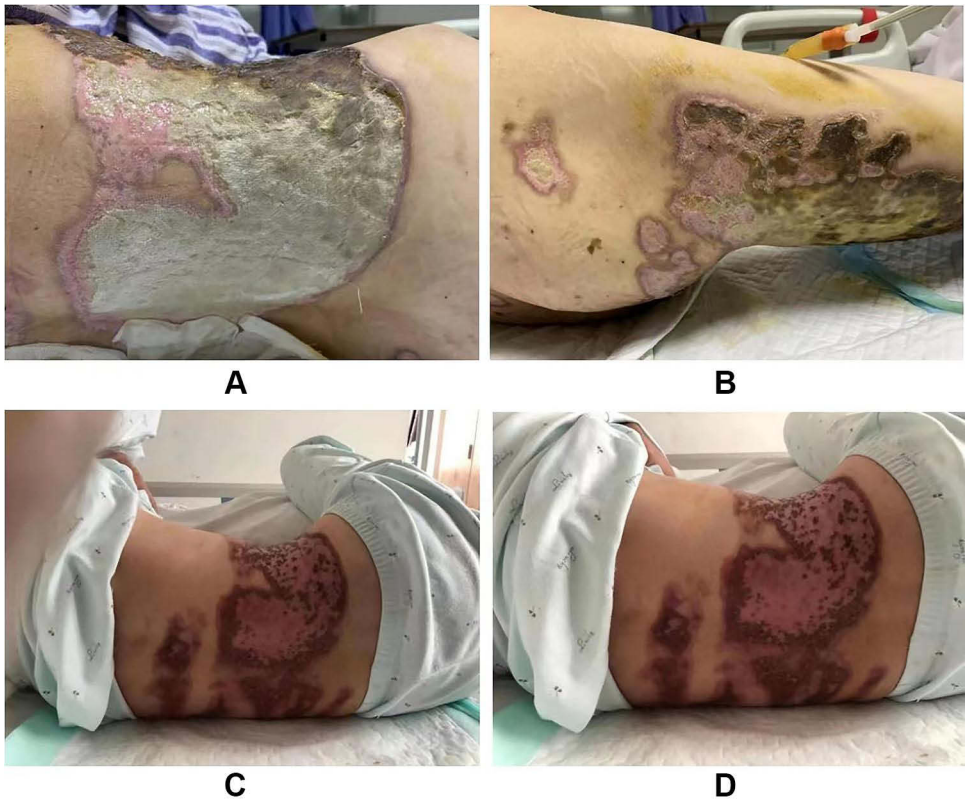

Figure 3 Wound situation of the patient before and after treatment. (**A**) Skin wound of the lower back; (**B**) skin wound of the right thigh; (**C** and **D**) Skin wound of the lower back after 2-months' treatment.

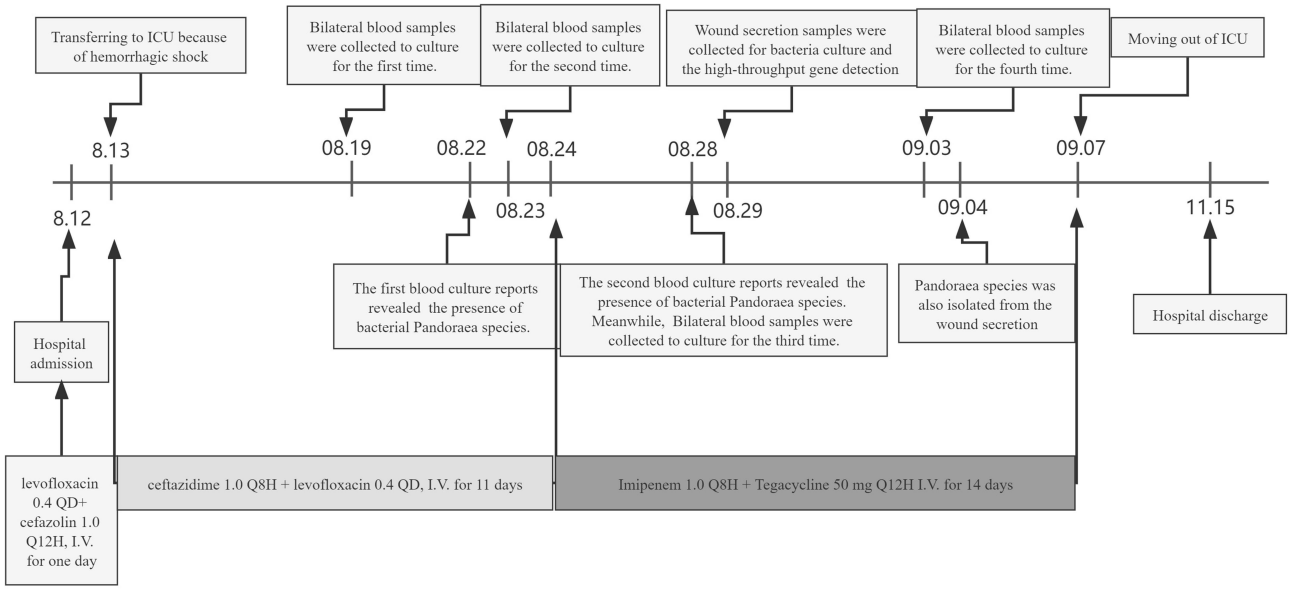

Figure 4 The treatment timeline showed clinical manifestations, significant examination results, and related antibiotic treatments of the case.

Recently, *Pandoraea* species was observed to be more likely to cause invasive infections in non-CF patients. Only 21 cases of *Pandoraea* infection (including ours) in non-CF patients were reported. Among the 21 isolated cases, seven were *Pandoraea pnomenusa*,[5,9–12] five were *Pandoraea* Apista,[5,13–15] three were *Pandoraea sputorum*,[16,17] two were *Pandoraea norimbergensis*,[1] and four were unidentified *Pandoraea* species.[5,18,19] Ten (47.6%) were isolated from blood, eight from the respiratory samples, one from the maxillary sinus,[5] one from the hemodialysis catheter,[16] and one from the skull base[14] (see Table 2). To our

Table 2 Case Reports Infected or Colonized with Pandoraea Species in Non-CF Patients

Author and Year	Age/ Gender/Country	Clinical Records (Underlying Diseases)	Strains	Sources	Antibiotic Susceptibility Test (Method/Interpretive Susceptibility Criteria)	Antibiotic Treatment	Outcomes
Coenye 2000[1]	NG/Belgium	NG	P. norimbergensis	Blood	NG	NG	NG
	NG/Sweden	NG	P. norimbergensis	BALF	NG	NG	NG
Daneshvar 2001[5]	66 y/ F/ USA	COPD	P. apista	Blood	NG	NG	NG
	75 y/ F/ USA	NG	P. apista	BALF	NG	NG	NG
	46 y/ M/ USA	Bacteremia	P. Pnomenusa	Blood	NG	NG	NG
	NG/ F/ USA	NG	Pandoraea species	Maxillary sinus	NG	NG	NG
	76y/ M/ USA	Bacteremia	P. Pnomenusa	Blood	NG	NG	NG
	49y/ M/ USA	NG	P. Pnomenusa	Blood	NG	NG	NG
	71y /F/ USA	Pneumonia	Pandorae species	Sputum	NG	NG	NG
Stryjewski 2003[9]	30y/M/ USA	Sepsis, MODS (lung transplantation)	P. Pnomenusa	Blood	S: IPM, MNO R: TEP, CAZ, MEM, AMK (KB/ NG)	IPM	Dead
Falces 2016[10]	10m/NG./Spain	Catheter-associated bacteremia (acute lymphoblastic leukemia)	P. Pnomenusa	Blood	NG	IPM	Alive
GAO 2018[18]	23 days/ M/China	Bacteremia (Neonatal jaundice)	Pandorae species	Blood	S: SXT, IPM, SAM, TCY I: TZP R: PIP, C, FEP, ATM, MEM, AMK, GEN, TOB, CIP, LVX (KB/ CLSI for Pseudomonas aeruginosa)	AMP+CSL	Alive
Monzón 2018[16]	71y /M / Spain	Colonization of CVC (Multiple myeloma, end-stage renal disease, hypertension, and type 2 diabetes mellitus)	P. sputorum	Hemodialysis catheter	NG	Removed the CVC	Alive
Lin 2019[13]	44y/M /China	Pneumonia (multiple injuries and coma after a brain injury)	P. Apista	Sputum	NG	MEM (2 g IV, 8 q8h) and VAN (1 million IU IV, q12h)	Dead
Xiao 2019[17]	43y/M /China	Bacteremia (Allogeneic liver transplantation with immunosuppressive therapy)	P. Sputorum	Blood	S: PIPP, TZP, CRO, IPM, CIP, LVX, TCY, MNO I: AMK R: CAZ, ATM, MEM, GEN, TOB, FEP (MIC/ other non-Enterobacteriaceae)	IPM (500 mg IV, q8 h) and TZC (2g IV, q12 h)	Alive
Patil 2021[14]	67/M /India	Skull base osteomyelitis	P. Apista	Skull base	NG	CIP (750mg, bid)	Alive

Singh 2021[15]	72y/M /India	Bacteremia (ARDS, COVID-19 Positive, diabetic)	P. Apista	Blood	S: SXT, IPM, MNO R: TEP, CAZ, MEM, AM (NG/ CLSI guidelines)	IPM (1g IV, q6 h)	Alive
Bodendoerfer 2021[11]	37y/M /Switzerland	CLABSI, PVE (injecting drug user)	P. pnomenusa	Blood	S: SXT, TZP, FEP, IPM, CIP, LVX, TCY, MNO R: PIP, AMP, CAZ, MEM, GEN, TGC (KB/EUCAST for Pseudomonas aeruginosa)	TZP *3 weeks; continuing with SXT*3 weeks	Alive
Dlewati 2021[19]	69y/M /USA	VAP, ARDS (COVID-19 positive ventilation)	Pandorae species	Respiratory secretion	S: IPM, AMK, CIP, LVX, MNO I: GEN R: CAZ, FEP, ATM, MEM (MIC/ CLSI guidelines)	MEM and MIF	Dead
Cubides-Diaz 2022[12]	55y/M /Colombia	Pneumonia (COVID-19 positive, ventilation)	P. pnomenusa	Respiratory secretion	S: SXT (KB/CLSI for Burkholderia cepacia complex) I: CIP (KB/CLSI for Pseudomonas aeruginosa)	CIP (400mg IV, bid) +SXT (240/1200 mg po, tid)*14 days	Alive
Our case	46y/F /China	Bacteremia with wound infection (multiple pelvic and rib fractures, acute traumatic brain injury, and multiple burns)	P. sputorum	Blood and wound secretion	Referring to Table 1 (MIC and KB/ CLSI for non-fermentative bacteria)	IPM (1.0q IV, 8h) + TGC (50mg IV, q12h)*14 days	Alive

Abbreviations: NG, not given; BALF, Bronchoalveolar Lavage Fluid; F, female; COPD, chronic obstructive pulmonary disease; M, male; MODS, multiple organ dysfunction syndromes; CVC, central venous catheter; IV, intravenous; ARDS, acute respiratory distress syndrome; CLABSI, central venous catheter-associated bloodstream infection; PVE, prosthetic valve endocarditis; VAP, ventilation-associated pneumonia; S, sensitive; R, resistant; I, intermediate; KB, Kirby-Bauer disk diffusion method; MIC, minimal inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee for Antimicrobial Susceptibility Testing; SXT, Trimethoprim/Sulfamethoxazole; PIP, Piperacillin; TZP, Piperacillin/tazobactam; CZO, Cefazolin; CXM, Cefuroxime; AMP, Ampicillin; CTL, Cefotetan; CAZ, Ceftazidime; CRO, Ceftriaxone; FEP, Cefepime; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; AMK, Amikacin; GEN, Gentamicin; TOB, Tobramycin; CIP, Ciprofloxacin; LVX, Levofloxacin; SAM, Ampicillin/sulbactam; TCY, Tetracycline; MNO, Minocycline; TGC, Tigecycline; AMP, Ampicillin; CSL, Cefoperazone/sulbactam; VAN, Vancomycin; TZC, Ceftriaxone/tazobactam; MIF, Micafungin.

https://doi.org/10.2147/IDR.S388520 DovePress

knowledge, only four non-CF patients infected by *Pandoraea* species were reported from China. Besides all the above information, our case was considered the third case of *Pandoraea sputorum*, isolated from non-CF patients worldwide. By reviewing all the cases reported to be infected by *Pandoraea* species from non-CF patients, we found that most of the cases had underlying susceptibility factors, which allow the host to enter into a situation of mild or severe immunosuppression (such as post-transplantation or underlying diseases), leading to disease susceptibility. Under specific conditions, *Pandoraea* species opportunistically invade the body and cause infections. However, the patient in our case was healthy and had no underlying disease before onset, which is different from the two cases of bloodstream infection of *Pandoraea sputorum* reported in previous literatures. Since *Pandoraea* species were isolated from the blood of the patient and wound secretion, her skin and soft tissue were infected by the microbes present in the environment, and they were considered the source of her sepsis.

By conventional culture medium method, *Pandoraea* species are frequently misdiagnosed and misidentified as Burkholderia or Ralstonia species.[4,5] Therefore, to avoid such confusion and misdiagnosis, molecular analysis such as 16S rRNA sequence analysis is recommended for further confirmation.[1] However, the limitation of this method has been reported when differentiating the *Pandoraea* species.[18] Other scientists have implemented gyrB gene sequences to identify species, but there is a lag in identifying closely related species as well.[2,20] Further, some researchers[21] have suggested the MALDI-TOF MS as a quicker, easier, and more reliable method for rapidly identifying closely related species. Through 16S rRNA analysis, our study confirmed that *Pandoraea* species is the causative pathogen. However, we could not further differentiate the species because of its close similarity with *P. sputorum* (99.89%), *P. vervacti* (99.66%), and *P. oxalalivorans* (99.66%). Finally, through the MALDI-TOF MS method, we identified and confirmed the causative strain as *Pandoraea sputorum*.

The pathogenesis and potential virulence of *Pandoraea* species are still unclear. Some researchers have found that chronic bronchopulmonary colonization by *P. apista* or *P. sputorum* had a close relationship with frequent exacerbations, and a decreased activity of lung function is observed in CF patients.[21] Further research has demonstrated that infection of *Pandoraea* species could induce a robust pro-inflammatory response in lung epithelial cells, with elevated inflammatory markers such as interleukin-6 (IL-6) and interleukin-8 (IL-8).[22]

Pandoraea species often resist antibiotics such as β-lactam agents, quinolones, and aminoglycosides. However, the susceptibility results vary between different stains. Some of the isolates were reported to be sensitive to trimethoprim-sulphamethoxazole,[11,12,15,18] minocycline,[17,19] piperacillin/tazobactam,[11,17] ampicillin/sulbactam,[18] ciprofloxacin,[11,17,19] and tetracycline.[11,17,18] Interestingly, almost all of the *Pandoraea* species were reported to be resistant to meropenem but sensitive to imipenem, which was the opposite of most Gram-negative non-fermenting bacteria (see Table 2). Biofilm formation and carbapenemase production by *Pandoraea* species may be related to increased antibiotic resistance.[23] When comparing the genome sequence of clinically isolated *P. pnomenusa* with the environmental isolates, researchers found that clinical isolates possessed many unique genes that differed from the environmental isolates, which could help to explain its resistance to multiple antibiotics.[24] To date, no recommended criteria guideline is available for the susceptibility of *Pandoraea* species. We used the 2018 CLSI criteria for non-fermentative isolates to judge the results. In our case, the susceptibility results were similar to the data available in the literature.

This case report highlights the potential virulence of *Pandoraea* species as an opportunistic pathogen from the environment in non-CF patients. Identification of these species needs molecular analysis, such as 16s RNA sequencing and MALDI-TOF MS analysis. The limitation is the lack of infection treatment guidelines and no appropriate CLSI susceptibility information for Pandoraea until now. Although different methods and interpretive susceptibility criteria result in unavoidable bias, the antimicrobial susceptibility profiles of *Pandoraea* species are often multidrug-resistant to antibiotics such as β-lactam agents, quinolones, and aminoglycoside. Almost all the Pandoraea species have a unique pattern of being resistant to meropenem but sensitive to imipenem, which might be a preferred practical treatment choice for *Pandoraea* species in the earliest identification steps.

Data Sharing Statement

The data is available upon reasonable request to the corresponding author.

Acknowledgment

We thank Medjaden Inc. for the scientific editing of this manuscript.

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

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