Case Report

Correspondence

Stephanie H. Stovall
stovallstephanieh@uams.edu

Received 22 January 2010

Accepted 24 March 2010

Nosocomial infection with *Asaia lannensis* in two paediatric patients with idiopathic dilated cardiomyopathy

Stefan Juretschko,^{1,2} Toni K. Beavers-May¹ and Stephanie H. Stovall^{1,2}

¹ACH, Arkansas Children's Hospital, Little Rock, AR 72202, USA

²UAMS, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

This is the first report, to our knowledge, of two temporally and geographically related nosocomial *Asaia lannensis* infections in a paediatric setting. Two patients with idiopathic dilated cardiomyopathy awaiting cardiac transplantation developed bacteraemia during their hospital stay. The physical location of both patients, the temporal association of infections, as well as the isolation of two identical pathogens suggested a nosocomial transmission. Commonly used identification methods and instruments failed to identify the isolated pathogens and only 16S rRNA gene sequencing provided definitive identification. These isolates of *A. lannensis* showed an unfavourable susceptibility pattern including resistance to carbapenems, all β -lactam agents and fluoroquinolones.

Introduction

In the year 2000, the genus *Asaia* was introduced by Yamada *et al.* (2000) with a single species, *Asaia bogorensis*. Two more species were added in 2001 and 2004, *Asaia siamensis* and *Asaia krungthepensis*, respectively (Katsura *et al.*, 2001; Yukphan *et al.*, 2004). A fourth species, *Asaia lannensis*, was described in 2008 (Malimas *et al.*, 2008). All species of the genus *Asaia* are described as acetic acid bacteria within the family *Acetobacteraceae* and the class *Alphaproteobacteria* (Huong *et al.*, 2007). No or weak oxidation of ethanol to acetic acid and no or very weak growth in the presence of 0.35 % acetic acid is common for all four species of *Asaia* (Malimas *et al.*, 2008; Yamada *et al.*, 2000). All species were originally isolated from tropical flowers or plant-derived materials in SE Asia, such as the orchid tree (*Bauhinia purpurea*), plumbago (*Plumbago auriculata*), heliconia flowers or fermented glutinous rice (Katsura *et al.*, 2001; Malimas *et al.*, 2008; Yamada *et al.*, 2000; Yukphan *et al.*, 2004). A case of spoilage of fruit-flavoured bottled water was attributed to *Asaia* species (Moore *et al.*, 2002). Strains of the genus *Asaia* have also been isolated from mosquito intestinal tracts and reported to be versatile symbionts in sugar-feeding insects (Crotti *et al.*, 2009).

The only two species that have been associated with human disease are *A. bogorensis* and *A. lannensis*. In 2004, *A. bogorensis* was isolated from a patient with peritonitis

(Snyder *et al.*, 2004), and a case of bacteraemia in a patient with a history of intravenous drug use was reported in 2006 (Tuuminen *et al.*, 2006). *A. lannensis* was isolated in 2009 in a case of a bloodstream infection after bone marrow transplantation (Abdel-Haq *et al.*, 2009).

In all cases, the final identification of *Asaia* species was done by molecular diagnostic methods with the help of 16S rRNA gene sequencing. This demonstrates the problem with identification of unusual environmental organisms not previously described as human pathogens. A lack of sufficient databases in automated identification systems contributes to this dilemma.

Here we describe what we believe to be the first report of two temporally and geographically related cases of paediatric nosocomial *A. lannensis* infections. The Institutional Review Board of the University of Arkansas for Medical Sciences classified this study as exempt from full review.

Case reports

Case 1

Patient A, a 15-month-old African American female with congestive heart failure due to idiopathic dilated cardiomyopathy, was admitted to Arkansas Children's Hospital (ACH) on 9 March 2007. Her initial presentation to a referring institution 2 weeks previously included symptoms consistent with a viral upper respiratory infection. Evaluation at that time suggested pneumonia and cardiomegaly. Subsequent evaluation revealed poor cardiac function by echocardiography. She was transferred to ACH for a higher level of cardiac care including cardiac

Abbreviations: ACH, Arkansas Children's Hospital; PICC, percutaneous intravascular central catheter.

The GenBank/EMBL/DDBJ accession numbers for the sequences obtained in this study are HM234122 (isolate H4348) and HM234123 (isolate H4349).

transplant evaluation. A percutaneous intravascular central catheter (PICC) was placed on hospital day 1 for infusion of inotropic medications. On hospital day 17 (25 March 2007), she developed fever (39.8 °C) and mild hypotension. A blood culture from the PICC was collected and incubated in BACTEC 9240 (Becton Dickinson). Over the subsequent 24 h, she developed a mild decrease in her total serum white blood cell count (6340 μl^{-1} ; normal for age 6000–17 500 μl^{-1}) compared to her baseline in the preceding 5 days (10 300 μl^{-1}) with an increase in band forms on manual differential counts (28 %). Meropenem and vancomycin were empirically administered for presumed bacteraemia. After 20 h, the aerobic blood culture bottle turned positive and Gram-negative bacilli were observed on Gram stain. Microbiological work-up, identification and susceptibility testing of this organism are described below. Vancomycin was immediately discontinued after Gram-stain results were reported. A second blood culture was obtained from the PICC 17 h after the initial culture became positive. After 38 h of incubation, this second blood culture also became positive depicting Gram-negative bacilli. The PICC was subsequently removed, but the catheter tip was not sent for culture. There was no clinical evidence of exit site infection. The patient became afebrile and the white blood cell count and differential returned to baseline within 24 h of PICC removal and within 48 h of antimicrobial treatment. All subsequent blood cultures ($n=3$) were negative. Meropenem treatment continued for 14 days.

Case 2

Patient B, a 5-year-old African American female with idiopathic dilated cardiomyopathy, was transferred to ACH for progressive myocardial dysfunction and cardiac transplantation evaluation. Her treatment included inotropic infusion via a double lumen PICC that was placed on 19 January 2007. On hospital day 99 (29 March 2007), she developed fever (39.9 °C) and mild hypotension (80/50 mmHg). Blood cultures were drawn from one lumen of the PICC and peripheral vein at that time. Vancomycin and meropenem were administered for presumed catheter-associated sepsis. Her complete blood count over the following 48 h revealed mild leukopenia (5000 μl^{-1} ; normal for age 5500–15 500 μl^{-1}) with numerous immature forms (up to 56 % band forms on manual differential counts) and thrombocytopenia (111 000 μl^{-1} ; normal 150 000–400 000 μl^{-1}). Blood cultures were repeated from one lumen of the PICC on March 30 and 31 (days 2 and 3). Aspiration of blood from the second lumen was attempted and failed. All blood culture bottles became positive within 30 h of incubation, with Gram-negative rods apparent on Gram stain. Vancomycin was discontinued and gentamicin was added. On March 31, the patient developed vomiting, severe abdominal pain, and progressive low cardiac output syndrome. She also had mildly elevated transaminases with alanine aminotransferase 53 IU l^{-1} (normal 10–25) and aspartate aminotransferase 72 IU l^{-1} (normal 15–50) and a

serum creatinine level of 0.8 mg dl^{-1} (normal 0.1–0.7 mg dl^{-1}), which was twice as high as her baseline creatinine. The PICC was subsequently removed; the catheter tip was not cultured. Signs of sepsis, including fever, resolved after removal of the PICC; however, the patient's cardiac status worsened requiring a centrifugal left ventricular assist device. Repeat blood cultures were negative after the PICC was removed, and antimicrobial therapy continued with both agents for 14 days.

Patients A and B resided in the same hospital unit but different private rooms. Both bloodstream infections occurred within a single week and caused similar symptoms and clinical signs.

Microbiological methods

Isolates from both patients showed the same micro- and macroscopic features. The non-haemolytic colonies grew on blood and chocolate agar, but not on Mueller–Hinton agar and initially not on MacConkey agar. The pink-pigmented colonies showed a convex appearance after 24 h growth and were 0.5–1 mm in diameter. Growth was observed at 25 °C and 35 °C, but not at 42 °C. Nutrient broth containing 6 % NaCl inhibited growth, and delayed growth was seen on nutrient broth without NaCl. On MacConkey agar, very small, light-pink colonies were observed after 72 h. Microscopic evaluation revealed short Gram-negative rods with rounded ends and no vacuoles. Motility was not observed.

The organisms were strictly aerobic, catalase-positive, and negative for oxidase, indole production, and urea, aesculin and gelatin hydrolysis. Nitrate and nitrite reactions were also negative. Triple-sugar iron reactions showed alkaline over alkaline slant/butt; H_2S production was negative. Acid production from D-glucose, D-xylose and D-mannitol was observed after 1–2 days; acid was produced from sucrose after 3–4 days. Weak acid production was seen on maltose after 1–2 days. Lactose showed an alkaline reaction.

Three commercially available identification systems were used for identification. The BD Phoenix (Becton Dickinson) identified both isolates as *Pseudomonas luteola* and, after repeat, *Acinetobacter baumannii* with confidence values of 99 % and 90 %, respectively. Antimicrobial susceptibilities could not be reported due to insufficient growth in the cartridge. These identifications were rejected due to incompatibilities with biochemical results. The Remel RapID NH kit identified the pathogen as *Gardnerella vaginalis* with a probability of >99.9 % in each case; however, biochemical results excluded this organism for true identification as well. Identification with the Vitek 2 system (bioMérieux) failed due to insufficient growth in the cartridge. Although the initial appearance resembled the genera *Roseomonas*, *Azospirillum* and *Methylobacterium*, mainly due to the pink pigment, definitive identification of these organisms was not possible with conventional bacteriological and phenotypic methods.

Thus, both isolates were submitted to the Centers for Disease Control and Prevention (CDC) for final identification. Extensive biochemical tests and 16S rRNA gene sequence analysis at CDC revealed that both strains were genotypically 100 % identical to each other based on 16S rDNA comparison. The closest phylogenetic match was the bacterium *Asaia lannensis* BCC 15733^T (AB286050) with 100 % sequence similarity. A 99.6 % similarity to *A. bogorensis* and 99.7 % to *A. krungthepensis* was observed (Fig. 1). The key nucleotide exchanges for the two isolates are on helix 28, position 838 ('T'→'C') and 848 ('A'→'G'), compared to *A. bogorensis*, thus building a strong stem connection to helix 22. An additional change in nucleotides was found in position 1274 ('G'→'A'). These three exchanges are consistent with the nucleotide composition of *A. lannensis* and differentiate between other species of *Asaia* (Malimas *et al.*, 2008; Yukphan *et al.*, 2006).

Results and Discussion

This report describes what we believe to be the first account of nosocomial transmission of *A. lannensis* occurring in two paediatric patients during concurrent hospital admissions. Both patients were treated with a 14 day regimen of meropenem; patient B also received gentamicin. No susceptibility guidelines are established for this organism, but to provide the clinicians with some treatment options, antibiotic susceptibility testing was performed by E-test (AB Biodisk; bioMérieux) on Mueller–Hinton plates with blood. The isolated strains of *A. lannensis* showed a remarkable resistance pattern. The MICs for all β -lactams, carbapenems and quinolones were extremely high, while lower MICs were observed for the tetracyclines and aminoglycosides (see Table 1). This is concordant with previous publications on similar species of the genus *Asaia* (Abdel-Haq *et al.*, 2009; Moore *et al.*, 2002; Snyder *et al.*, 2004; Tuuminen *et al.*, 2006). The isolated organisms grew sufficiently on Mueller–Hinton plates with blood, but failed to grow on BBL Haemophilus Test medium Agar (Abdel-Haq *et al.*, 2009), although Tuuminen *et al.* (2006) reported this to be a suitable medium for susceptibility

testing. A summary of tested antibiotics and a comparison to other isolates of *Asaia* species is depicted in Table 1. Despite the notable resistance of the organism to multiple antibiotics, neither patient had prolonged bacteraemia. Due to their clinical instability, both patients had their PICC removed very soon after they were found to be bacteraemic. Although the resistance to meropenem was not known at the time of administration, all cultures cleared and both patients recovered very quickly. This could be attributed primarily to the removal of the PICC, since patient A received only meropenem for treatment. Because patient B received both gentamicin and meropenem, determination of the efficacy of meropenem alone was not possible. Fortunately, both patients resolved signs of sepsis after PICC removal and did not have recurrence of bacteraemia with *A. lannensis* during the remainder of their hospital admission. Patient A was subsequently discharged from the ACH inpatient service in October 2007 with no recurrence of *Asaia* bacteraemia, and patient B was discharged in July 2007 with no further episodes of *Asaia* bacteraemia.

This report describes the occurrence of two hospital-associated and likely catheter-related infections with *A. lannensis*. Both cases occurred within the same week when both patients resided in the same hospital unit. The patients were housed in private rooms about 20 feet apart from each other. Both patients were African American girls without known underlying immunosuppression. Patients A and B developed similar symptoms and signs during their infections including mild leukopenia, bandaemia, fever and hypotension.

The Infection Control team initiated an immediate investigation after the organisms were identified as the same species. Unfortunately, the delay in identification of these organisms prevented the investigation from including equipment such as infusion pumps, pulse oximeter machines and feeding pumps because the patients and equipment had been physically relocated. A thorough investigation of the personal belongings and documentation in nursing notes, bedside charting and medical records revealed no distinct source connecting the two patients.

Fig. 1. Phylogenetic affiliation of patient isolates H4348 (patient A) and H4349 (patient B) among various species of the genus *Asaia* as inferred by neighbour-joining analysis on the basis of 16S rRNA gene sequence similarity values. Bootstrap values greater than 50 % for 1000 replicates are indicated at branch nodes. Strains of *Acetobacter* species and *Gluconobacter* species are placed as the outgroup. The scale bar is equal to 1 % estimated sequence divergence.

Table 1. Antimicrobial susceptibilities of *Asaia* species isolates according to Table 2B-5 in CLSI (2008) for non-*Enterobacteriaceae*, in MIC ($\mu\text{g ml}^{-1}$), with 0.5 McFarland after 24 h incubation

R, Resistant; I, intermediate; S, susceptible.

	<i>Asaia lannensis</i>						<i>Asaia bogorensis</i>	
	Patient A		Patient B		Abdel-Haq <i>et al.</i> (2009)		Tuuminen <i>et al.</i> (2006)	
Aztreonam	>256	R	>256	R	>256	R	>256	R
Cefepime	>256	R	>256	R	>256	R	>32	R
Ceftazidime	>256	R	>256	R	>256	R	64	R
Ciprofloxacin	>32	R	>32	R	>32	R	>32	R
Colistin	>256	R	>256	R	>256	R	1024	R
Imipenem	8	I	4	S	>32	R	—	—
Meropenem	>32	R	>32	R	>32	R	>32	R
Piperacillin/tazobactam	>256	R	>256	R	>256	R	>256	R
Ticarcillin/clavulanate	96	R	256	R	—	—	—	—
Tobramycin	3	S	4	S	0.5	S	8	I
Trimethoprim/sulfamethoxazole	>32	R	>32	R	>32	R	>32	R
Doxycycline	0.75	S	0.75	S	—	—	1.5	S
Minocycline	1	S	0.75	S	—	—	64	R
Vancomycin	>256	R	>256	R	—	—	—	—
Gentamicin	—	—	—	—	0.25	S	3	S
Ceftriaxone	14 mm	R*	12 mm	R*	>32	R	12	R

*Based on disc-diffusion values for *Acinetobacter* species (Table 2B-2 in CLSI, 2008).

Different staff placed all catheters and different individuals performed collection of blood cultures. Since the natural environment for *A. lannensis* is lilies (Malimas *et al.*, 2008), the presence of flowers in the unit during the time was ruled out. Moore *et al.* (2002) described *Asaia* species as the spoilage organism of fruit-flavoured bottled water. During the time of these infections, no beverages of this kind were discovered at the nurses' station, patients' rooms or visitors' waiting room. Contamination of inotropic infusion fluids could not be excluded, because the infusion bags were not available for subsequent testing.

The most likely source of infection was contamination of the PICC, since the removal of the indwelling device led to immediate relief. All previous reported cases with *A. bogorensis* and *A. lannensis* have one fact in common: the probable source of infection was an indwelling device, either a PICC, a central venous line catheter or other catheter (Abdel-Haq *et al.*, 2009; Snyder *et al.*, 2004; Tuuminen *et al.*, 2006). The patients in these cases also showed immediate improvement and resolution after removal of devices, despite inferior and perhaps inappropriate treatment (Abdel-Haq *et al.*, 2009; Snyder *et al.*, 2004).

This report shows the unfortunate situation that recently identified species and genera, such as *Asaia* species, are not included in the databases of automated identification systems such as BD Phoenix and Vitek 2. Rapid site-specific updates of databases with novel species and their biochemical composition is not possible and places the users of automated systems at the discretion of the

manufacturers' automated updates for these changes. When a laboratory encounters the same organism, prior to a manufacturer's update, the automated system repeats its initial error and provides no or inaccurate results.

This is demonstrated by the finding that all reported cases of infection with *Asaia* species required 16S rRNA gene sequence analysis for final identification. Using conventional methods for microbial work-up, misidentification with the genera *Roseomonas*, *Azospirillum* and *Methylobacterium* can occur. Limited information is available and only one current microbiology textbook describes the genus *Asaia* and its differentiation from similar genera, although not the species *A. lannensis* (Schreckenbeger *et al.*, 2007). However, at the time of isolation of these two strains this textbook was not yet available. Hence, we propose an additional flowchart for the work-up of pink-pigmented organisms, which is depicted in Table 2. *Asaia* species can be identified as non-fermentative, Gram-negative rods with pink-pigmented colonies. Their key biochemical characteristics are: catalase-positive, indole-negative, motility-positive, oxidase-negative and negative for hydrolysis of urea (Table 2).

A recent epidemiological study showed that the incidence of paediatric idiopathic dilated cardiomyopathy is higher in males (0.66/100 000) than in females (0.47/100 000), in black people (0.98/100 000) than in white (0.33/100 000) and in those residing in the Northeastern United States (0.64/100 000) compared to those in the central Southwestern United States (0.54/100 000) (Towbin *et al.*, 2006). The fact that our patients were African American

Table 2. Laboratory identification scheme and differential characteristics for pink-pigmented, non-fermentative, Gram-negative bacilli

Catalase	Indole production	Urea hydrolysis	Growth at 42 °C	Aesculin hydrolysis	Oxidase	Growth on MacConkey agar	Identification
Positive	Negative	Positive	Positive	Positive	Positive	Variable	<i>Azospirillum</i> species
				Negative	Negative*	Positive	<i>Roseomonas</i> species
		Negative	Negative	Negative	Negative	Positive	<i>Asaia</i> species
					Positive	Negative	<i>Methylobacterium</i> species

*Negative or weakly positive.

probably reflects the population of paediatric idiopathic dilated cardiomyopathy of our geographical region and not any predisposition relating to their race or underlying disease. Because of the temporal nature of these cases, it is likely that the infections were related to temporal and geographical events and not any underlying epidemiological factor.

Treatment including antimicrobial therapy and catheter removal was successful for both patients in this series. Unfortunately, no source for the infections could be found. More research is needed to determine the ultimate source of this organism in hospitalized patients and to develop better methods for quicker identification of the organism.

Acknowledgements

The authors would like to thank Leta Helsel and Arnold Steigerwalt from the Centers for Disease Control and Prevention, Atlanta, GA, USA, for providing expert guidance and genetic sequencing, which allowed for final identification of the organisms. Preliminary data were presented as a poster at the 2008 ECCMID meeting in Barcelona, Spain (<http://www.blackwellpublishing.com/eccmid18/abstract.asp?id=69042>).

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