


A population-based study of aerococcal bacteraemia in the MALDI-TOF MS-era

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Abstract The purpose of this study was to determine the incidence of aerococcal bacteraemia in the MALDI-TOF MS-era, to describe the clinical presentation and to determine the MIC values of aerococci for ten antibiotics. Aerococci in blood cultures were identified through searches in the laboratory database for the years 2012–2014. MALDI-TOF MS, sequencing of the 16S rRNA gene and a PYR test were used for species identification. Patients' medical charts were systematically reviewed. Etests were used to determine MIC values. Seventy-seven patients were identified (*Aerococcus urinae* $n=49$, *Aerococcus viridans* $n=14$, *Aerococcus sanguinicola* $n=13$ and *Aerococcus christensenii* $n=1$) corresponding to incidences of 14 cases per 1,000,000 inhabitants per year (*A. urinae*) and 3.5 cases per 1,000,000 inhabitants per year (*A. sanguinicola* and *A. viridans*). *A. urinae* was in pure culture in 61 %, *A. sanguinicola* in 46 % and *A. viridans* in 36 % of the cases. The *A. urinae* and *A. sanguinicola* patients were old and many had urinary tract disorders, and a majority had a suspected urinary tract focus of the bacteraemia. Eighty percent of the *A. urinae* patients were men. Five *A. urinae* patients were diagnosed with infective endocarditis. Six patients died within 30 days. Most isolates had low MICs to penicillins and carbapenems. MALDI-TOF MS has led to an increased identification of aerococcal bacteraemia. *A. urinae* remains the most common *Aerococcus* in blood cultures and in aerococcal IE.

Introduction

Aerococci have received increasing recognition as clinically important pathogens during recent years [1–5]. These catalase negative, Gram-positive cocci cause urinary tract infection (UTI) [6] and invasive infections like bacteraemia and infective endocarditis (IE) [1, 7–10]. Aerococci have often been misidentified with conventional methods of identification based on morphology and biochemistry [8, 11]. Matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS)—has been shown to be a reliable species identification method for aerococci [12, 13] and its introduction has drawn attention to aerococci as important human pathogens [5]. Two species, *Aerococcus urinae* and *Aerococcus sanguinicola*, are consistently reported from clinical samples whereas other species are rare causes of human infections [8, 9, 11, 14]. The incidence of *A. urinae* and *A. sanguinicola* bacteraemia have been proposed to be 0.5–3 cases per 1,000,000 inhabitants per year [7, 8, 15] and 1.4 cases per 1,000,000 inhabitants per year [9], respectively, but these estimates are limited by the low number of cases in previous studies and their retrospective study design. The role of *A. viridans* as a human pathogen is less clear, and many reports about cases of *A. viridans* infections likely represent misidentification of *A. sanguinicola* [16]. The incidence of aerococcal IE is not known, and early studies indicated that IE was common in aerococcal bacteraemia [1, 7, 17]. Several recent studies have however indicated that IE complicate aerococcal bacteraemia only in a small proportion of cases [4, 8, 9]. Early case reports also suggested a poor prognosis of aerococcal IE [1, 7, 15, 17], whereas a recent large case series of aerococcal IE indicates a more favorable prognosis [10].

Most aerococcal isolates are sensitive to penicillins and carbapenems with very low minimal inhibitory concentrations

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(MICs) whereas MICs towards cephalosporins are typically higher [3, 9, 14, 18, 19]. Most aerococcal isolates have low MICs for vancomycin [8, 9, 19] whereas high MIC-values against clindamycin [3, 20] and quinolones [11, 21] have been reported. The aim with this study was to determine the incidence of aerococcal bacteraemia after the introduction of MALDI TOF-MS, to describe the clinical presentation of such infections and to determine the MIC distributions of the aerococci for relevant antibiotics.

Material and methods

Bacterial isolates

We retrospectively identified all blood cultures that grew aerococci for the years 2012–2014 through searches in the laboratory database of the two clinical microbiology laboratories located in Malmö and Lund. All clinical samples from a region with 1,288,908 inhabitants (on December 31, 2014, Statistics Sweden, www.scb.se) were sent to either one of these two laboratories. The BacT/Alert blood culture system (bioMérieux, Marcy l'Etoile, France) was used at both laboratories from 2012 through November 2014. In December 2014, the BacT/Alert system was replaced by the BACTEC FX blood culture system (Becton Dickinson, Franklin Lakes, USA). Gram staining of blood culture broth was used for preliminary identification. Definite species identification relied on analysis of bacterial colonies with Microflex, UltrafleXtreme or Autoflex Speed MALDI-TOF MS (Bruker Daltronics, Bremen, Germany) with the FlexControl and MALDI Biotyper RTC (Realtime Classification) software (version 3.1 with reference database MBT-BDAL-5627). The direct transfer method was used as described in [22]. The isolates were stored at $-80\text{ }^{\circ}\text{C}$ in medium containing horse serum and glycerol. Four isolates were missing in the freezer (*A. urinae* $n=3$, *A. sanguinicola* $n=1$).

If a MALDI-TOF MS score >2.0 was obtained in the routine work, we considered identification to be reliable to the species level. In cases where a score <2.0 had been recorded, isolates were subjected to renewed analysis with MALDI-TOF MS by the authors. If a score <2.0 was again obtained, species identification was considered not to be reliable and isolates were subjected to sequencing of the 16S rRNA gene essentially as previously described [23] using primers fD1 mod [24] and P911 [25]. MacVector version 14.0.6 (MacVector, Inc., Apex, USA) and Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST) were used when comparing sequences. A test for detection of pyrrolidonyl arylamidase (PYR test, Remel Inc., Lenexa, USA) was used.

Patient information

The patients' medical charts were reviewed and information regarding clinical presentation, underlying diseases, antibiotic treatment and outcome was noted. Systemic inflammatory response syndrome (SIRS) and organ dysfunction was defined according to the Swedish Society of Infectious Diseases' guidelines [8]. Urinary tract disorders included cancer, stone formation or conditions causing obstruction in the urinary tract. Urinary tract symptoms included flank pain, suprapubic pain, frequency, urgency, obstruction, urination pain, foul-smelling urine or hematuria. The focus of bacteraemia was considered to be the urinary tract if a urinary tract symptom was present or if urine grew aerococci. Respiratory tract symptoms included coughing or dyspnea. Pneumonia was regarded as the focus of bacteraemia if both radiological and clinical evidence of pneumonia was present. GraphPad Prism v 6.0 was used for statistical analyses. The local research ethical committee approved this study (registration number 2013/31).

Antibiotic susceptibility testing

Etests (bioMérieux, Marcy l'Etoile, France) were used according to the manufacturer's instructions to determine MIC values. Muller-Hinton agar plates, supplemented with 5 % horse blood, were used. The plates were incubated at $35\text{ }^{\circ}\text{C}$ in 5 % CO_2 for 24–48 hours before MIC values were determined.

Results

Bacterial blood isolates

We identified 77 patients with aerococcal bacteremia during the three-year period. The isolates had been identified either as *A. urinae* ($n=49$), *A. sanguinicola* ($n=11$), *A. viridans* ($n=10$) or as *Aerococcus* species ($n=7$). In some cases, only one pair of blood culture bottles was sent to the laboratory, but a majority had two pairs of blood cultures drawn on the same date. For nine isolates, a MALDI-TOF MS score >2.0 was not obtained and 16S rRNA gene sequencing identified two of the isolates as *A. sanguinicola* (797/797 and 872/873 nucleotides respectively identical to accession number AY837833.1), one to be *A. urinae* (790/793 nucleotides identical to accession number NR113770) and one to be *A. christensenii* (799/800 nucleotides identity with accession number KP192302.1). In five isolates the 16S rRNA gene sequence could not discriminate *A. viridans* and *A. urinaeequi* (99 % identity to sequences from both species). Positive PYR tests identified these five isolates as *A. viridans* [26]. In total, 49 patients with *A. urinae* bacteraemia, 14 with *A. viridans* bacteraemia, 13

with *A. sanguinicola* bacteraemia and one with *A. christensenii* bacteraemia were identified (Table 1), corresponding to incidences of 14 cases per 1,000,000 inhabitants per year (*A. urinae*) and 3.5 cases per 1,000,000 inhabitants per year (*A. sanguinicola* and *A. viridans*). One patient had two episodes and one patient had three episodes of *A. urinae* bacteraemia, both within three months. One patient with an episode of *A. urinae* bacteraemia in January 2012 had been diagnosed with the same condition in December 2011 (before the start of the study-period). These three patients were all diagnosed with IE. In 30 of 49 patients *A. urinae* was in pure culture (Table 1). Twenty-four of the 49 patients had growth in two out of two blood cultures, three patients had growth in one out of one culture and 22 patients had growth in one out of two blood cultures. Sixteen patients with *A. urinae* bacteraemia had one additional organism isolated and three patients had two additional organisms in their blood. In six of 13 patients *A. sanguinicola* was in pure culture, while seven patients had additional species isolated. Five of the 14 patients had *A. viridans* in pure culture.

Other microbiological findings

Urine samples were collected from 42 of the 49 patients with *A. urinae* bacteraemia. Seven of these patients had aerococcal bacteruria (*A. urinae* $n=6$, *A. sanguinicola* $n=1$) (Table 1). Twenty-two patients had other organisms isolated from the urine and 15 patients had sterile urine. One patient with *A. sanguinicola* bacteraemia had *A. sanguinicola* in the urine culture, three patients had other organisms isolated and five

patients had no growth of microorganisms. None of the 14 patients with *A. viridans* bacteraemia had *A. viridans* isolated in a urine culture.

Patient characteristics

The median age was 82 years (range 59–98) for patients with *A. urinae* bacteraemia, 86 years (range 67–100) for those with *A. sanguinicola* bacteraemia and 64 years (range 1–87 years) for those with *A. viridans* bacteraemia (Table 2) ($p<0.0001$ for difference using the Kruskal-Wallis test). The patient with *A. christensenii* bacteraemia was a 22-year-old woman. Eighty percent of the *A. urinae* patients were male, whilst a more even gender distribution was seen in the other groups ($p=0.09$ for difference using the chi-square test).

Underlying diseases

Thirty-two of the patients with *A. urinae* bacteraemia had urinary tract disorders (of which 16 had prostate disease) and 20 patients had a urinary tract catheter (UC) (Table 2). Three of the *A. sanguinicola* patients had urinary tract disorders and five had UC, whereas none of the patients with *A. viridans* had UC and three had a urinary tract disorder. For other diseases and statistical comparison see Table 2.

Clinical presentation

The clinical presentations of patients are summarized in Table 2. The most common sign was fever. Urinary tract

Table 1 Bacterial blood isolates and other microbiological findings

Culture/measure	<i>A. urinae</i> (N=49)	<i>A. sanguinicola</i> (N=13)	<i>A. viridans</i> (N=14)
Pure culture ^a	30 (61 %)	6 (46 %)	5 (36 %)
Growth in			
2/2 cultures	24 (49 %)	5 (38 %)	
1/1 culture	3 (6 %)	1 (8 %)	2 (14 %)
1/2 culture	22 (45 %)	7 (54 %)	12 (86 %)
One additional organism in blood	CoNS ^b $n=4$ <i>Actinobaculum schaalii</i> $n=2$ <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Citrobacter freundii</i> , <i>Proteus vulgaris</i> , <i>Enterococcus faecium</i> , <i>Enterococcus faecalis</i> , <i>Oligella urethralis</i> , <i>Acinetobacter</i> species, <i>Bacteroides fragilis</i> , <i>Staphylococcus aureus</i> $n=1$	<i>E. coli</i> $n=2$ <i>Proteus mirabilis</i> $n=1$ <i>A. schaalii</i> $n=1$	CoNS $n=4$ <i>Acinetobacter</i> species $n=1$ <i>E. coli</i> $n=1$
Two additional species in blood	<i>E. faecalis</i> and <i>Pseudomonas aeruginosa</i> <i>S. aureus</i> and <i>P. aeruginosa</i> <i>E. coli</i> and a <i>Streptococcus</i> species	<i>S. aureus</i> and <i>P. aeruginosa</i> <i>P. mirabilis</i> and <i>E. faecalis</i> <i>A. schaalii</i> and a <i>Streptococcus</i> species	<i>Micrococcus</i> species and <i>Staphylococcus</i> species ($n=2$) <i>S. aureus</i> and <i>Streptococcus</i> species
Aerococci in urine	<i>A. urinae</i> $n=6$ <i>A. sanguinicola</i> $n=1$	<i>A. sanguinicola</i> $n=1$	

^a $p=0.2$ using the chi-square test

^b Coagulase-negative staphylococci

Table 2 Characteristics of 76 patients with aerococcal bacteraemia

Characteristic	<i>A. urinae</i> (N=49)	<i>A. sanguinicola</i> (N=13)	<i>A. viridans</i> (N=14)	Comparison with chi ² if not KW ^a
Median age (range)	82 years (59–98)	86 years (67–100)	64 years (1–87)	$p < 0.0001$ (KW)
Male sex	39 (80 %)	7 (54 %)	8 (57 %)	$p = 0.09$
Underlying conditions				
Indwelling urinary catheter	20 (41 %)	5 (38 %)	0	$p = 0.02$
Urinary tract disorders	32 (65 %)	3 (23 %)	3 (21 %)	$p = 0.002$
Cardiovascular ^b	30 (61 %)	8 (62 %)	7 (50 %)	<i>ns</i> ^c
Diabetes mellitus	8 (16 %)	4 (31 %)	2 (14 %)	<i>ns</i>
Dementia	7 (14 %)	2 (15 %)	0	<i>ns</i>
Clinical presentation				
Fever	29 (59 %)	9 (69 %)	9 (64 %)	<i>ns</i>
Urinary tract symptoms	25 (51 %)	7 (54 %)	1 (7 %)	$p = 0.01$
Respiratory tract symptoms	16 (33 %)	3 (23 %)	1 (7 %)	$p = 0.2$
SIRS (>2 criteria)	38 (78 %)	13 (100 %)	10 (71 %)	<i>ns</i>
Severe sepsis	25 (51 %)	9 (69 %)	3 (21 %)	$p = 0.04$
Focus of bacteraemia				
Urinary tract	26 (53 %)	7 (54 %)	1 (7 %)	$p = 0.01$
Pneumonia	1 (2 %)	1 (8 %)	1 (7 %)	<i>ns</i>
IE	5 ^d (10 %)	0	0	<i>ns</i>
Other ^e	0	1 (8 %)	2 (14 %)	<i>ns</i>
Unknown	19 (39 %)	4 (31 %)	10 (71 %)	$p = 0.06$
Antibiotic treatment				
Ctx ^f initially	28 (57 %)	8 (62 %)	8 (57 %)	<i>ns</i>
Median length of Iv ^g treatment (range)	7 days (0–38)	7 days (0–14)	5 days (0–12)	$p = 0.12$ (KW)
Median total length of treatment (range)	14 days (3–38)	15 days (0–22)	11 days (0–17)	$p = 0.06$ (KW)
Outcome				
ICU ^h	3 (6 %)	0	1 (7 %)	<i>ns</i>
30-day mortality	3 (6 %)	2 (15 %)	1 (7 %)	<i>ns</i>

^a Kruskal-Wallis test^b Ischemic heart disease, atrial fibrillation, stroke^c No statistical significance^d Three patients fulfilled Duke's criteria^e Gallstone, diverticulitis, flegmone^f Cefotaxime^g Intra-venous^h Intensive care unit

symptoms were common in patients with *A. urinae* and *A. sanguinicola* bacteraemia, whereas only one patient with *A. viridans* had such symptoms. Three patients had clinical and radiological evidence of pneumonia. A majority of all patients fulfilled the SIRS criteria and more than half of the patients with *A. urinae* and *A. sanguinicola* bacteraemia also had signs of organ dysfunctions, thus fulfilling the criteria for severe sepsis. Kidney failure was the most common organ dysfunction. Five patients received a clinical diagnosis of IE (Table 3), of whom three fulfilled Duke's criteria [27]. All IE patients had *A. urinae* in pure culture and none had aerococci in the urine. One patient

had three episodes of *A. urinae* bacteraemia during a 3-month period and was treated for suspected IE twice. This patient had significant co-morbidity and died one week after discharge from the third episode. Another IE patient had two episodes of *A. urinae* bacteraemia, and died within 30 days after admission the second time. Transthoracic or transoesophageal echocardiogram was performed without evidence for IE on 12 other patients. Three patients with *A. urinae*, two patients with *A. sanguinicola* and one patient with *A. viridans* died within 30 days after first blood culture positivity. The patient who died having *A. viridans* bacteraemia was a young patient with

Table 3 Clinical presentation of five cases of aerococcal infective endocarditis (IE)

Age	Sex	Underlying conditions	Symptoms	Duke’s criteria fulfilled	Treatment	Outcome	Comments
87	M	AF, CHF, larynx cancer, tracheostomy	Fever fatigue	Yes	Cef+ tob	CA, ICU Died on day 21 of the second hospitalization	TEE MV AuB 2 months before
77 ^a	M	Liver failure, alcoholism	Fatigue, confusion, urinary obstruction, receives UC.	Yes	Pip/taz PcG + gent	Recovery after 27 days in-hospital treatment	TEE: new AVI
83 ^a	M	AF, HT, UC	Fever for 1 month, fatigue	Yes	Pip/taz PcG + tob	Recovery after 40 days in-hospital treatment	TEE: MV BVP Valve culture negative, 16S ^b positive (<i>A. urinae</i>)
73 ^a	M	DM, MS, SUC	Fever, dyspnoea	No	Cef Pip/taz PcG + gent	Died at home 90 days after the first culture positivity	Three episodes TTE normal TEE could not be performed
88	F	AS, dementia, scleroderma	Fever, nausea	No	Pcg + tob	Recovery after 41 days in-hospital treatment	TTE: severe AS AuB and IE one month earlier (year 2011)

M male, F female, AF atrial fibrillation, CHF congestive heart failure, Cef cefotaxime, tob tobramycin, CA cardiac arrest, ICU intensive care unit, TEE transoesophageal echocardiogram, MV mitral valve vegetation, AuB Aerococcus urinae bacteraemia, UC urinary catheter, Pip/taz piperacillin/tazobactam, PcG benzylpenicillin, gent gentamicin, AVI aortic valve insufficiency, HT hypothyreosis, BVP biological valve prosthesis, DM diabetes mellitus, MS multiple sclerosis, SUC suprapubic urinary catheter, TTE transthoracic echocardiogram, AS aortic stenosis^a Indicates that the patient was included in a previous work on aerococcal IE (7)^b Sequencing of the 16S rRNA gene

subarachnoid bleeding, and one patient with *A. sanguinicola* bacteraemia suffered from a pelvic fracture.

Antibiotic susceptibility and antibiotic treatment

The *A. urinae* and *A. sanguinicola* isolates displayed low MICs for benzylpenicillin, piperacillin/tazobactam, imipenem, vancomycin and rifampicin (Tables 4 and 5).

Most isolates had low MIC values also for cefotaxime. Several *A. urinae* and *A. sanguinicola* isolates displayed high MICs against ciprofloxacin and clindamycin. *A. viridans* had higher MIC values than *A. urinae* and *A. sanguinicola* for the

beta-lactam antibiotics in general, except for imipenem (Table 6). *A. viridans* isolates had low MICs for vancomycin and rifampicin. The *A. christensenii* isolate had low MIC values for all antibiotics tested (Table 7).

Cefotaxime was the initial empiric antibiotic treatment in 57 % of cases. The length of the intra-venous antibiotic treatment expressed as median (range), was 6 days (0–38) and the total antibiotic treatment length was 13 days (0–38) for all cases (Table 2). Most patients received per oral treatment as follow-up, most commonly with amoxicillin. The patient with *A. christensenii* bacteraemia received amoxicillin only for ten days.

Table 4 MIC-values (mg/L) for 46 *A. urinae* isolates

Antibiotic	MIC-values																	
	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>256
Penicillin G	1	2	10	17	14	2												
Cefotaxime				1	1	16	8	13	6		1							
Vancomycin									13	26	7							
Ciprofloxacin					1	1	7	11	8	10	1		1	2	4 (>32)			
Gentamicin							1				1	1	6	10	15	8	1	3
Clindamycin					1	5	7	6	10	12	2			1				2
Piperacillin/tazobactam				1	9	17	15	4										
Imipenem		3	9	18	16													
Linezolid							1	2	6	11	24	2						
Rifampicin	6	16	15	9														

Table 5 MIC-values (mg/L) for 12 *A. sanguinicola* isolates

Antibiotic	MIC-values																		
	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>256	
Penicillin G				2	1	5	3	1											
Cefotaxime						1		2	4	4	1								
Vancomycin									3	7	2								
Ciprofloxacin								1	1	2		2	1		5 (>32)				
Gentamicin											2	2	2	3	3				
Clindamycin					1	1	3		2	2	2							1	
Piperacillin/tazobactam						3		7	1	1									
Imipenem				2	3	6	1												
Linezolid								1	1	3	7								
Rifampicin	1	1		1	4	3	2												

Discussion

This study on aerococcal bacteraemia is the largest one to date. Although it is retrospective, the methods used in the laboratory for species identification are well validated and have been consistent during the study period and the population-base is well defined. In line with previous reports, *A. urinae* was found to be the most common *Aerococcus* in blood cultures and the most common cause of aerococcal IE [7–9, 15]. Male gender, old age and urological conditions, such as prostate disease or the presence of a urinary tract catheter, seem to be predisposing factors for *A. urinae* bacteraemia [7, 8]. The patients with *A. sanguinicola* bacteraemia were old, but the gender distribution was more even than in previous studies [9, 28]. In these studies, several cases of IE are reported, but no patients with *A. sanguinicola* in our study were however diagnosed with IE. The incidence of *A. urinae* and *A. sanguinicola* bacteraemia demonstrated in this study is several times higher than previously reported [7–9, 15]. This is likely explained by the

introduction of a species determination system (MALDI-TOF MS), which properly identifies aerococci. However, a true increase cannot be excluded since the number of old persons is increasing. Many patients in our study had organ dysfunction due to their blood stream infections, hence aerococci have potential to give rise to serious conditions in the elderly population.

Five patients with *A. urinae* bacteraemia died, of which three were within 30 days. Four of them were diagnosed with severe infections, and two of them were admitted to the intensive care unit. The patient with *A. viridans* bacteremia had however a cause of death unrelated to the bacteraemia. Five of 49 patients with *A. urinae* bacteraemia were diagnosed with IE but a trans-thoracic or transoesophageal echocardiogram was performed in only 12 other patients. Two IE patients did not fulfill Duke's criteria and the clinical diagnosis was thus questionable. One had severe aortic valve stenosis, which made the TTE hard to interpret, and the other three had episodes of *A. urinae* bacteraemia, but no signs of IE in repeated

Table 6 MIC-values (mg/L) for 14 *A. viridans* isolates

Antibiotic	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>256
Penicillin G							1	8	5									
Cefotaxime								3	4	4	2	1						
Vancomycin							2	1	7	4								
Ciprofloxacin									1	7	4		1		1 (>32)			
Gentamicin											10	3	1					
Clindamycin							2	3	4	2	1							2
Piperacillin/tazobactam						1			4	5	4							
Imipenem					1	8	5											
Linezolid											5	8	1					
Rifampicin							8	6										

Table 7 MIC-values (mg/L) for the *A. christensenii* isolate

PcG	Cefotaxime	Vancomycin	Ciprofloxacin	Gentamicin	Clindamycin	Piperacillin/tazobactam	Imipenem	Linezolid	Rifampicin
0.008	0.032	1	0.25	32	0.25	0.064	0.016	1	0.008

TTEs. We suggest that the possibility of IE must be taken into consideration in all cases of aerococcal bacteraemia, especially with *A. urinae*. However, the risk for IE in aerococcal bacteraemia has been over-estimated previously, most likely due to a bias to publish dramatic presentations of infection.

As suggested by us previously [16, 29], *A. viridans* seem to be a contaminant in most cases. It was isolated less frequently in pure culture and often together with other low-grade pathogens. Underlying urinary tract diseases or symptoms were uncommon in patients with *A. viridans* bacteremia. In a majority of cases, the focus of bacteraemia was unknown. *A. viridans* is found in dust and hospital environments [30] and can possibly lead to contamination of blood cultures. The significance of *A. viridans* in a blood culture must thus always be interpreted with caution. *A. christensenii* was originally isolated from vaginal flora [31] and accordingly our case of *A. christensenii* bacteremia as well as a very recent report from Denmark [32] concerns a pregnant woman with fever.

Very few patients had aerococci isolated from urine and this has been reported previously [8, 9]. This may be because mediums or incubation conditions used for urinary cultures are not optimal for aerococci. Though clinical characteristics of patients often strongly indicate a urinary tract focus, it is possible that some aerococcal blood stream infections originate from other sites of the body. Three patients in this study had pneumonia, but the causative role of aerococci was not confirmed by cultures from the airways, and thus no firm evidence of aerococcal etiology could be established. Many patients with *A. urinae* and *A. sanguinicola* bacteraemia had other bacteria species isolated in their blood cultures that are typically part of the intestinal flora. This may suggest that *A. urinae* and *A. sanguinicola* also have their normal habitat in the intestinal tract.

No clinical breakpoints have been established for aerococci. Based on MIC-values, benzylpenicillin or ampicillin seems to be the treatment of choice for *A. urinae* and *A. sanguinicola*. For penicillin allergic patients, vancomycin could be used. Most patients received cefotaxime as empiric treatment and the result of treatment was generally favorable, indicating that this also might be a reasonable treatment regime.

In conclusion, the introduction of MALDI-TOF MS has had a major impact on the number of detected cases of aerococcal bacteraemia in our healthcare region.

Our results also demonstrate that *A. urinae* and *A. sanguinicola* bacteremia indicate true and sometimes severe infection while *A. viridans* bacteremia often represents contamination. A careful clinical assessment, including the

possibility of IE, is called for when a blood culture yields aerococci.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The local research ethical committee in Lund, Sweden, approved this study (registration number 2013/31).

Informed consent Not applicable

References

- Rasmussen M (2013) Aerococci and aerococcal infections. *J Infect* 66(6):467–474. doi:10.1016/j.jinf.2012.12.006
- Rasmussen M (2015) Aerococcus: an increasingly acknowledged human pathogen. *Clin Microbiol Infect* 22(1):22–27. doi:10.1016/j.cmi.2015.09.026
- Humphries RM, Hindler JA (2014) In vitro antimicrobial susceptibility of *Aerococcus urinae*. *J Clin Microbiol* 52(6):2177–2180. doi:10.1128/JCM.00418-14
- Yasukawa K, Afzal Z, Mbang P, Stager CE, Musher DM (2014) Aerococcal infection at three US Tertiary Care Hospitals. *South Med J* 107(10):642–647. doi:10.14423/SMJ.000000000000158
- Opota O, Prod'homme G, Andreutti-Zaugg C, Dessauges M, Merz L, Greub G et al (2015) Diagnosis of *Aerococcus urinae* infections: importance of MALDI-TOF MS and broad-range 16S rDNA PCR. *Clin Microbiol Infect* 22(1):e1–2. doi:10.1016/j.cmi.2015.08.026
- Christensen JJ, Kerner B, Kjaergaard H (1989) *Aerococcus*-like organism—an unnoticed urinary tract pathogen. *APMIS* 97(6):539–546
- Christensen JJ, Jensen IP, Faerk J, Kristensen B, Skov R, Kerner B (1995) Bacteremia/septicemia due to *Aerococcus*-like organisms: report of seventeen cases. Danish ALO Study Group. *Clin Infect Dis* 21(4):943–947
- Senneby E, Petersson AC, Rasmussen M (2012) Clinical and microbiological features of bacteraemia with *Aerococcus urinae*. *Clin Microbiol Infect* 18(6):546–550. doi:10.1111/j.1469-0691.2011.03609.x

9. Senneby E, Eriksson B, Fagerholm E, Rasmussen M (2014) Bacteremia with *Aerococcus sanguinicola*: case series with characterization of virulence properties. *Open Forum Infect Dis* 1(1): ofu025. doi:10.1093/ofid/ofu025
10. Sunnerhagen T, Nilson B, Olaison L, Rasmussen M (2015) Clinical and microbiological features of infective endocarditis caused by aerococci. *Infection*. doi:10.1007/s15010-015-0812-8
11. Cattoir V, Kobal A, Legrand P (2010) *Aerococcus urinae* and *Aerococcus sanguinicola*, two frequently misidentified uropathogens. *Scand J Infect Dis* 42(10):775–780. doi:10.3109/00365548.2010.485576
12. Christensen JJ, Dargis R, Hammer M, Justesen US, Nielsen XC, Kemp M et al (2012) Matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of Gram-positive, catalase-negative cocci not belonging to the *Streptococcus* or *Enterococcus* genus and benefits of database extension. *J Clin Microbiol* 50(5):1787–1791. doi:10.1128/JCM.06339-11
13. Senneby E, Nilson B, Petersson AC, Rasmussen M (2013) Matrix-assisted laser desorption ionization-time of flight mass spectrometry is a sensitive and specific method for identification of aerococci. *J Clin Microbiol* 51(4):1303–1304. doi:10.1128/JCM.02637-12
14. Senneby E, Petersson AC, Rasmussen M (2015) Epidemiology and antibiotic susceptibility of aerococci in urinary cultures. *Diagn Microbiol Infect Dis* 81(2):149–151. doi:10.1016/j.diagmicrobio.2014.11.009
15. de Jong MF, Soetekouw R, ten Kate RW, Veenendaal D (2010) *Aerococcus urinae*: severe and fatal bloodstream infections and endocarditis. *J Clin Microbiol* 48(9):3445–3447. doi:10.1128/JCM.00835-10
16. Rasmussen M (2014) Letter to the editor. *Can J Infect Dis Med Microbiol* 25(4):232
17. Ebnother C, Altwegg M, Gottschalk J, Seebach JD, Kronenberg A (2002) *Aerococcus urinae* endocarditis: case report and review of the literature. *Infection* 30(5):310–313. doi:10.1007/s15010-002-3106-x
18. Skov R, Christensen JJ, Komer B, Frimodt-Moller N, Espersen F (2001) In vitro antimicrobial susceptibility of *Aerococcus urinae* to 14 antibiotics, and time-kill curves for penicillin, gentamicin and vancomycin. *J Antimicrob Chemother* 48(5):653–658
19. Lupo A, Guilarte YN, Droz S, Hirzel C, Furrer H, Endimiani A (2014) In vitro activity of clinically implemented beta-lactams against *Aerococcus urinae*: presence of non-susceptible isolates in Switzerland. *New Microbiol* 37(4):563–566
20. Facklam R, Lovgren M, Shewmaker PL, Tyrrell G (2003) Phenotypic description and antimicrobial susceptibilities of *Aerococcus sanguinicola* isolates from human clinical samples. *J Clin Microbiol* 41(6):2587–2592
21. Cattoir V, Kobal A, Legrand P (2011) First molecular characterization of fluoroquinolone resistance in *Aerococcus* spp. *Antimicrob Agents Chemother* 55(1):451–452. doi:10.1128/AAC.01065-10
22. Bizzini A, Durussel C, Bille J, Greub G, Prod'homme G (2010) Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 48(5):1549–1554. doi:10.1128/JCM.01794-09
23. Kahn F, Linder A, Petersson AC, Christensson B, Rasmussen M (2010) Axillary abscess complicated by venous thrombosis: identification of *Streptococcus pyogenes* by 16S PCR. *J Clin Microbiol* 48(9):3435–3437. doi:10.1128/JCM.00373-10
24. Jalava J, Kotilainen P, Nikkari S, Skurnik M, Vanttinen E, Lehtonen OP et al (1995) Use of the polymerase chain reaction and DNA sequencing for detection of *Bartonella quintana* in the aortic valve of a patient with culture-negative infective endocarditis. *Clin Infect Dis* 21(4):891–896
25. Fredricks DN, Relman DA (1998) Improved amplification of microbial DNA from blood cultures by removal of the PCR inhibitor sodium polyanethanesulfonate. *J Clin Microbiol* 36(10):2810–2816
26. Vela AI, Garcia N, Latre MV, Casamayor A, Sanchez-Porro C, Briones V et al (2007) *Aerococcus suis* sp. nov., isolated from clinical specimens from swine. *Int J Syst Evol Microbiol* 57(Pt 6): 1291–1294. doi:10.1099/ijs.0.64537-0
27. Durack DT, Lukes AS, Bright DK (1994) New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. *Am J Med* 96(3):200–209
28. Ibler K, Truberg Jensen K, Ostergaard C, Sonksen UW, Bruun B, Schonheyder HC et al (2008) Six cases of *Aerococcus sanguinicola* infection: clinical relevance and bacterial identification. *Scand J Infect Dis* 40(9):761–765. doi:10.1080/00365540802078059
29. Rasmussen M (2012) *Aerococcus viridans* is not a matter of opinion. Comment on: An unusual microorganism, *Aerococcus viridans*, causing endocarditis and aortic valvular obstruction due to a huge vegetation (*Turk Kardiyol Dem Ars* 2011;39:317–319). *Turk Kardiyol Dem Ars* 40(1):112
30. Williams RE, Hirsch A, Cowan ST (1953) *Aerococcus*, a new bacterial genus. *J Gen Microbiol* 8(3):475–480. doi:10.1099/00221287-8-3-475
31. Collins MD, Jovita MR, Hutson RA, Ohlen M, Falsen E (1999) *Aerococcus christensenii* sp. nov., from the human vagina. *Int J Syst Bacteriol* 49(Pt 3):1125–1128. doi:10.1099/00207713-49-3-1125
32. Carlstein CSL, Christensen J (2015) *Aerococcus christensenii* as part of severe polymicrobial chorioamnionitis in a pregnant woman. *Open Microbiol J* 9:1–4