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Original article

Impact of the MALDI-TOF as a tool for bacterial identification in the frequency of isolation of *Aerococcus* spp and *Actinotignum schaalii* in urinary tract infection

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ABSTRACT

Background: *Actinotignum schaalii* and the genus *Aerococcus* are considered emerging pathogens, since their isolation has been rising the last years thanks to the improvement of diagnosis techniques, such as the implementation of MALDI-TOF in microbiology laboratories for routine. Their patogenicity is nowadays well described in urinary tract infections affecting susceptible individuals, although both have been isolated from other biological samples. The aim of our study is to evaluate the impact of using mass spectrometry technology on the frequency of isolation of *Aerococcus* spp and *A. schaalii* in our hospital.
Methods: From January 2014 and December 2015 44.654 urines were collected in our laboratory from patients that were expected to have an UTI. Samples were processed using a flow cytometer and cultured if applicable. After 48 h, microbial growth was assessed. Due to the suspicion of an *Aerococcus* spp or *A. schaalii* infection identification test was performed using Vitek2 until 2014 and MALDI-TOF from 2015.
Results: Between the period of study, a total of 35 *Aerococcus* spp/*A. schaalii* isolates were collected from 34 patients. Six isolates were identified by Vitek2 and the other 29 were identified by MALDI-TOF. Out of 34 patients, 33 had at least one risk factor including age >65 years, immunosuppression or cancer, abnormality of the genitourinary tract, recurrent UTI, diabetes or a catheterization.
Conclusions: Since the implementation of the MALDI-TOF in the laboratory the isolation of *Aerococcus* spp/*A. schaalii* has increased almost five times. The most frequent patient corresponds to an elderly patient with recurrent UTI and cancer.

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Impacto del MALDI-TOF como herramienta de identificación bacteriana en la frecuencia de aislamiento de *Aerococcus* spp y *Actinotignum schaalii* en infecciones del tracto urinario

Palabras clave:
Infección del tracto urinario
Aerococcus spp
Actinotignum schaalii
MALDI-TOF

RESUMEN

Introducción: *Actinotignum schaalii* y el género *Aerococcus* son considerados patógenos emergentes debido al aumento en los últimos años de su aislamiento gracias a la mejora de las técnicas diagnósticas, como la implementación del MALDI-TOF en la rutina de los laboratorios de microbiología. Su patogenicidad está bien descrita en las infecciones del tracto urinario (ITU) en individuos susceptibles, aunque los dos géneros se han aislado también en otras muestras biológicas. El objetivo de nuestro estudio es evaluar el impacto del uso de la espectrometría de masas en la frecuencia de aislamiento de *Aerococcus* spp/*A. schaalii* en nuestro hospital.
Métodos: Desde enero 2014 a diciembre 2015 se recibieron 44.654 orinas en nuestro hospital procedentes de pacientes con sospecha de ITU. Las muestras fueron procesadas por citometría de flujo y sembradas según criterios establecidos. Pasadas 48 h, se evaluó el crecimiento. Ante la sospecha de infección por

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Aerococcus spp/*A. schaalii,* se realizó un test de identificación con Vitek2® hasta 2014 y con MALDI-TOF desde 2015.
Resultados: Durante el periodo de estudio se registraron 35 aislamientos de *Aerococcus* spp/*A. schaalii* correspondientes a 34 pacientes. Seis aislados se identificaron por Vitek2® y 29 por MALDI-TOF. De los 34 pacientes, 33 tenían como mínimo un factor de riesgo (>65 años, inmunosupresión o cáncer, anormalidades del tracto urinario, ITU recurrente, diabetes o cateterismo).
Conclusiones: Desde la implementación del MALDI-TOF en el laboratorio, el aislamiento de *Aerococcus* spp/*A. schaalii* ha aumentado 5 veces. El perfil más afectado es el de un individuo de edad avanzada con ITU recurrentes y cáncer.
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Introduction

Urinary tract infections (UTI) affect 150 million people each year worldwide[1]; and about 1 out 3 women will have had at least 1 episode of UTI requiring antimicrobial treatment by the age of 24 years.[2] The most common pathogens are gram-negative bacteria found in 75–90% of cases. Among these, the most frequent causative agent is *Escherichia coli* followed by *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Gram-positive bacteria including *Staphylococcus saprophyticus*, *Enterococcus faecalis* and group B *Streptococcus* are responsible for the remaining proportion. Nevertheless, they are particularly common among the elderly, pregnant women or in individuals who have other risk factors for UTI such as structural or functional alterations of the urinary tract (often associated to catheterization), or other underlying renal, metabolic or immunological disorders.[3,4]

Nowadays, other rare, misclassified and underreported gram-positive bacteria are emerging, coinciding with the implementation of a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) system in the microbiology laboratories for routine identification.[5–9] *Aerococcus* spp and *Actinotignum schaalii* (formerly *Actinobaculum schaalii*[10]) exemplify this fact.

Several causes can explain why these organisms have been overlooked. *A. schaalii* is a gram-positive and facultative anaerobic coccoid rod and it is part of the urogenital flora and skin of healthy patients.[11] It has a slowly (48 h) and tedious growth requiring blood-enriched media and an anaerobic or 5% CO_2-supplemented atmosphere. These conditions are not usually achieved in routine isolation, especially from urine. Indeed, nitrate reductase activities are negative. In addition, its tiny and gray colonies could be dismissed as a contaminant or overgrown by other bacteria.[3,5–7]

Aerococcus spp are gram-positive cocci showing a cluster organization in Gram stain although before catalase test they look like streptococci. The normal habitat of the human pathogenic aerococci is not known, but they occur both as a part of the normal flora of the human urinary tract and of the human oral flora of patients receiving cytostatic drugs. By the morphology of their colonies they can be confused with viridans streptococci.[9]

Moreover, in the pre-molecular diagnostic era identification was performed using standard phenotypic methods such as API system and Vitek 2 Compact (bioMérieux, Marcy-l'Étoile, France) which did wrongly distinguish the two species.[6,9] Indeed, some authors consider biochemical reactions inappropriate for aerococci determination.[9]

Performing direct examination of urine samples with Gram stain when there is significant leukocyturia allows microbiologists to detect the presence of gram-positive bacteria in order to cultivate them in suitable conditions (i.e. onto gram-positive selective media, colistin-nalidixic acid agar, incubated under 5% CO_2).[5]

The aim of our study was to evaluate the utility of MALDI-TOF to identify *A. schaalii* and *Aerococcus* spp by comparing the frequency of infection due to these bacteria over 2 years in Germans Trias i Pujol University Hospital (HUGTiP, Badalona, Spain) before and after its introduction in our laboratory and to describe demographic data of the patients.

Material and methods

Study design

This is a retrospective observational study designed to assess the evolution of the diagnosis of UTI at the Microbiology Department of the HUGTiP with two different techniques: Vitek 2 Compact (BioMérieux) until 2014 and MALDI-TOF (Bruker Daltonics) from 2015.

Study population and clinical samples

During the period January 2014–December 2015, our hospital's clinical microbiology laboratory received 44.654 urines to be processed. These samples were collected from patients that were expected to have an UTI (i) coming from the emergency department or hospitalized at HUGTiP (21.722 urines) and (ii) from primary care centers (22.932 urines). All urine samples were collected in 5 mL sterile tubes and stored without preservatives for a maximum of 4 h at 4 °C before processing.

Urine samples processing

Urine samples were processed using the Sysmex UF-1000*i* flow cytometer (TOA Medical Electronics, Kobe, Japan). This stains the urine particles with a fluorescent dye, allowing them to be classified into white blood cells (WBC), red blood cells (RBC), epithelial cells (EC), bacteria (BAC) and yeasts (YEA) by impedance, scattering and fluorescence. This version has also an independent channel for bacteria.

All the samples from the HUGTiP were cultured with a 10 μL loop onto chromogenic chromID® CPS® Elite plates (BioMérieux, Marcy-l'Étoile, France) whereas only those samples considered positives (>40 WBC/μL or 500 BAC/μL by flow cytometry as established by previous studies[12]) from primary care centers were cultured. All samples were incubated for 24–48 h at 37 °C under aerobic conditions. Additionally, samples from the HUGTiP with >40 WBC/μL by flow cytometry were Gram stained. When gram-positive bacteria with clinical significance were detected selective plates such as colistin-nalidixic acid plate were cultivated 48 h under 5% CO_2.

After this time, microbial growth was assessed by counting the number of colony-forming units per milliliter (CFU/mL) and by making a morphological recognition of the colonies. Clinical reports

were collected to determine the pathogenic role of the isolate. Due to the suspicion of an *Aerococcus* spp or *A. schaalii* infection an identification test was performed: until 2014 was used the Vitek 2 (Bio-Mérieux, France) and from 2015 is used the MALDI-TOF mass spectrometry (Bruker Daltonics, Leipzig, Germany).

Ethical approval from the Ethics Committee Research of Germans Trias i Pujol University Hospital Ethics Committee was obtained (PI-17-165) and the need for informed consent was waived.

Results

Between January 2014 and December 2015, a total of 35 *Aerococcus* spp or *A. schaalii* isolates were collected from 34 patients. Six isolates were identified by Vitek2 (Bio-Mérieux, France) in 2014, and the other 29 were identified by MALDI-TOF mass spectrometry (Bruker Daltonics, Germany) in 2015. Out of 35 episodes, 4 correspond to patients from primary care centers and 31 were HUGTiP patients. The evolution in the frequency of *Aerococcus* spp or *A. schaalii* isolates before and after the introduction of the MALDI-TOF mass spectrometry (Bruker Daltonics, Germany) is summarized in Table 1.

The study population was made up of 25 women and 9 men. The mean age was 75 years, ranging from 39 to 92 years. Patient demographic data for this cohort is shown in Table 2.

All the samples corresponding to the 35 episodes presented pathological sediment (only in 5 cases were counted <40 leukocytes/μL, but in all those cases they were >500 BAC/μL). Plate counting of colony-forming units was in 33 of 35 episodes over 100.000, in 2 cases from 10.000 to 50.000 and in 1 case from 1.000 to 10.000.

Gram stain was performed in 26 of 35 urine samples, observing in all the cases cocci or bacilli forms. Bacteria in study were isolated from selective plates and in 100% of the cases were consistent with the observed form in the Gram stain.

Out of 34 patients, 33 had at least one risk factor, including age >65 years (76% of the individuals), immunosuppression or cancer (26%), abnormality of the genitourinary tract or recurrent UTI (24%), diabetes (15%) or a catheterization (12%). In 18 of the 35 documented episodes infections were monomicrobial (only *A. schaalii* or *Aerococcus* spp were isolated); while in the remaining cases[17] urine samples harbored in addition other common uropathogenic bacteria (*Enterobacteriaceae* in more than 75% of samples).

Table 1
Total of positive urine samples and *Aerococcus* spp or *A. schaalii* isolates.

	2014 (pre-MALDI-TOF era)	2015 (post-MALDI-TOF era)
HUGTiP	5/4547 (0.11[a])	26/5029 (0.52[a])
Primary care centers	1/1216 (0.08[a])	3/1611 (0.19[a])

[a] Percentage over positive urines.

Table 2
Clinical characteristics of pacients.

Episode	Year	Age	Risk factors	Isolate	Other isolates	Criteria for Gram stain	Gram stain	Symptoms of UTI	Urinary catheter
1	2014	81	Age, REC UTI, DM	*A. urinae*	*K. pneumoniae*	Yes	GPCstaph, GNB	No	No
2	2014	89	Age, cancer	*A. urinae*	*S. gallolyticus*	Yes	GPCstaph	No	No
3	2014	81	Age, cancer	*A. urinae*	No	Yes	GPCstaph	Yes	No
4	2014	39	Cancer	*A. urinae*	*E. coli*	Yes	GPCstaph, GNB	No	No
5	2014	92	Age	*A. viridans*	*E. coli*	No	N/A	N/A	No
6	2014	53	ALT, CAT	*A. viridans*	No	Yes	GPCstaph	Yes	Yes
7	2015	69	Age, cancer	*A. schaalii*	No	Yes	GPB	Yes	No
8	2015	75	Age, DM	*A. schaalii*	No	Yes	GPB	Yes	No
9	2015	84	Age, ALT VIA, REC UTI	*A. schaalii*	*P. aeruginosa*	Yes	GPB	Yes	No
10	2015	82	Age, CAT	*A. schaalii*	*C. amycolatum*	Yes	GPB	No	Yes
11	2015	49	REC UTI	*Aerococcus* spp	*E. coli*	Yes	GPCstaph GNB	No	No
12	2015	88	Age, REC UTI	*Aerococcus* spp	No	Yes	GPC	No	No
13	2015	80	Age, DM, REC UTI	*Aerococcus* spp	No	Yes	GPC	Yes	No
14	2015	70	Age, cancer	*Aerococcus* spp	*E. coli, P. mirabilis*	N/A	GPC, GNB	Yes	No
15	2015	78	Age	*A. urinae*	*E. coli*	Yes	GPCstaph GNB	Yes	No
16	2015	79	Age	*A. urinae*	*P. stuartii*	Yes	GPCstaph, GNB	No	No
17	2015	88	Age, REC UTI	*A. urinae*	No	Yes	GPCstaph	Yes	No
18	2015	59	Cancer	*A. urinae*	No	Yes	GPC	N/A	No
19	2015	40	ALT, REC UTI	*A. urinae*	No	No	N/A	Yes	No
20	2015	44	ALT	*A. urinae*	No	Yes	GPC	Yes	No
21	2015	61	ALT	*A. urinae*	No	Yes	GPC	N/A	N/A
22	2015	78	Age	*A. urinae*	No	No	N/A	No	No
23	2015	55	Cancer	*A. urinae*	*S. oralis*	No	N/A	Yes	No
24	2015	68	Age, cancer	*A. urinae*	*E. coli*	Yes	GPCstaph, GNB	No	No
25	2015	83	Age	*A. urinae*	*E. coli*	No	N/A	N/A	No
26	2015	67	Age, ALT	*A. urinae*	No	N	N/A	N/A	No
27	2015	80	Age	*A. urinae*	No	N	N/A	N/A	No
28	2015	66	Age, ALT	*A. urinae*	No	Yes	GPCstaph	Yes	No
29	2015	61	None	*A. urinae*	No	Yes	GPC staph	Yes	No
30	2015	41	REC UTI	*A. urinae*	*E. coli*	No	N/A	No	No
31	2015	75	Age, cancer	*A. urinae*	No	Yes	GPCstaph	No	No
32	2015	79	Age	*A. urinae*	No	No	N/A	No	No
33	2015	73	Age, CATH	*A. urinae*	*S. anginosus, Enterobacteriaceae*	Yes	GPC, GNB	Yes	Yes
34	2015	83	Age, REC UTI, DM	*A. urinae*	*E. coli*	Yes	GPC, GNB	No	No
35	2015	80	Age, DM, ALT	*A. viridans*	*E. coli*	Yes	GPC, GNB	No	No

REC: recurrent UTI, DM: diabetes mellitus, ALT: urinary tract alteration, CATH: use of urinary catheter, GPC: Gram-positive cocci, GPCstaph: Gram-positive cocci staphylococcus-like, GNB: Gram-negative bacilli.

Discussion

The current study focuses in UTIs because MALDI-TOF was firstly introduced in the urine culture section of the laboratory for routine identification. In reviewing the literature, UTIs are the most frequent infection reported caused by aerococci and *A. schaalii*, however *A. schaalii* has been associated with sepsis, osteomyelitis, endocarditis and Fournier's gangrene[13–16] among other and *Aerococcus* spp has been described to also cause urosepsis, infective endocarditis and osteoarticular infection.[9,17,18] This highlights the invasive potential of these microorganisms. Fimbrial genes coding for attachment pili, recently revealed through the study of the genome of *A. schaalii*, can intervene in the colonization.[19]

The ongoing introduction of MALDI-TOF in other areas of the microbiology laboratory of HUGTiP permitted to identify *Aerococcus* spp and *A. schaalii* from other samples than urine. In the period of study, *Aerococcus* spp was present in 2 blood cultures and *A. schaalii* was isolated from one blood culture and 3 collections in the groin area. This result suggests that this organism can be found as a commensal on skin, as other studies concluded.[11] Respecting the sepsis, in one case *Aerococcus* spp was the only bacteria isolated from blood culture and urine and in the other 2 episodes another recognized pathogen such as *E. coli* was isolated both from blood and urine cultures.

Some authors[5] suggest to perform Gram stain to urine samples when the sediment is considered pathological in order to culture urines onto selective media. According to them, our results show that in all the cases of *A. schaalii* or *Aerococcus* spp infection optimal conditions of growth were achieved after performing a Gram stain. Thus, no *A. schaalii* or aerococci were ignored in urines with pyuria, but neither a selective plate was cultured unjustifiably.

The increase in the frequency of *A. schaalii* or *Aerococcus* spp isolates from urine samples in our laboratory coincides with the introduction of MALDI-TOF MS in clinical practice for routine identification of causative agents of UTI. It seems possible that this was due to a lack on the database included on biochemical tests used before 2015, which did not permit to identify these organisms.[4,6,7] Mass spectrometry technique and molecular-based methods are more accurate since they allow distinguishing species with similar characteristics.

As Vouga and Greug have described in a recent review,[20] the improvement in diagnosis tools is one of the reasons that have lead to the discovery of previously underreported preexisting prokaryotes, not only pathogenic microbes but also beneficial or harmless bacteria. The authors add the increase of human exposure to organisms due to social and environmental changes, and the increased susceptibility of people and virulence of bacteria. Further, knowledge of the invasive capacity of these emerging prokaryotes has increased the interest of microbiologists to isolate them.

As announced previously, UTIs are the most common presentation of an *Aerococcus* spp or *A. schaalii* infection. Although all the episodes of isolation of these organisms form urine samples were accompanied by pathological sediment, only 15 patients presented obvious symptoms of urinary tract infection (e.g. dysuria, pollakyuria). It is important to remember, on one hand, that at advanced ages such as our cohort symptoms of UTI can be very non-specific (confusion, malaise). On the other hand, asymptomatic bacteriuria can be accompanied by pyuria in 90% of the elderly.[21] Thus, clinicians have to assess the role of the isolate to decide whether or not to treat it.

In our study we found 17 monomicrobial urine samples, and 18 cultures with a classical uropathogen in addition to aerococci or *A. schaalii.* Monomicrobial cultures of gram-positive bacteria were deemed as responsible for infection with the exception of 2 cases of urinary catheterized-patients where the presence of the microorganism was interpreted as colonization and malfunction of the catheter.

More controversial is to assess a mixed culture with a classical pathogen. Since the literature recognizes the uropathogenic potential of these organisms, in our laboratory we did not underestimate the infective role of *Aerococcus* spp or *A. schaalii*, especially if they are found in the same proportion as the classical uropathogen. Nevertheless, some authors with similar results consider the other possibly associated bacteria a commensal and they fail to identify it.[7]

At least one risk factor is found in 33 about 34 patients, which reveal their opportunistic behavior. Advanced age is the most frequent risk factor in our cohort, coinciding to other studies.[7,11,22,23] An explanation is that the humid environment generated by urinary incontinence in elderly patients facilitates the colonization for *A. schaalii*. Following a recent study[24] a similar reasoning can be made for small children, who can likely harbor the bacteria as they have enuresis and wear diapers.

Suffering from recurrent UTI is another common risk factor found in patients with aerococci/*A. schaalii* urinary tract infection. Uncomplicated UTI caused by classical uropathogen are usually treated orally with fluoroquinolones (such as ciprofloxacin) or trimethoprim/sulfamethoxazole, however both *Aerococcus* spp and *A. schaalii* are resistant to these antibiotics to a slightly different extent, hence they can outlast to infections. Furthermore, its tedious culture and identification can prolong the detection.

Urologic-related predisposing conditions are also associated to UTI by gram-positive bacteria.[3] In some cases, isolating *Aerococcus* spp/*A. schaalii* from a patient suffering chronic UTI could serve as an indicator of an alteration of the urinary tract.

Summarizing, microbiologists have to suspect an *Aerococcus* spp/*A. schaalii* infection before an elderly patient undergoing persistent UTI together with leukocyturia and negative cultures, now that literature recognizes its potential disease-causing capacity. New diagnostic tools such as MALDI-TOF have permitted to identify emerging prokaryotes. The role of these bacteria in normal microbiota and the incidence of asymptomatic bacteriuria remain to be studied.

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Conflict of interest

None.

References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2016;**13**:269–84, http://dx.doi.org/10.1038/nrmicro3432
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002;**113**:5–13.
3. Lewis AL, Kline KA. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Microbiol Spectr* 2016;**4**:1–54, http://dx.doi.org/10.1128/microbiolspec.UTI-0012-2012
4. Edwards MS, Baker CJ. Group B streptococcal infections in elderly adults. *Clin Infect Dis* 2006;**41**:839–47, http://dx.doi.org/10.1086/432804
5. Lotte R, Lotte L, Ruimy R. *Actinotignum schaalii* (formerly *Actinobaculum schaalii*): a newly recognized pathogen—review of the literature. *Clin Microbiol Infect* 2016;**22**:28–36, http://dx.doi.org/10.1016/j.cmi.2015.10.038
6. Stevens RP, Taylor PC. *Actinotignum* (formerly *Actinobaculum*) schaalii: a review of MALDI-TOF for identification of clinical isolates, and a proposed method for presumptive phenotypic identification. *Pathology* 2016;**48**:367–71, http://dx.doi.org/10.1016/j.pathol.2016.03.006
7. Prigent G, Perillaud C, Amara M, Coutard a, Blanc C, Pangon B. *Actinobaculum schaalii*: a truly emerging pathogen?: *Actinobaculum schaalii:* un pathogène réellement émergent? *New Microb New Infect* 2016;**11**:8–16, http://dx.doi.org/10.1016/j.nmni.2015.10.012

8. Opota O, Prod'hom G, Andreutti-Zaugg C, Dessauges M, Merz L, Greub G, et al. Diagnosis of *Aerococcus* urinae infections: importance of matrix-assisted laser desorption ionization time-of-flight mass spectrometry and broad-range 16S rDNA PCR. *Clin Microbiol Infect* 2016;**22**:e1–2, http://dx.doi.org/10.1016/j.cmi.2015.08.026
9. Rasmussen M. *Aerococcus*: an increasingly acknowledged human pathogen. *Clin Microbiol Infect* 2016;**22**:22–7, http://dx.doi.org/10.1016/j.cmi.2015.09.026
10. Yassin AF, Spröer C, Pukall R, Sylvester M, Siering C, Schuman P. Dissection of the genus *Actinobaculum*: reclassification of *Actinobaculum schaalii* Lawson et al., 1997 and *Actinobaculum urinale* Hall et al., 2003 as *Actinotignum schaalii gen. nov*., comb. nov. and *Actinotignum urinale comb. nov*., description of *Actinotignum*. *Int J Syst Evol Microbiol* 2015;**65**:614–24, http://dx.doi.org/10.1099/ijs.0.069294-0
11. Olsen AB, Andersen PK, Bank S, Søby KM, Lund L, Prag J. *Actinobaculum schaalii*, a commensal of the urogenital area. *BJU Int* 2013;**112**:394–7, http://dx.doi.org/10.1111/j.1464-410X.2012.11739.x
12. Íñigo M, Coello A, Fernández-Rivas G, Carrasco M, Marcó C, Fernández A, et al. Evaluation of the SediMax automated microscopy sediment analyzer and the Sysmex UF-1000i flow cytometer as screening tools to rule out negative urinary tract infections. *Clin Chim Acta* 2016;**456**:31–5, http://dx.doi.org/10.1016/j.cca.2016.02.016
13. Vanden Bempt I, Van Trappen S, Cleenwerck I, De Vos PCK, Camps K, Celens A, et al. *Actinobaculum schaalii* causing Fournier's gangrene. *J Clin Microbiol* 2011;**49**:2369–71, http://dx.doi.org/10.1128/JCM.00272-11
14. Le Brun C, Robert S, Bruyere F, Tanchoux CLP. Urinary tract infection caused by *Actinobaculum schaalii*: a urosepsis pathogen that should not be underestimated. *JMM Case Rep* 2015;**2**.
15. Hoenigl M, Leitner E, Valentin T, Zarfel G, Salzer HJ, Krause R, et al. Endocarditis caused by *Actinobaculum schaalii*. *Emerg Infect Dis* 2010;**16**:1171–3, http://dx.doi.org/10.3201/eid1607.100349
16. Haller P, Bruderer T, Schaeren S, Laifer G, Frei R, Battegay M, et al. Vertebral osteomyelitis caused by *Actinobaculum schaalii*: a difficultto-diagnose and potentially invasive uropathogen. *Eur J Clin Microbiolgy Infect Dis* 2007;**26**:667–70, http://dx.doi.org/10.1007/s10096-007-0345-x
17. Senneby E, Eriksson B, Fagerholm ERM. Bacteremia with *Aerococcus sanguinicola*: case series with characterization of virulence properties. *Open Forum Infect Dis* 2014;**1**, http://dx.doi.org/10.1093/ofid/ofu025
18. De Jong MFC, Soetekouw R, Kate ten RWVD. *Aerococcus* urinae: severe and fatal bloodstream infections and endocarditis. *J Clin Microbiol* 2010;**48**:3445–7, http://dx.doi.org/10.1128/JCM.00835-10
19. Kristiansen R, Dueholm MS, Bank S, Nielsen PH, Karst SM, Cattoir V, et al. Complete genome sequence of *Actinobaculum schaalii* strain CCUG 27420. *Genome Announ* 2014;**2**:e00880–914, http://dx.doi.org/10.1128/genomeA.00880-14
20. Vouga M, Greub G. Emerging bacterial pathogens: the past and beyond. *Clin Microbiol Infect* 2016;**22**:12–21, http://dx.doi.org/10.1016/j.cmi.2015.10.010
21. Andreu Domingo A, Cacho J, Coira Nieto A, Lepe Jiménez JA. Diagnóstico microbiológico de las infecciones del tracto urinario. *SEIMC* 2010:2.
22. Bank S, Jensen A, Hansen TM, Søby KM, Prag J. *Actinobaculum schaalii*, a common uropathogen in elderly patients, Denmark. *Emerg Infect Dis* 2010;**16**:76–80, http://dx.doi.org/10.3201/eid1601.090761
23. Nielsen HL, Søby KM, Christensen JJ, Prag J. *Actinobaculum schaalii*: a common cause of urinary tract infection in the elderly population. Bacteriological and clinical characteristics. *Scand J Infect Dis* 2010;**42**:43–7, http://dx.doi.org/10.3109/00365540903289662
24. Andersen LB, Bank S, Hertz B, Søby KM, Prag J. *Actinobaculum schaalii*, a cause of urinary tract infections in children? *Acta Paediatr* 2012;**101**:e232–4, http://dx.doi.org/10.1111/j.1651-2227.2011.02586.x