UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTIFICA E INTELECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:
https://www.clinicalkey.com/#!/content/playContent/1-s2.0-S1201971209002136

DOI: 10.1016/j.ijid.2009.04.016

Direitos autorais / Publisher's copyright statement:
©2010 by Elsevier. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo
CEP 13083-970 – Campinas SP
Fone: (19) 3521-6493
http://www.repositorio.unicamp.br

International Journal of Infectious Diseases 14 (2010) e344–e347

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Balanoposthitis caused by *Pseudomonas aeruginosa* co-producing metallo-β-lactamase and 16S rRNA methylase in children with hematological malignancies

Nilton Lincopan [a,b,*], Patricia Neves [b], Elsa M. Mamizuka [b], Carlos E. Levy [c]

[a] Department of Microbiology, Institute of Biomedical Sciences, Universidade de São Paulo, CEP 05508-900, São Paulo, Brazil
[b] Department of Clinical Analysis, School of Pharmacy, Universidade de São Paulo, São Paulo, Brazil
[c] Department of Clinical Pathology, School of Medicine, Universidade de Campinas, Campinas, Brazil

ARTICLE INFO

Article history:
Received 15 January 2009
Received in revised form 13 April 2009
Accepted 20 April 2009

Corresponding Editor: Mark Holodniy, California, USA

Keywords:
Balanitis
Multidrug-resistant
Pseudomonas aeruginosa
Neutropenic patients

ABSTRACT

Balanoposthitis is defined as the inflammation of the glans penis and its foreskin. In the presence of other underlying medical conditions, this localized infection may spread systemically, serving as a source of fever and bacteremia in neutropenic males. Two rare cases of balanoposthitis caused by a clonally related *Pseudomonas aeruginosa* isolate co-producing the SPM-1 metallo-β-lactamase and the novel 16S rRNA methylase RmtD are described. Four multidrug-resistant (MDR) *P. aeruginosa* isolates were successively recovered from glans/foreskin swabs and urine cultures from two uncircumcised pediatric patients, one with Burkitt's non-Hodgkin's lymphoma and one with acute lymphoblastic leukemia. Clinically, preputial colonization by MDR *P. aeruginosa* evolved to severe balanoposthitis with glans/foreskin lesions as a source of fever. Combination therapy of ciprofloxacin and/or aztreonam (systemic) plus polymyxin B (topical) was effective once reversion of the neutropenic condition was achieved. Although *P. aeruginosa* remains an unusual cause of balanoposthitis, these cases should alert the physician to the potential pathogenicity of this bacterium. Furthermore, co-production of metallo-β-lactamase and 16S rRNA methylase has a potential impact on the empirical management of complicated infections caused by *P. aeruginosa*.

Crown Copyright © 2009 Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. All rights reserved.

1. Introduction

Balanoposthitis is defined as the inflammation of the glans penis and its foreskin. A wide variety of infectious causes and predisposing factors have been described, and the condition is more common among uncircumcised men, possibly as a result of poorer hygiene, limited retraction of the foreskin, or due to irritation by smegma.[1] In the presence of other underlying medical conditions, this localized infection may spread systemically, serving as a source of fever and bacteremia in neutropenic males.[2] Even though the cause often remains undiagnosed, it is known that many cases are caused by infection with *Candida spp*. On the other hand, infection with *Gardnerella vaginalis* and anaerobes are common, especially when sexually transmitted.[1,3] Group B and group A streptococci have also been reported to cause balanoposthitis. Other known causes include *Staphylococcus aureus*, *Treponema pallidum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma spp*, plus some viral and parasitic causes.[1,3–6] As to balanoposthitis caused by *P. aeruginosa*, there are only three case reports that can be found in the medical literature (PubMed database). One report describes balanoposthitis due to *P. aeruginosa* diagnosed in a neutropenic patient who underwent immunosuppressive therapy,[2] whereas the other reports describe a case of erosive pseudomonal balanitis that developed during treatment with topical antibacterial, antifungal, and corticosteroid agents, and isolated gangrenous lesions in a male child with acute leukemia and granulocytopenia in the setting of *P. aeruginosa* bacteremia.[7,8] Herein we report two rare cases of balanoposthitis caused by multidrug-resistant (MDR) *P. aeruginosa*, in uncircumcised pediatric patients with underlying hematological malignancy.

2. Case reports

The Centro Infantil Boldrini (CIB) is a pediatric hematology–oncology hospital in Campinas, Southeastern Brazil. This institution,

* Corresponding author. Tel.: +55 11 3091 7296; fax: +55 11 3091 7354.
E-mail address: lincopan@usp.br (N. Lincopan).

1201-9712/$36.00 – see front matter. Crown Copyright © 2009 Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. All rights reserved.
doi:10.1016/j.ijid.2009.04.016

with 77 beds, is the pediatric hematology–oncology reference center for the region. The majority of patients hospitalized at the CIB are on chemotherapy for solid tumors and leukemias and the neutropenic condition of these in-patients is associated with various levels of myelosuppression resulting from the disease and/or indirectly induced by chemotherapy.

2.1. Case 1

On May 3, 2004, a 5-year-old male patient with Burkitt's non-Hodgkin's lymphoma, on consolidation chemotherapy, was admitted to the CIB due to septic shock. He had previously been hospitalized at the same center for short-term chemotherapy and because of two episodes of fever and neutropenia, which were treated empirically with a 10-day course of ceftriaxone (80 mg/kg every 24 h) and a 7-day course of ceftazidime (50 mg/kg every 8 h). On physical examination on this admission, hypotension, tachycardia, anuria, jaundice, and foreskin edema and erythema were noticed, and severe neutropenia was observed. A set of blood and surveillance swab cultures, including of the foreskin, were collected and the patient was transferred to the pediatric intensive care unit (PICU). Treatments involved volume replacement without vasoactive drugs, which was effective in improving the hemodynamic parameters and in re-establishing diuresis, and empiric antibiotic therapy with ceftazidime (50 mg/kg every 8 h), amikacin (20 mg/kg every 24 h), and vancomycin (10 mg/kg every 6 h). On the second day after admission, the patient developed severe balanoposthitis with cellulitis, erythema and swelling of the glans/foreskin, and persistent fever. On the third day after admission, without antibiotic change, the patient showed clinical improvement as a result of bone marrow recuperation, monitored by white blood cell (WBC) count. An MDR *P. aeruginosa* strain, sensitive only to aztreonam (strain CIB 518), was unexpectedly recovered from the foreskin swab culture (Table 1). Blood cultures were negative. During the following three days, the patient did not present fever and the swelling of the glans/foreskin began to subside, thus, he was transferred to the ward before being discharged.

On June 11, after a new session of chemotherapy he was readmitted to the CIB presenting with neutropenia, fever, and upper respiratory tract infection. Fever was attributed to viral infection and the patient, being considered in the low-risk group, was treated with ceftriaxone (100 mg/kg every 24 h). On the second day, swelling, redness, pain, and purulent secretion on the foreskin were noted and antibiotic therapy was changed to ceftazidime (50 mg/kg every 8 h). Samples from glans secretion and midstream urine were collected for culture, and two further MDR *P. aeruginosa* isolates (strains CIB 704 and 904) were recovered (Table 1). Due to the scarcity of an antimicrobial option, combination therapy using ciprofloxacin (10 mg/kg every 12 h) plus topical polymyxin B was initiated. This therapeutic regimen proved to be clinically successful; however, it must be pointed out that during this combination therapy the patient was not neutropenic, marrow recovery having been achieved during consolidation chemotherapy, as observed by hematological evaluation. Finally, on June 17, the patient was discharged home having successfully completed chemotherapy.

2.2. Case 2

On July 12, 2004, a 9-year-old boy with acute lymphoblastic leukemia (ALL), on a second induction chemotherapy regimen due to high risk for early relapse of his ALL, was admitted to the CIB with neutropenic fever and pain on his penis. During the previous months he had been hospitalized three times at the CIB. The first admission was for neutropenic fever associated with cellulitis and bacteremia caused by *Staphylococcus aureus*, which was successfully treated with cefazolin and amikacin. The latter two admissions were due to mucositis and herpes zoster infection plus bacteremia with *Acinetobacter baumannii*. Mucositis was treated with cefazolin and amikacin (20 mg/kg every 8 h and 20 mg/kg every 24 h, respectively), whereas the *A. baumannii* bacteremia was treated with amikacin and ceftazidime (20 mg/kg every 24 h and 50 mg/kg every 8 h, respectively). On this admission, two blood samples were collected for bacteriological culture, and antibiotic therapy was started with cefazolin (20 mg/kg every 8 h) and amikacin (20 mg/kg every 24 h). Forty-eight hours after admission, the patient developed severe balanoposthitis with cellulitis and erythema. Moreover, the local lesion began to extend to the pubic region. Even though blood cultures were negative, fever and neutropenia were persistent, thus, additional blood samples and glans/foreskin swabs were collected and the antibiotic regimen was changed to ceftazidime (50 mg/kg every 8 h) and metronidazole (7.5 mg/kg every 6 h). On the fifth day after admission, glans and foreskin necrotizing lesions were observed (Figure 1) and his clinical condition progressively worsened. On physical examination, hypotension, tachycardia, and oliguria were noted. When the patient developed septic shock related to Fournier's gangrene and neutropenia, he was transferred to the PICU, where antibiotic therapy was changed to imipenem (15 mg/kg every 6 h), and volume replacement therapy and dopamine (5 mg/kg/min) were initiated. From the second set of samples collected for microbiological culture, an MDR *P. aeruginosa* isolate (strain CIB 940) with susceptibility only to aztreonam and colistin was recovered exclusively from the glans and penis foreskin; blood samples remained negative (Table 1). On the sixth day, secretion with blood from the foreskin necrotic lesion was observed and the local foreskin/glans swelling extended to the total pubic and scrotal area. Due to scarcity of a therapeutic option, imipenem was changed to a combination therapy of ciprofloxacin and aztreonam (systemic administration of 10 mg/kg every 12 h and 30 mg/kg every 6 h, respectively) and topical polymyxin B ointment, in an attempt to achieve a synergistic effect. At this point, the patient presented an increase in WBC count indicating bone marrow

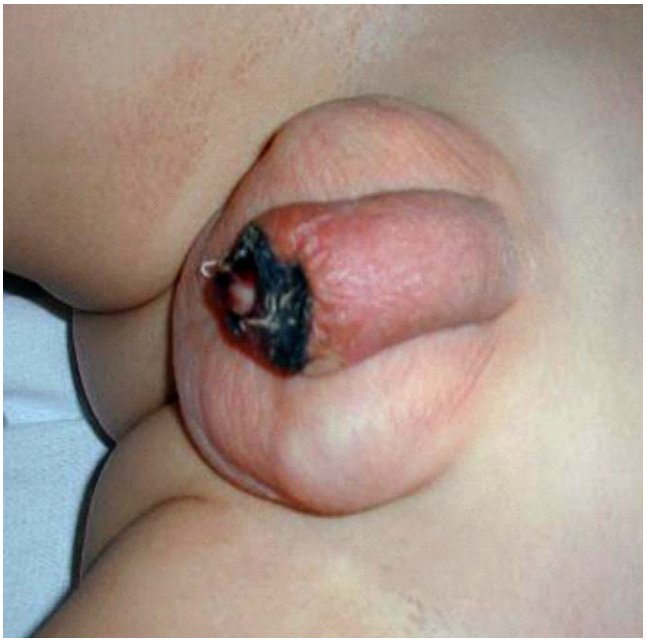

Figure 1. Necrotizing balanoposthitis due to *Pseudomonas aeruginosa* co-producing SPM-1 metallo-β-lactamase and 16S rRNA methylase RmtD in a pediatric patient with acute lymphoblastic leukemia (case 2). In the picture, a dark black necrotizing ring at the tip of foreskin is observed.

Table 1
Epidemiological and microbiological data for the multidrug-resistant *Pseudomonas aeruginosa* isolates

Strain	Source	Date (m/d/y)	Minimum inhibitory concentration (μg/ml)							PCR assay	ERIC profile
			IMP	IMP/EDTA	CAZ	ATM	AMK	CIP	PXB		
CIB 518	Glans swab	05/04/04	>256	6	>256	2	>256	>32	<4	bla_{SPM-1}, *rmtD*	A
CIB 704	Urine[a]	06/14/04	>256	6	>256	3	>256	>32	<4	bla_{SPM-1}, *rmtD*	A
CIB 904	Glans swab	06/14/04	>256	4	>256	4	>256	>32	<4	bla_{SPM-1}, *rmtD*	A
CIB 940	Glans swab	07/16/04	128	2	>256	3	>256	>32	<4	bla_{SPM-1}, *rmtD*	A

AMK, amikacin; ATM, aztreonam; CIP, ciprofloxacin; CAZ, ceftazidime; EDTA, ethylenediaminetetraacetic acid; IPM, imipenem; PXB, polymyxin B.
[a] Urine, 50 000 cfu/ml.

recovery. The administration of dopamine was brought to an end and the patient was transferred to the ward due to fever not being present during the latter 48 hours. On July 26 (at day 14 after admission), the patient, being considered clinically recovered, was discharged home. Two months later, complete healing of the foreskin/glans lesions was attained.

2.3. Isolates identified

The four isolates of *P. aeruginosa* were identified by the Vitek system (BioMérieux, Hazlewood, MO, USA). Minimum inhibitory concentrations (MICs) were determined by E-test (AB Biodisk, Solna, Sweden). Next, metallo-β-lactamase (MBL) production was screened by a double disk-synergy test using ceftazidime and imipenem as substrates and ethylenediaminetetraacetic acid (EDTA) and thiol compounds [2-mercaptoacetic acid (2-MAA) and 2-mercaptopropionic acid (2-MPA)] as β-lactamase inhibitors. The enhancement of inhibition zone or the presence of a key hole (or ghost zone) between imipenem- or ceftazidime-containing disk and EDTA- or thiol compound-containing disk was interpreted as a positive result to MBL production.[9] DNA amplification by PCR was used to search for specific bla_{IMP}, bla_{VIM}, and bla_{SPM} MBL genes.[10] Additionally, aminoglycoside-resistance was investigated by PCR for the *armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD* 16S rRNA methylase genes.[11]

All isolates recovered presented the unusual antibiogram phenotype of resistance to all β-lactams except aztreonam, and were also resistant to all the other antibiotics available such as aminoglycosides and fluoroquinolones. The high levels of imipenem resistance (≥128 μg/ml) observed in these *P. aeruginosa* isolates suggested the presence of an MBL, since these enzymes are considered to be the most frequent cause of carbapenem resistance in these organisms.[12] MBL activity was confirmed by the double-disk test, but ghost zones were observed exclusively between 2-MAA-containing disks and ceftazidime-containing disks. Moreover, there was a reduction in the MIC of imipenem in the presence of EDTA, from a range of ≥128 down to ≤6 μg/ml, indicating MBL activity (Table 1). PCR analysis showed that all isolates harbored both the bla_{SPM} and *rmtD* genes, which were responsible for resistance to all beta-lactams and aminoglycosides, respectively. Using the ERIC-2 primer,[13] genotyping revealed that the isolates were clonally related (Table 1), all presenting identical band profiles.

3. Discussion

Pseudomonas aeruginosa is one the most frequent pathogens in neutropenic patients.[14] Combination therapy is normally recommended for the treatment of these infections because this organism displays a decreased susceptibility to the currently used anti-pseudomonal agents.[15] Although the preferred combination remains β-lactams and aminoglycosides, over the past few years the frequency of resistance to these antibiotics has been higher, especially in Brazil.[16] In this regard, the emergence of isolates producing carbapenemases, mainly MBL, have limited the use of β-lactams;[12] SPM-1 producing *P. aeruginosa* has been reported to be endemic in Brazilian hospitals since 2003.[10,17] Indeed, a fatal outbreak of infection due to clonally related SPM-1 producing *P. aeruginosa* was documented at our institution[18] at the time when these two cases of balanoposthitis occurred, and the four *P. aeruginosa* isolates studied here were part of the cluster of the SPM-1 producing *P. aeruginosa*. On the other hand, production of 16S rRNA methylase has emerged recently as a mechanism of high-level resistance to all 4,6-disubstituted deoxystreptamine aminoglycosides, such as amikacin, tobramycin, and gentamicin.[11] Thus, co-production of novel 16S rRNA methylases and MBL would render ineffective a potent double-coverage regimen of carbapenem plus aminoglycoside, contributing to the emergence of multidrug-resistant phenotypes.[17]

In the present study three main findings are reported. First, balanoposthitis due to *P. aeruginosa* infection occurs in neutropenic patients and can be recurrent.[3] In this regard, it is likely that the initial colonization of the glans and its foreskin resulted from exposure of the preputial mucosa to contaminated surfaces, or just as likely, contaminated hands (particularly healthcare workers), evolving to severe balanoposthitis with glans/foreskin lesions as a source of fever. Secondly, co-production of MBL and 16S rRNA methylase has a potential impact on the empirical management of complicated infections caused by *P. aeruginosa*, which are usually treated with cephalosporins and aminoglycosides. Thirdly, combined treatment may be helpful but it should be correlated with the reversion of the neutropenic condition. In fact, MDR *P. aeruginosa* sepsis in the neutropenic patient has been successfully treated with serial granulocyte transfusions.[19] On the other hand, MDR *P. aeruginosa* infection in neutropenic patients has been successfully treated with combination therapy using polymyxin B.[20] Interestingly, none of the MBLs hydrolyze aztreonam.[12] In this regard, aztreonam has been proved to be an effective alternative for combined treatment of Gram-negative infections and fever of unknown origin in cancer patients,[21] including those infections caused by MBL-producing *P. aeruginosa* and *Klebsiella pneumoniae*.[22–24] Likewise, ciprofloxacin has been effective against infection with *P. aeruginosa* carrying bla_{IMP} MBL in an endogenous bacteremia model.[25] However, all these reports must be regarded as preliminary, and large, randomized controlled trials are required to define the optimal treatment for these infections.

4. Conclusions

We report two rare cases of balanoposthitis caused by *P. aeruginosa* co-producing MBL and 16S rRNA methylase. Although *P. aeruginosa* remains an unusual cause of balanoposthitis, these cases should alert the physician to the potential pathogenicity of this bacterium. Furthermore, co-production of MBL and 16S rRNA methylase has a potential impact on the empirical management of complicated infections caused by *P. aeruginosa*.

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Conflict of interest: No conflict of interest to declare.

References

1. Edwards S. Balanitis and balanoposthitis: a review. *Genitourin Med* 1996; **72**:155–9.
2. Manian FA, Alford RH. Nosocomial infectious balanoposthitis in neutropenic patients. *South Med J* 1987;**80**:909–11.
3. Lisboa C, Ferreira A, Resende C, Rodrigues AG. Infectious balanoposthitis: management, clinical and laboratory features. *Int J Dermatol* 2009;**48**:121–4.
4. English JC, 3rd, Laws RA, Keough GC, Wilde JL, Foley JP, Elston DM. Dermatoses of the glans penis and prepuce. *J Am Acad Dermatol* 1997;**37**:1–24.
5. Herieka E, Fisk P. Methicillin resistant *Staphylococcus aureus* (MRSA) balanoposthitis in an insulin dependent diabetic male. *Sex Transm Infect* 2001;**77**:223.
6. Alsterholm M, Flytström I, Leifsdottir R, Faergemann J, Bergbrant IM. Frequency of bacteria, *Candida* and *Malassezia* species in balanoposthitis. *Acta Derm Venereol* 2008;**88**:331–6.
7. Petrozzi JW, Erlich A. Pseudomonal balanitis. *Arch Dermatol* 1977;**113**:952–3.
8. Rabinowitz R, Lewin EB. Gangrene of the genitalia in children with *Pseudomonas* sepsis. *J Urol* 1980;**124**:431–2.
9. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing Gram-negative bacteria by using thiol compounds. *J Clin Microbiol* 2000;**38**:40–3.
10. Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-β-lactamase. *J Antimicrob Chemother* 2003;**52**:699–702.
11. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. *Clin Infect Dis* 2007;**45**:88–94.
12. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005;**18**:306–25.
13. Pellegrino FL, Teixeira LM, Carvalho Md Mda G, Aranha Nouér S, Pinto De Oliveira M, Mello Sampaio JL, et al. Occurrence of a multidrug-resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brazil. *J Clin Microbiol* 2002;**40**:2420–4.
14. Wang FD, Lin ML, Liu CY. Bacteremia in patients with hematological malignancies. *Chemotherapy* 2005;**51**:147–53.
15. Bassetti M, Righi E, Viscoli C. *Pseudomonas aeruginosa* serious infections: mono or combination antimicrobial therapy? *Curr Med Chem* 2008;**15**:517–22.
16. Andrade SS, Jones RN, Gales AC, Sader HS. Increasing prevalence of antimicrobial resistance among *Pseudomonas aeruginosa* isolates in Latin American medical centres: 5 year report of the SENTRY Antimicrobial Surveillance Program (1997-2001). *J Antimicrob Chemother* 2003;**52**:140–1.
17. Doi Y, Ghilardi AC, Adams J, de Oliveira DG, Paterson DL. High prevalence of metallo-beta-lactamase and 16S rRNA methylase coproduction among imipenem-resistant *Pseudomonas aeruginosa* isolates in Brazil. *Antimicrob Agents Chemother* 2007;**51**:3388–90.
18. Lincopan N, Leis R, Gallo LB, Rossi F, Levy CE, Mamizuka EM. Fatal outbreak of SPM-1 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in a pediatric hematology–oncology care center in Campinas, Brazil. *Int J Infect Dis* 2006;**10**(Suppl):S150.
19. Lin YW, Adachi S, Watanabe K, Umeda K, Nakahata T. Serial granulocyte transfusions as a treatment for sepsis due to multidrug-resistant *Pseudomonas aeruginosa* in a neutropenic patient. *J Clin Microbiol* 2003;**41**:4892–3.
20. Ostronoff M, Ostronoff F, Sucupira A, Souto Maior AP, Caniza M, Florêncio R, et al. Multidrug-resistant *Pseudomonas aeruginosa* infection in neutropenic patients successfully treated with a combination of polymyxin B and rifampin. *Int J Infect Dis* 2006;**10**:339–40.
21. Bodey G, Reuben A, Elting L, Kantarjian H, Keating M, Hagemeister F, et al. Comparison of two schedules of cefoperazone plus aztreonam in the treatment of neutropenic patients with fever. *Eur J Clin Microbiol Infect Dis* 1991;**10**:551–8.
22. Guerin F, Henegar C, Spiridon G, Launay O, Salmon-Ceron D, Poyart C. Bacterial prostatitis due to *Pseudomonas aeruginosa* harbouring the bla_{VIM-2} metallo-β-lactamase gene from Saudi Arabia. *J Antimicrob Chemother* 2005;**56**:601–2.
23. Panagiotakopoulou A, Daikos GL, Miriagou V, Loli A, Tzelepi E, Tzouvelekis LS. Comparative in vitro killing of carbapenems and aztreonam against *Klebsiella pneumoniae* producing VIM-1 metallo-beta-lactamase. *Int J Antimicrob Agents* 2007;**29**:360–2.
24. Weile J, Ohler S, Schönthal S, Knabbe C. A VIM-1 metallo-beta-lactamase-producing *Klebsiella pneumonia*e clinical isolate in an acute hospital in Germany. *Int J Antimicrob Agents* 2009;**33**:389–91.
25. Aoki S, Hirakata Y, Kondoh A, Gotoh N, Yanagihara K, Miyazaki Y, et al. Virulence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in vitro and in vivo. *Antimicrob Agents Chemother* 2004;**48**:1876–8.