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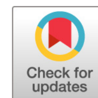
LETTER TO THE EDITOR



Detection of OXA-58-Producing *Acinetobacter seifertii* Recovered from a Black-Necked Swan at a Zoo Lake

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Acinetobacter species are ubiquitous pathogens that are broadly encountered in the environment, with some species, like *Acinetobacter baumannii*, *A. pittii*, and *A. nosocomialis*, more frequently detected in nosocomial settings (1). During the last decades, carbapenem resistance rates among such microorganisms have increased mainly because of the acquisition of carbapenem-hydrolyzing class D β-lactamase (CHDL)-encoding genes. The dissemination of bla_{OXA-23}, $bla_{OXA-143}$, and more recently bla_{OXA-72} is the main cause of carbapenem resistance among Brazilian *A. baumannii* clinical isolates (2). In contrast, bla_{OXA-58} has been rarely reported in clinical isolates of *Acinetobacter* species in Brazil (3–5). However, Cayô and colleagues recently described two OXA-58-producing *A. seifertii* isolates from patients hospitalized at a tertiary-care hospital in São Paulo, Brazil. Interestingly, these isolates were recovered more than 20 years ago (1993 and 1997) (3). Here, we describe an OXA-58-producing *A. seifertii* isolate colonizing a black-necked swan residing in the lakes of the São Paulo zoo.

During a surveillance study, 37 black-necked swans (*Cygnus melanocoryphus*) residing in the lakes of the São Paulo zoo were screened for colonization by carbapenem-resistant Gram-negative bacilli. Swabs of both the choana and the cloaca were collected. The swabs were streaked onto MacConkey agar supplemented with imipenem at 1 μg/ml (Sigma-Aldrich, St. Louis, MO), followed by Gram staining. We recovered a cloacal carbapenem-resistant Gram-negative coccobacillus (Ac-12.1) that was initially identified by matrix-assisted laser desorption ionization time of flight mass spectrometry as a member of the *Acinetobacter calcoaceticus-baumannii* complex. Subsequently, Ac-12.1 was identified to the species level as *A. seifertii* by *rpoB* sequencing (6). Antimicrobial susceptibility testing was performed by broth microdilution and interpreted according to the EUCAST guidelines (7). Ac-12.1 showed high piperacillin-tazobactam (>256 and 4 μg/ml), ampicillin-sulbactam (32 and 16 μg/ml), cefepime (16 μg/ml), ceftazidime (32 μg/ml), and ceftriaxone (64 μg/ml) MICs. It was resistant to imipenem (MIC, 16 μg/ml), meropenem (MIC, 8 μg/ml), amikacin (MIC, >256 μg/ml), and polymyxin B (MIC, 4 μg/ml). In contrast, Ac-12.1 was susceptible to gentamicin (MIC, 4 μg/ml), ciprofloxacin (MIC, 1 μg/ml), levofloxacin (MIC, 0.25 μg/ml), and sulfamethoxazole-trimethoprim (MICs, 2 and 38 μg/ml).

Screening for carbapenemase production by Blue Carba test was positive with 2 h of incubation, as previously described (8). Molecular characterization of carbapenemase-encoding genes was performed by PCR, followed by DNA sequencing with specific primers (9, 10), and demonstrated that Ac-12.1 carried the bla_{OXA-58} gene. No additional

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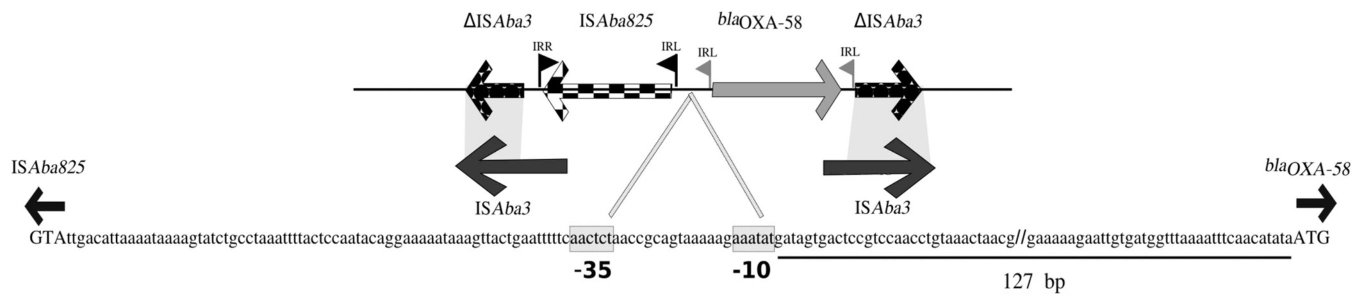

FIG 1 Genetic context of bla_{OXA-58} in *A. seifertii* isolate Ac-12.1. Genes are shown as labeled arrows; inverted repeats (IRs) of IS*Aba3* and IS*Aba825* are shown as flags (gray flags, IS*Aba3*; black flags, IS*Aba825*). The presence of a left IR (IRL) of IS*Aba3* upstream of bla_{OXA-58} suggests that insertion of IS*Aba825* occurred after transposon formation (ΔIS*Aba3*–bla_{OXA-58}–ΔIS*Aba3*). Integral IS*Aba3* sequences are represented below the genetic context, and regions with similarity to ΔIS*Aba3* are highlighted in light gray. The nucleotide sequence of the promoter region is boxed. The double bars in the127-bp nucleotide sequence represent the nucleotides omitted. Uppercase letters represent the start codons of IS*Aba825* and bla_{OXA-58}.

acquired β-lactamase-encoding genes were detected. The clonal relationship of Ac-12.1 and the two historical OXA-58-producing *A. seifertii* strains (Asp-70064 and Asp-1069) previously detected in Brazil (3) was analyzed by pulsed-field gel electrophoresis (PFGE) with the ApaI restriction enzyme (11). All OXA-58-producing *A. seifertii* isolates showed the same PFGE pattern (data not shown), despite being collected more than 20 years apart. Plasmid profiling and Southern blot hybridization were performed by the Kieser methodology and with the DIG DNA Labeling and Detection kit (Roche Diagnostics GmbH, Penzberg, Germany), respectively. Our results showed that bla_{OXA-58} was located on a plasmid of ~59 kb in strain Ac12.1, which is similar to the size of plasmids carried by clinical strains Asp-70064 and Asp-1069 (3). Transfer of bla_{OXA-58} by transformation assays with *A. baumannii* ATCC 19606 as the recipient strain was unsuccessful.

To determine the genetic surroundings of the bla_{OXA-58} gene, PCR, followed by sequencing by DNA walking, was performed with specific primers designed for IS*Aba3*, as previously published (12). The sequence analyses revealed a 2.8-kb structure (GenBank accession number MF417790) in which IS*Aba825* was located upstream of bla_{OXA-58} in the opposite direction. This IS*Aba825*–bla_{OXA-58} structure was located within a composite transposon formed by two truncated copies of IS*Aba3*, as shown in Fig. 1. It has been already reported that IS*Aba825* was associated with bla_{OXA-58} overexpression (13, 14). The genetic background found in the present study was identical to those observed for plasmid pAb242, which was previously detected in an OXA-58-producing *A. baumannii* clinical isolate from Argentina (unpublished data; accession number KR055667.1). Distinct genetic backgrounds have been described for bla_{OXA-58} genes so far, demonstrating that complex transposable elements are associated with this resistance determinant (15).

To date, *A. seifertii* has been isolated in hospital and community settings (door handle and game console), illustrating its ubiquitous nature (3, 12, 16). These isolates retained susceptibility to quinolones and aminoglycosides and also showed susceptibility to most β-lactam agents when they did not carry a CHDL (3, 16). To our knowledge, this is the first report of OXA-58-producing *Acinetobacter* species isolated from an environmental source. The detection of an identical OXA-58-producing *A. seifertii* clone nearly 25 years after its first description highlights the adaptation of this fit clone in this geographic region. In addition, since only one bird was colonized by OXA-58-producing *A. seifertii*, it seems that this microorganism does not belong to the normal microbiota of *C. melanocoryphus*. Although we did not investigate the source of *A. seifertii* acquisition, it seems that local environmental contamination did not play an important role because other birds were not colonized by this clone. The lakes of the São Paulo zoo are often visited by migratory birds, which may have acted as vectors transmitting OXA-58-producing *A. seifertii* from other environmental sources. There is little evidence of the clinical relevance of *A. seifertii*. However, Na and colleagues (17)

demonstrated that *A. seifertii* isolates had consistently high virulence-associated phenotypes, with no significant difference from those showed by *A. baumannii* (with the exception *A. baumannii* clone ST110) and *A. nosocomialis* (17). Thus, we believe that *A. seifertii* is capable of causing infections similar to those caused by *A. baumannii*. Lastly, the acquisition of a carbapenemase-encoding gene by *A. seifertii* is very worrisome, since this species seems to be naturally resistant to polymyxins, drastically limiting the therapeutic options available for the treatment of infections with this bacterium.

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REFERENCES

1. Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M. 2016. Reservoirs of non-baumannii Acinetobacter species. Front Microbiol 7:49. https://doi.org/10.3389/fmicb.2016.00049.
2. Vasconcelos AT, Barth AL, Zavascki AP, Gales AC, Levin AS, Lucarevschi BR, Cabral BG, Brasiliense DM, Rossi F, Furtado GH, Carneiro IC, da Silva JO, Ribeiro J, Lima KV, Correa L, Britto MH, Silva MT, da Conceição ML, Moreira M, Martino MD, de Freitas MR, Oliveira MS, Dalben MF, Guzman RD, Cayô R, Morais R, Santos SA, Martins WM. 2015. The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. Diagn Microbiol Infect Dis 83:382–385. https://doi.org/10.1016/j.diagmicrobio.2015.08.006.
3. Cayô R, Rodrigues-Costa F, Pereira Matos A, Godoy Carvalhaes C, Dijkshoorn L, Gales AC. 2016. Old clinical isolates of *Acinetobacter seifertii* in Brazil producing OXA-58. Antimicrob Agents Chemother 60:2589–2591. https://doi.org/10.1128/AAC.01957-15.
4. Figueiredo DQ, Santos KR, Pereira EM, Schuenck RP, Mendonça-Souza CR, Teixeira LM, Mondino SS. 2011. First report of the bla(OXA-58) gene in a clinical isolate of *Acinetobacter baumannii* in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 106:368–370. https://doi.org/10.1590/S0074-02762011000300019.
5. de Souza Gusatti CS, Bertholdo LM, Otton LM, Marchetti DP, Ferreira AE, Corção G. 2012. First occurrence of bla_{OXA-58} in *Acinetobacter baumannii* isolated from a clinical sample in southern Brazil. Braz J Microbiol 43:243–246. https://doi.org/10.1590/S1517-83822012000100027.
6. La Scola B, Gundi VA, Khamis A, Raoult D. 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J Clin Microbiol 44:827–832. https://doi.org/10.1128/JCM.44.3.827-832.2006.
7. European Committee on Antimicrobial Susceptibility Testing. 2017. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2017. European Committee on Antimicrobial Susceptibility Testing, Basel, Switzerland. http://www.eucast.org/clinical_breakpoints/. Accessed 30 March 2017.
8. Pires J, Novais A, Peixe L. 2013. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol 51:4281–4283. https://doi.org/10.1128/JCM.01634-13.
9. Higgins PG, Lehmann M, Seifert H. 2010. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 35:305. https://doi.org/10.1016/j.ijantimicag.2009.10.014.
10. Viana AL, Cayô R, Avelino CC, Gales AC, Franco MC, Minarini LAR. 2013. Extended-spectrum β-lactamases in Enterobacteriaceae isolated in Brazil carry distinct types of plasmid-mediated quinolone resistance genes. J Med Microbiol 62(Part 9):1326–1331. https://doi.org/10.1099/jmm.0.055970-0.
11. Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, Heersma H, Dijkshoorn L. 2005. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. J Clin Microbiol 43:4328–4335. https://doi.org/10.1128/JCM.43.9.4328-4335.2005.
12. Marti S, Sánchez-Céspedes J, Blasco MD, Espinal P, Ruiz M, Alba V, Vila J. 2008. Characterization of the carbapenem-hydrolyzing oxacillinase OXA-58 in an *Acinetobacter phenon* 6/ct13TU clinical isolate. Diagn Microbiol Infect Dis 61:468–470. https://doi.org/10.1016/j.diagmicrobio.2008.03.014.
13. Ravasi P, Limansky AS, Rodriguez ER, Viale AM, Mussi MA. 2011. IS*Aba825*, a functional insertion sequence modulating genomic plasticity and bla_{OXA-58} expression in *Acinetobacter baumannii*. Antimicrob Agents Chemother 55:917–920. https://doi.org/10.1128/AAC.00491-10.
14. Lopes BS, Al-Hassan L, Amyes SG. 2012. ISAba825 controls the expression of the chromosomal bla(OXA-51-like) and the plasmid borne bla(OXA-58) gene in clinical isolates of *Acinetobacter baumannii* isolated from the USA. Clin Microbiol Infect 18:E446–E451. https://doi.org/10.1111/j.1469-0691.2012.03979.x.
15. Evans BA, Amyes SG. 2014. OXA β-lactamases. Clin Microbiol Rev 27:241–263. https://doi.org/10.1128/CMR.00117-13.
16. Choi JY, Kim Y, Ko EA, Park YK, Jheong WH, Ko G, Ko KS. 2012. *Acinetobacter* species isolates from a range of environments: species survey and observations of antimicrobial resistance. Diagn Microbiol Infect Dis 74:177–180. https://doi.org/10.1016/j.diagmicrobio.2012.06.023.
17. Na IY, Chung ES, Jung CY, Kim DH, Shin Y, Kang K, Kim ST, Ko KS. 2016. Comparison of the virulence-associated phenotypes of five species of *Acinetobacter baumannii* complex. J Microbiol Biotechnol 26:171–179. https://doi.org/10.4014/jmb.1507.07076.