Infection Control & Hospital Epidemiology (2022), **43**, 245–247
doi:10.1017/ice.2021.39

SHEA

Concise Communication

Nosocomial transmission of a *bla*$_{VIM-2}$ carbapenemase integron between isolates of two different *Pseudomonas* species

Andrea C. Büchler MD[1], Daniel Wüthrich PhD[2,3], Melanie Wicki Jauslin PhD[1], Adrian Egli MD, PhD[2,3] and Andreas F. Widmer MD, MSc[1]

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland, [2]Division of Clinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland and [3]Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland

Abstract

We report the first documented in-hospital patient-to-patient-transmission of a *bla*$_{VIM-2}$ integron between isolates of *Pseudomonas alcaligenes* and *P. aeruginosa*. Molecular typing looking only for difference within species may fail to detect nosocomial transmission of resistance genes.

(Received 19 May 2020; accepted 24 January 2021; electronically published 19 February 2021)

Carbapenemase-producing gram-negative bacteria are an emerging problem worldwide.[1] In low-endemicity countries, the spread of carbapenemase-producing microorganisms is frequently related to outbreaks in hospitals, *Pseudomonas aeruginosa* is frequently transmitted by contact with the hands of healthcare workers or environmental sources.[2-5] To our knowledge, we report the first well-documented in-hospital patient-to-patient-transmission of a *bla*$_{VIM-2}$ integron between isolates belonging to 2 different species, *P. aeruginosa* and *P. alcaligenes,* with indirect transmission to patients, likely by environmental contamination.

A 72-year-old male (patient Z) was transferred to our hospital for diabetic foot syndrome and chronic osteomyelitis after being previously hospitalized in Southeast Asia. He had received multiple antibiotic treatment courses, not recorded in the transfer protocol. At day 15 of the hospitalization in at the University Hospital Basel, a first screening for carbapenemase-producing gram-negatives, consisting of swabs of the throat and rectum as well as a urine sample, showed colonization with several isolates: a *P. aeruginosa* harboring a *bla*$_{VIM-2}$ carbapenemase-encoding gene; *Citrobacter koseri* and *Klebsiella pneumoniae,* both with a *bla*$_{NDM-1}$ carbapenemase-encoding genes; and *Escherichia coli* with a *bla*$_{OXA-181}$ carbapenemase-encoding gene. In addition to these strains, culture of the bone biopsy revealed isolates of *Proteus mirabilis*, *Enterococcus faecalis*, and *Morganella morganii. P. alcaligenes* was not identified in either the screening or the biopsies. An amputation of the right lower limb was performed for treatment of the chronic polymicrobial osteomyelitis. Antibiotic treatment was stopped after cultures of the bone from the proximal part of the amputation site remained negative, and there were no signs of osteomyelitis in the pathology specimen. The patient was put under strict contact precautions. All contact patients who shared the same room during or after hospitalization of the index case were screened for carbapenemase-producing gram-negative pathogens using the chromID CARBA SMART Bi-Plate Agar (bioMérieux, Marcy-l'Étoile, France) and, if cultures were positive, the Xpert Carba-R (Cepheid, Sunnyvale, CA) was used to identify carbapenemases.

A 90-year-old women (patient Y) was hospitalized in room 1 five days after the index patient Z had moved to a different room (Fig. 1). The patient was hospitalized for a soft-tissue infection of her left lower leg with bacteremia with *Staphylococcus aureus* and *Enterococcus faecalis* secondary to a leg injury. She was treated with debridement of the left lower leg. After a negative rectal screening for carbapenemase-producing microorganisms on day 4 of her hospitalization, she tested positive for *bla*$_{VIM-2}$ containing *P. alcaligenes* in a rectal swab taken 5 days later. The species was routinely determined by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) and later confirmed by 16S rRNA gene identity and ribosomal multilocus sequence typing (rMLST). She did not experience any infection due to *Pseudomonas alcaligenes*, and they are rarely reported in the literature as well.[6] She lived at home with her husband and denied any recent travel or hospitalization abroad or even within Switzerland. She had a history of myelodysplastic syndrome treated symptomatically with erythropoietin and danazolum. All other contact patients were negative for carbapenemase-producing gram-negative microorganisms.

In our institution, the first isolated strains from each patient harboring carbapenemases are routinely typed by whole-genome sequencing. We sequenced isolates 807875-6-18 and 808084-18 on both an Illumina NextSeq500 device (Illumina, San Diego, CA; 56x and 87x mean coverage, respectively) and an Oxford Nanopore MinION device (Oxford Nanopore, Oxford, UK; 104x and 128x mean coverage, respectively) in a hybrid assembly approach using Unicycler v 0.3.0b software[7] to reconstruct the genomes into 30 and 9 scaffolds, respectively. All reads have been submitted to the European Nucleotide Archive (project no. PRJEB34600). We scanned the 2 assemblies for resistance genes using ABRicate software[7] to search the NCBI AMR gene database (PRJNA313047). In both genomes, we identified a *bla*$_{VIM-2}$ gene. A

Author for correspondence: Andreas F. Widmer, E-mail: andreas.widmer@usb.ch
Cite this article: Büchler AC, *et al.* (2022). Nosocomial transmission of a *bla*$_{VIM-2}$ carbapenemase integron between isolates of two different *Pseudomonas* species. *Infection Control & Hospital Epidemiology*, 43: 245–247, https://doi.org/10.1017/ice.2021.39

CrossMark

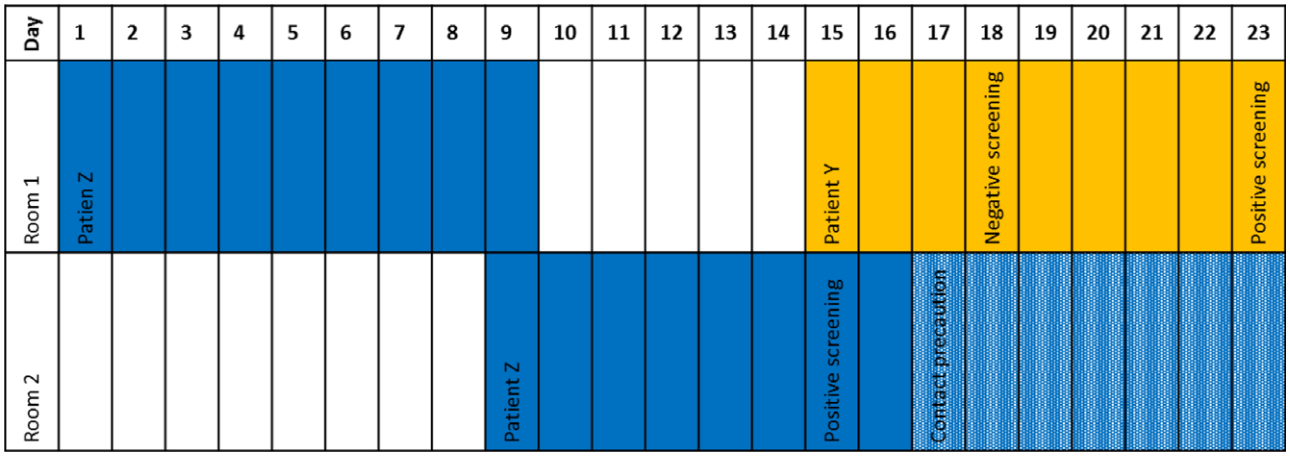

Fig. 1. Room occupancy of patient Z and patient Y during hospitalization.

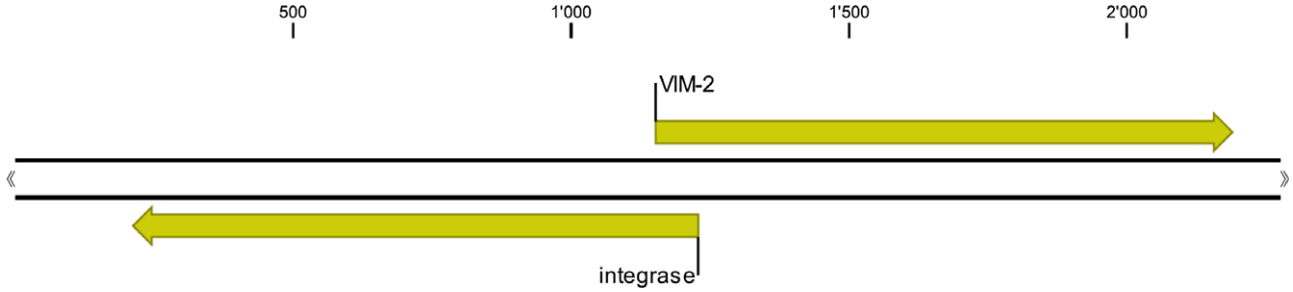

Fig. 2. Coding sequences of the integron carrying bla_{VIM-2}.

closer comparison of the genes and their genomic surroundings using Artemis software[8] showed that the bla_{VIM-2} genes are located in a 2,264-bp integron in both strains. The integron carries an integrase and a bla_{VIM-2} gene (Fig. 2). The sequence comparison, performed in SeaView[9] shows that the elements in the 2 strains differ by 2 single-nucleotide polymorphisms (SNPs). The bla_{VIM-2} genes are identical. By searching the NCBI database, we found that the found integron is almost identical (1 SNP difference) to the integron In76 that was described in the context of the discovery of the first bla_{VIM-2} gene in a *P. aeruginosa* isolate in Latin America.[8] We found that the integron in *P. aeruginosa* isolate 807875-6-18 (patient Z) is located on a 1,238,389-bp contig (chromosome fragment), and in *P. alcaligenes* isolate 808084-18 (patient Y) it is located on a 77,160-bp circular contig, representing a plasmid (rep_cluster_777, accession number JQ432564 (https://github.com/jrober84/mob-typer)). This contig shows identity (58% coverage and 98% sequence identity) to the pKS208 plasmid previously described in an uncultured bacterium that does not carry the bla_{VIM-2} gene.[9]

The evidence of horizontal gene transfer between 2 species is supported by (1) a very strong epidemiological link between the 2 patients, and (2) detection of identical bla_{VIM-2} that are extremely rare in our patient population.[10] In 2018, bla_{VIM-2} occurred in only 16 cases in Switzerland, of which 15 were in non–*Escherichia coli* or non–*Klebsiella pneumoniae* bacteria.[11] Molecular typing to detect transmission is commonly performed within species, but it may be extended to detect transmission of key factors between species if the epidemiological link is strong. A strong epidemiological link should trigger an extended workup with not only next-generation sequencing of the core genome but also include transposons to prove or to exclude nosocomial transmission.

Acknowledgments. We thank Dr Helena Seth-Smith for critical reading of the manuscript, and Elisabeth Schultheiss, Rosa-Maria Vesco and the team of Prof Richard Neher for excellent technical support. Dr Alfredo Mari performed additional plasmid analysis. Assemblies were performed at sciCORE (http://scicore.unibas.ch) scientific computing center at University of Basel, Switzerland.

Financial support. The study was supported by the University of Basel, Switzerland.

Conflict of interests. All authors report no conflicts of interest relevant to this article.

References

1. Baxter R, Hastings N, Law A, Glass EJ. *Guidelines for the Prevention and Control of Carbapenem-Resistant Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa in Health Care Facilities*. Geneva: World Health Organization; 2017.
2. Voor AF, Severin JA, Hagenaars MBH, Goeij I De, Gommers D, Vos MC. VIM-positive *Pseudomonas aeruginos*a in a large tertiary-care hospital: matched case- control studies and a network analysis. *Antimicrob Resist Infect Control* 2018;7(32):1–10.
3. Verfaillie C, Bruno M, F. Voor in't holt A, *et al.* Withdrawal of a novel-design duodenoscope ends outbreak of a VIM-2-producing *Pseudomonas aeruginosa*. *Endoscopy* 2015;47:493–502.
4. Price D, Ahearn DG. Incidence and persistence of *Pseudomonas aeruginosa* in whirlpools. *J Clin Microbiol* 1988;26:1650–1654.

5. Kossow A, Kampmeier S, Willems S, *et al.* Control of multidrug-resistant *Pseudomonas aeruginosa* in allogeneic hematopoietic stem cell transplant recipients by a novel bundle including remodeling of sanitary and water supply systems. *Clin Infect Dis* 2017;65:935–942.
6. Suzuki M, Suzuki S, Matsui M, Hiraki Y, Kawano F, Shibayama K. Genome sequence of a strain of the human pathogenic bacterium *Pseudomonas alcaligenes* that caused bloodstream infection. *Genome Announc* 2013;1(5):1–2.
7. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13(6):1–22.
8. Rutherford K, Parkhill J, Crook J, *et al.* Artemis: sequence visualization and annotation. *Bioinformatics* 2000;16:944–945.
9. Gouy M, Guindon S, Gascuel O. Sea view version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010;27:221–224.
10. Erb S, Frei R, Dangel M, Widmer AF. Multidrug-resistant organisms detected more than 48 hours after hospital admission are not necessarily hospital acquired. *Infect Control Hosp Epidemiol* 2017;38:18–23.
11. Carbapenem resistenz. Anresis - Schweizerisches Zentrum für Antibiotikaresistenzen website. https://www.anresis.ch/de/antibiotikaresistenz/resistance-data-human-medicine/. Accessed October 14, 2020.

https://doi.org/10.1017/ice.2021.39 Published online by Cambridge University Press