Infection, Genetics and Evolution 28 (2014) 276–277

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Letter To the Editor

Human infections due to *Pseudomonas chlororaphis* and *Pseudomonas oleovorans* harboring new bla_{VIM-2}-borne integrons

Pseudomonas spp has the innate ability to use a wide range of organic and inorganic compounds and to survive under diverse environmental conditions (Moore et al., 2006). Most species of *Pseudomonas* grow rapidly and are particularly renowned for their ability to metabolize an extensive number of substrates. They are often resistant to antibiotics, disinfectants, detergents, heavy metals, and organic solvents. *Pseudomonas aeruginosa* is the most clinically important species that cause different kinds of human infections. *Pseudomonas oleovorans* and *Pseudomonas chlororaphis* are considered to be environmental species, and are phylogenetically related to *P. aeruginosa* group and *P. chlororaphis* group, respectively (Anzai et al., 2000). To the best of our knowledge these species have never been described to cause human infections.

Metallo-β-lactamases (MBLs) are a growing group of enzymes with a strong carbapenemase activity, which are inhibited by chelating agents such as ethylenediaminetetraacetic acid (EDTA). However, they are not affected by clinical β-lactamase inhibitors (e.g. clavulanic acid) and retain susceptibility against monobactams (Corgnaglia et al., 2011). IMP and VIM are the most common MBLs mostly detected in non-fermenters, especially in *P. aeruginosa*. bla_{VIM} and bla_{IMP} are mobile gene cassettes inserted on integrons, which are associated with transposons and plasmids, increasing the spread of several antimicrobial resistance determinants (Corgnaglia et al., 2011). Forty-one variants of bla_{VIM} gene were described, with bla_{VIM-2} being the most common allele.

Two clinical isolates, named M11740 (03/19/2010) and M13320 (02/19/2011), initially identified as *Pseudomonas putida* by automated systems were submitted from Mendoza and Córdoba provinces, Argentina, to the National Reference Laboratory in Antimicrobial Resistance as possible MBL-producers.

Isolate M11740 was identified twice by the Bruker MALDI Biotyper, based in matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics Co), as *P. chlororaphis*, high score: 2.271 (scores >2.0 provides accurate identification). Partial DNA sequence of 16S rRNA subunit (accession number KM877268) showed a similarity higher than 99% with *P. chlororaphis* subsp. *aurantiaca* Pr7 reference strain. *P. chlororaphis* M11740 was recovered from a 59-year old male from blood culture. The patient suffered prolonged febrile syndrome and was suspected of having endocarditis.

For isolate M13320, two different results with high scores were obtained using MALDI-TOF: *Pseudomonas alcaliphila* (score: 2.109) and *P. oleovorans* (score: 2.101). The 16S rRNA sequence (accession number KM877267) analysis showed a >99% similarity with *P. oleovoans* ATCC 8062 (former *Pseudomonas pseudoalcaligenes*). *P. oleovorans* M13320 was recovered from a 3-year old female from blood culture. This child had fever and was a chronic patient with an external ventricular bypass valve.

MBL activity was confirmed by synergism between carbapenems and EDTA (750 μg) plus sodium mercaptoacetate (1900 μg) discs and by the modified Hodge test according CLSI guidelines for *P. aeruginosa* (CLSI, 2013). By using Vitek2C system (Biomerieux®), isolates M11740 and M13320 displayed the following MIC (mg/L) values: imipenem, ⩾16 and ⩾16; meropenem, ⩾16 and 8; ceftazidime, ⩾64 and ⩾64; cefepime, ⩾64 and 8, amikacin, ⩽2 and ⩽2; gentamicin, ⩽1 and 4; ciprofloxacin, ⩾4 and ⩾4; and colistin ⩽0.5 and ⩽0.5, respectively. The use of EDTA (0.4 mM) reduced the carbapenem MICs (agar dilution) by at least three dilutions in both strains, also suggesting the expression of MBLs.

Screening of bla_{VIM}, bla_{IMP} and bla_{NDM} MBL genes was performed by PCR and positive results were confirmed by DNA sequencing. Integron array was evaluated by PCR using different set of primers (5CS [5′-GGCATCCAAGCAGCAAG-3′], 3CS [5′-AAGCAGACTTGACC TGA-3′], tniC-F [5′-CGATCTCTGCGAAGAACTCG-3′], VIM-F [5′-AGTGGTGAGTATCCGACAG-3′], VIM-R [5′-ATGAAAGTGCGTGGA-GAC-3′]) and DNA sequencing. *P. chlororaphis* M11740 harbored the In899 (accession number KJ668595), an unusual class 1 integron, which lacks the 3′CS but has the *tni*C gene of the Tn*402*-like transposon and contains the bla_{VIM-2} gene as unique cassette (Fig. 1). This integron was identified on a 55 Kb plasmid when the S1 nuclease digestion protocol plus Southern blot (bla_{VIM} probe) was performed (Gomez et al., 2013). *P. oleovoans* M13320 harbored a class 1 integron named In984 (accession number KJ668596) according to The Integron Database (Integrall, available at http://integrall.bio.ua.pt/) containing bla_{VIM-2} and *aacA27* cassettes. Two plasmids (20 and 350 Kb) were observed in this isolate and the smaller one yielded positive hybridization with bla_{VIM} probe.

Since 2002 more than 70 isolates of *Pseudomonas* spp. other than *P. aeruginosa* were recovered from >30 hospitals of our country, all containing bla_{VIM} gene (unpublished data). *P. putida* was the most frequent species of this group. Interestingly, the first detection of MBL from the referring hospital in Mendoza was the *P. chlororaphis* M11740 isolate. Conversely, several bla_{VIM}-producing *Pseudomonas* isolates were observed in the Córdoba Hospital, seven of which were contemporary to M13320 isolate (recovered between 11/11/2010 and 09/29/2011). These isolates were identified by MALDI-TOF as *P. putida* (4), *Pseudomonas monteilii* (2), and *P. putida* group (1). Three of these isolates harbored the same In984 integron as *P. oleovorans* M13320, two of them yielded positive hybridization with a 75 Kb plasmid and the remaining with a 65 Kb plasmid. The other four *Pseudomonas* isolates harbored a different integron array and yielded positive hybridization with 65 Kb (2), 70 Kb (1) and 100 Kb (1) plasmids (Data not shown). These results suggest a complex distribution of bla_{VIM} genes on plasmids of different sizes.

http://dx.doi.org/10.1016/j.meegid.2014.10.012

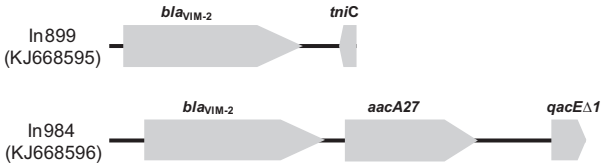

Fig. 1. Schematic representation of In899 and In984 integrons. In899 is a Tn*402*-like class 1 integrons (1118 bp) containing bla_{VIM-2} as unique cassette. In984 is a class 1 integron (2166 bp) containing both bla_{VIM-2} and *aacA27* cassettes.

To our knowledge this is the first report of MBL-producing *P. chlororaphis* and *P. oleovoans* clinical isolates recovered from human infections. Additionally, new integron arrays were detected, including one containing bla_{VIM-2} as a unique gene cassette into an unusual class 1 integron. The finding of this same integron in different *Pseudomonas* species indicates the ability of these species to act as unexplored reservoirs of multiresistance genes. This report highlights the potential of unusual environmental species of *Pseudomonas* as opportunistic human pathogens, as well as, an actual reservoir for MBLs and other resistance genes. Therefore, it would be important to perform screening for MBL and other carbapenemases production for clinical isolates of all species of *Pseudomonas*.

Funding

This work was supported by the regular federal budget of the National Ministry of Health of Argentina.

Acknowledgements

Integron numbers were assigned by INTEGRALL (Bioinformatics. 2009 15;25(8):1096-8).

References

Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H., Oyaizu, H., 2000. Phylogenetic affiliation of the pseudomonas based on 16S rRNA sequence. Int. J. Syst. Evol. Microbiol. 50, 1563–1589.

Clinical and Laboratory Standards Institute (CLSI), 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.

Corgnaglia, G., Giamarellou, H., Rossolini, G.M., 2011. Metallo-β-lactamases: a last frontier for β-lactams? Lancet Infect. Dis. 11, 381–393.

Gomez, S., Pasteran, F., Faccone, D., Bettiol, M., Veliz, O., De Belder, D., Rapoport, M., Gatti, B., Petroni, A., Corso, A., 2013. Intrapatient emergence of OXA-247: a novel carbapenemase found in a patient previously infected with OXA-163-producing *Klebsiella pneumoniae*. Clin. Microbiol. Infect. 19, E233–235.

Moore, E.R.B., Tindall, B.J., Martins dos Santos, V.A.P., Pieper, D.H., Ramos, J.L., Palleroni, N.J., 2006. Nonmedical: *Pseudomonas*. Prokaryotes 6, 646–703.

Diego Faccone
Fernando Pasteran
Ezequiel Albornoz
Servicio Antimicrobianos, National Reference Laboratory in Antimicrobial Resistance, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina

Liliana Gonzalez
Hospital Infantil Municipal, Córdoba, Argentina

Omar Veliz
Servicio Antimicrobianos, National Reference Laboratory in Antimicrobial Resistance, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina

Monica Prieto
Servicio Bacteriología Especial, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina

Ricardo Bucciarelli
Hospital Español, Mendoza, Argentina

Raquel Callejo
Servicio Bacteriología Especial, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina

Alejandra Corso *
Servicio Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbrán", Av. Velez Sarsfield 563 (1281), Buenos Aires, Argentina
* Tel./fax: +54 11 4303 2812.
E-mail address: acorso@anlis.gov.ar

Available online 18 October 2014