Infection, Genetics and Evolution 12 (2012) 2003–2005

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

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

Letter to the Editor

First clonal spread of KPC-producing *Pseudomonas aeruginosa* in Buenos Aires, Argentina

Sir:

Pseudomonas aeruginosa is one of the leading gram negative bacteria associated with hospital acquired infections, causing life threatening processes particularly in compromised patients at intensive care units (www.aam.org.ar). It may express a wide repertoire of resistance mechanisms and is able to acquire additional resistance genes (Bebrone, 2007; Juan Nicolau and Oliver, 2008). The increasing detection of carbapenemases, which exhaust therapeutic options avoiding carbapenem activity, is of great concern although the loss of the outer membrane protein OprD, overexpression of efflux pumps and hyperproduction of chromosomal class C β-lactamase may also contribute to develop full resistance to carbapenems (Quale et al., 2006; Santella et al., 2010a,b).

KPC enzymes hydrolyze at significant levels penicillins, cephalosporins, monobactams and carbapenems, and are weakly inhibited by clavulanic acid and tazobactam (Sacha et al., 2009; Walsh, 2010). Since the first detection of KPC-1/2 in *Klebsiella pneumoniae* in 2001 in USA, they have been encountered in many other *Enterobacteriaceae* from the Americas, Europe, Asia and Middle East (Yigit et al., 2001; Nordmann et al., 2009). Their wide propagation across multiple continents has been related to a mobile genetic element, Tn4401 (Naas et al., 2008) and to the successful dissemination of a single *K. pneumoniae* sequence type (ST) 258 clone (Kitchel et al., 2009; Cejas et al., 2012).

In 2007, KPC-2 emerged in a clinical *P. aeruginosa* isolate recovered in Colombia (Villegas et al., 2007), soon after, this enzyme was reported from *P. aeruginosa* from Trinidad y Tobago (Alpaka et al., 2009), USA (Poirel et al., 2010) and China (Ge et al., 2011). KPC-5 was described in *P. aeruginosa* isolated at Puerto Rico (Wolter et al., 2009).

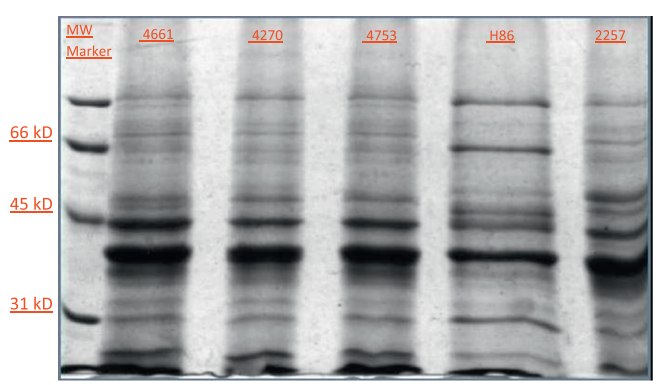
Although KPC-2 producing *K. pneumoniae* are wide distributed in Buenos Aires hospitals and other regions of Argentina (Pasterán et al., 2008; Cejas et al., 2012), the presence of this enzyme has been only once reported from *P. aeruginosa* isolated in Bariloche placed 1700 km from Buenos Aires (Pasterán et al., 2011).

We report the first outbreak of clonally related KPC-producing *P. aeruginosa* in Buenos Aires, Argentina, which belong to the same sequence type of those isolates recovered previously in our country.

There were included three carbapenem resistant *P. aeruginosa* isolates from patients at the Intensive Care Unit (ICU) of a single hospital at Buenos Aires, Argentina, from July to September 2010. Susceptibilities to piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacina, colistin, gentamicin and amikacin were determined by the disk diffusion test according to the CLSI recommendations (Clinical and Laboratory Standards Institute, 2010). Screening for metallo-β-lactamases (MBL) and KPC β-lactamases were performed by a double disk diffusion method using 1 μmol EDTA (Santella et al., 2010a,b) and 300 μg phenyl boronic acid containing disks, respectively. PCR amplification of MBL coding genes and *kpc* were carried out as previously described (Bradford et al., 2004; Ellington et al., 2007). Amplified fragments were sequenced in both strands using an ABI Prism DNA 3700 sequencer and compared with databases using the NCBIś Basic Local Alignment Search Tool (Blast). Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA of *P. aeruginosa* isolates was carried out with a CHEF-DRIII system (Bio-Rad, Hemel Hempstead, UK), as described previously (Tsakris et al., 2000). Multi-Locus Sequence-Typing (MLST) with seven housekeeping genes (*acs*, *aro*, *gua*, *mut*, *nuo*, *pps*, *trp*) was performed according to published protocols (Curran et al., 2004). Sequence type (ST) numbers were assigned using the pubMLST database (http://pubmlst.org/paeruginosa).

Epidemiological data and antimicrobial susceptibility patterns of the isolates are shown in Table 1. The three isolates were susceptible to gentamicin, amikacin and colistin, intermediate to ceftazidime and resistant to piperacillin, piperacillin/tazobactam, cefepime, aztreonam, carbapenemes and fluoroquinolones. Synergy tests with EDTA were negative in all cases, also the multiplex PCR perfomed did not detect any alleles encoding VIM, IMP, SIM, SPM or GIM families of acquired MBLs. Screening of KPC enzymes using phenyl boronic acid was not reproducible even when it was performed with the same strain; however, the presence of *kpc*-2 was confirmed in all isolates.

Outer membrane proteins were extracted and resolved in 12% SDS–PAGE (Lambert, 1988). Previously characterized, *P. aeruginosa* H86 that produced OprD and *P. aeruginosa* 2257 that lack this protein were included as controls (Santella et al., 2010a,b). KPC producing isolates included in this study displayed an altered outer membrane protein profiles, lacking OprD.



Outer membrane protein profiles: 2257: *P. aeruginosa* that lack OprD; H86: *P. aeruginosa* that produce OprD; 4661, 4270 and 4753: this study.

1567-1348/$ - see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.meegid.2012.03.022

Table 1
Epidemiological data and antimicrobial susceptibility profile of KPC-2 producing *P. aeruginosa*.

Isolate number	Culture source	Date of isolation	Antimicrobial susceptibility (inhibition zone: mm)											Therapeutic treatment	Outcome
			PIP	PTZ	CAZ	FEP	AZT	IMI	MER	COL	AKN	GEN	CIP	LEV		
4661	Respiratory secretions /Blood	22/07/10	13	13	15	6	6	6	6	15	23	19	6	6	COL	Favorable
4270	Respiratory secretions	30/08/10	13	13	15	6	6	6	6	15	23	19	6	6	COL	Favorable
4753	Respiratory secretions	05/09/10	13	13	14	6	6	6	6	15	24	19	6	6	COL	Favorable

PIP: piperacillin, PTZ: piperacillin-tazobactam, CAZ: ceftazidime, FEP: cefepime, AZT: aztreonam, IMI: imipenem, MER: meropenem, CIP: ciprofloxacin, LEV: levofloxacina, COL: colistin, GEN: gentamicin, AKN: amikacin.

PFGE profiles were identical, revealing clonal relatedness. Allele sequences and STs were verified at http://pubmlst.org/paeruginosa and corresponded to sequence type ST 654 (allelic profile: *acs*:17, *aro*: 5, *gua*: 26, *mut*: 3, *nuo*: 4, *pps*: 4, *trp*:26).

We report the first outbreak of clonally related KPC-producing *P. aeruginosa* in Buenos Aires, Argentina. Since phenotypic detection of this resistance marker is still difficult and has not been yet standardized (Cuzon et al., 2011; Pasterán et al., 2011), it remains uncertain to what extent KPC-producing *P. aeruginosa* have spread in our country. Some epidemiological studies performed in carbapenemase producing *P. aeruginosa* indicate that some sequence types (ST) like ST654, ST175, ST235, ST621, among others, have been associated to the presence of MBL (Koh et al., 2010; Samuelsen et al., 2010; Santella et al., 2010a,b). To our knowledge, little is known about the clonal relationship and genetic background of KPC-producing *P. aeruginosa* isolates. ST654 (clonal complex CC244) correspond to an internationally disseminated clone previously associated to both IMP and VIM enzymes (Samuelsen et al., 2010), however *P. aeruginosa* ST654 has been also reported as a KPC producer in those isolates recovered in 2008 in Bariloche, Argentina.

Recent studies have provided evidence that KPC producing *P. aeruginosa* have a non-clonal population structure interspersed by geographically distributed clones (Cuzon et al., 2011). The dissemination of *kpc* into genetically unrelated *P. aeruginosa* isolates has already been described in Puerto Rico and Colombia (Wolter et al., 2009; Cuzon et al., 2011). However our findings indicate the presence of a successful *P. aeruginosa* ST 654 clone disseminated in our country.

Acknowledgements

This work was partially supported by grants from UBACyT and ANPCyT to G. Gutkind. G. Gutkind and M. Radice are members of Carrera del Investigador Científico (CONICET). M. Papalia is recipient of a doctoral fellowship from UBA.

References

Alpaka, P.E., Swanston, W.H., Ihemere, H.N., Correa, A., Torres, J.A., Tafur, J.D., Montealegre, M.C., Quinn, J.P., Villegas, M.V., 2009. Emergence of KPC-producing Pseudomonas aeruginosa in Trinidad and Tobago. J. Clin. Microbiol. 47, 2670–2671.

Bebrone, C., 2007. Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem. Pharmacol. 74 (12), 1686–1701.

Bradford, P.A., Bratu, S., Urban, C., Visalli, M., Mariano, N., Landman, D., Rahal, J.J., Brooks, S., Cebular, S., Quale, J., 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin. Infect. Dis. 39, 55–60.

Cejas, D., Fernández Canigia, L., Nastro, M., Rodríguez, C., Tanco, A., Rodriguez, H., Vay, C., Maldonado, I., Famiglietti, A., Giovanakis, M., Magarinos, F., Berardinelli, E., Neira, L., Mollerach, M., Gutkind, G., Radice, M., 2012. Hyperendemic clone of KPC producing Klebsiella pneumoniae ST 258 in Buenos Aires hospitals. Infect. Genet. Evol 12, 499–501.

Clinical and Laboratory Standards Institute, 2010. Performance standards for antimicrobial susceptibility testing; twenty informational supplement M100–S20. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Curran, Jonas, D., Grundmann, H., Pitt, T., Dowson, C.G., 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. 42, 5644–5649.

Cuzon, G., Naas, T., Villegas, M.V., Correa, A., Quinn, J.P., Nordmann, P., 2011. Wide dissemination of *Pseudomonas aeruginosa* producing-beta-lactamase bla_{KPC-2} gene in Colombia. Antimicrob. Agents Chemother. 55, 5350–5353.

Ellington, M.J., Kistler, J., Livermore, D., Woodford, N., 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J. Antimicrob. Chemother. 59, 321–322.

Ge, C., Wei, Z., Jiang, Y., Shen, P., Yu, Y., Li, L., 2011. Identification of KPC-2-producing *Pseudomonas aeruginosa* isolates in China. J. Antimicrob. Chemother. 66, 1184–1186.

Juan Nicolau, C., Oliver, A., 2008. Carbapenemases in *Pseudomonas* spp.. Enferm. Infecc. Microbiol. Clin. 28 (Suppl. 1), 19–28.

Kitchel, B., Rasheed, J.K., Patel, J.B., Srinivasan, A., Navon-Venezia, S., Carmeli, Y., Brolund, A., Giske, C.K., 2009. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob. Agents Chemother. 53, 3365–3370.

Koh, T.H., Khoo, C.T., Tan, T.T., Arshad, M.A., Ang, L.P., Lau, L.J., Hsu, L.Y., Ooi, E.E., 2010. Multilocus sequence types of carbapenem-resistant *Pseudomonas aeruginosa* in Singapore carrying metallo-beta-lactamase genes, including the novel *bla*(IMP-26) gene. J. Clin. Microbiol. 48, 2563–2564.

Lambert, P.A., 1988. Isolation and purification of outer membrane proteins from Gram-negative bacteria. In: Hancock, I., Poxton, I. (Eds.), Bacterial Cell Surface Techniques. John Wiley and Sons, New York, pp. 110–121.

Naas, T., Cuzon, G., Villegas, M.V., Lartigue, F., Quinn, J.P., Nordmann, P., 2008. Genetic structures at the origin of acquisition of the beta-lactamase *bla* KPC gene. Antimicrob. Agents Chemother. 52, 1257–1263.

Nordmann, P., Cuzon, G., Naas, T., 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect. Dis. 9, 228–236.

Pasterán, F., Veliz, O., Faccone, D., Guerriero, L., Rapoport, M., Méndez, T., Corso, A., 2011. A simple test for the detection of KPC and metallo-beta-lactamase carbapenemase-producing *Pseudomonas aeruginosa* isolates with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin. Microbiol. Infect. 17, 1438–1441.

Pasterán, F.G., Otaegui, L., Guerriero, L., Radice, G., Maggiora, R., Rapoport, M., Faccone, D., Di Martino, A., Galas, M., 2008. *Klebsiella pneumoniae* Carbapenemase-2, Buenos Aires, Argentina. Emerg. Infec.t Dis. 14, 1178–1180.

Poirel, L., Nordmann, P., Lagrutta, E., Cleary, T., Munoz-Price, L.S., 2010. Emergence of KPC-producing *Pseudomonas aeruginosa* in the United States. Antimicrob. Agents Chemother. 54, 3072.

Quale, J., Bratu, S., Gupta, J., Landman, D., 2006. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. Antimicrob. Agents Chemother. 50, 1633–1641.

Sacha, P., Ostas, A., Jaworowska, J., Wieczorek, P., Ojdana, D., Ratajczak, J., Tryniszewska, E., 2009. The KPC type beta-lactamases: new enzymes that confer resistance to carbapenems in Gram-negative bacilli. Folia Histochem. Cytobiol. 47, 537–543.

Samuelsen, O., Toleman, M.A., Sundsfjord, A., Rydberg, J., Leegaard, T.M., Walder, M., Lia, A., Ranheim, T.E., Rajendra, Y., Hermansen, N.O., Walsh, T.R., Giske, C.G., 2010. Molecular epidemiology of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolates from Norway and Sweden shows import of international clones and local clonal expansion. Antimicrob. Agents Chemother. 54, 346–352.

Santella, G., Cuirolo, A., Almuzara, M., Palombarani, S., Sly, G., Radice, M., Gutkind, G., 2010a. Full resistance and decreased susceptibility to carbapenems in IMP-13-producing *Pseudomonas aeruginosa* isolates from an outbreak. Antimicrob. Agents Chemother. 54, 1381–1382.

Santella, G., Pollini, S., Docquier, J.D., Mereuta, A.I., Gutkind, G., Rossolini, G.M., Radice, M., 2010b. Intercontinental dissemination of IMP-13-producing *Pseudomonas aeruginosa* belonging in sequence type 621. J. Clin. Microbiol. 48, 4342–4343.

Tsakris, A., Pournaras, S., Woodford, N., Palepou, M.F., Babini, G.S., Douboyas, J., Livermore, D.M., 2000. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. J. Clin. Microbiol. 38, 1290–1292.

Villegas, M.V., Lolans, K., Correa, A., Kattan, J.N., Lopez, J.A., Quinn, J.P., 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob. Agents Chemother. 51, 1553–1555.

Walsh, T.R., 2010. Emerging carbapenemases: a global perspective. Int. J. Antimicrob. Agents 36 (Suppl. 3), 8–14.

Wolter, D.J., Kurpiel, P.M., Woodford, N., Palepou, M.F., Goering, R.V., Hanson, N.D., 2009. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. Antimicrob. Agents Chemother. 53, 557–562.

Yigit, H., Queenan, A.M., Anderson, G.M., Domenech-Sanchez, A., Biddle, J.W., Steward, C.D., Alberti, S., Bush, K., Tenover, F.C., 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 45, 1151–1161.

Gisela Santella
Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Ciudad Autónoma de Buenos Aires, CP 1113, Argentina

Roxana Cittadini
Sanatorio Mater Dei, San Martín de Tours 2952, Ciudad Autónoma de Buenos Aires, CP 1425, Argentina

Mariana Papalia
Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Ciudad Autónoma de Buenos Aires, CP 1113, Argentina

Cecilia Vera Ocampo
Sanatorio Mater Dei, San Martín de Tours 2952, Ciudad Autónoma de Buenos Aires, CP 1425, Argentina

Marcelo Del Castillo
Sanatorio Mater Dei, San Martín de Tours 2952, Ciudad Autónoma de Buenos Aires, CP 1425, Argentina

Carlos Vay
Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Ciudad Autónoma de Buenos Aires, CP 1113, Argentina
Sanatorio Mater Dei, San Martín de Tours 2952, Ciudad Autónoma de Buenos Aires, CP 1425, Argentina

Gabriel Gutkind
Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Ciudad Autónoma de Buenos Aires, CP 1113, Argentina
Tel.: +5411 49648285; fax: +5411 45083645.
E-mail address: ggukind@ffyb.uba.ar

Marcela Radice
Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Ciudad Autónoma de Buenos Aires, CP 1113, Argentina

Available online 5 April 2012