

Escherichia coli with bla_{IMP-8} in Singapore

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n imipenem-resistant Escherichia coli strain was isolated from A the urine of a local 72-year-old female Chinese patient in 2006. The carbapenem MICs by Etest (bioMérieux SA, Marcy l'Etoile, France) were ertapenem at 2 μ g/ μ l, meropenem at 4 μ g/ μ l, and imipenem at >32 μ g/ μ l. A modified Hodge test was weakly positive. The presence of a metallo- β -lactamase (MBL) was suspected because of enhancement of the zone of inhibition around ertapanem, imipenem, and meropenem antimicrobial susceptibility testing disks with the addition of EDTA (1). A multiplex PCR for detection of carbapenemase genes was positive for $bla_{\rm IMP}$ (2). A 2,847-bp partial integron sequence was amplified using the IntA primer described by Rosser and Young (3) and an in-house primer, tniRR (5'-GGC AAG CTT GTG TTC GGT AT-3'). This sequence (GenBank accession number KF534724) contained *intI1*, *bla*_{IMP-8}, an aminoglycoside 6'-N-acetyltrasferase gene [aac(6')-IId], and tniR (a putative resolvase possibly involved in transposition). This result was identical to that determined for Klebsiella oxytoca (GenBank accession number HQ651093.1) from Fujian in China, except the intI1 partial sequence was not disrupted by IS26, though we have not excluded the possibility that this could have been inserted further downstream in the sense of *intI1* (4). This structure is carried on a plasmid of approximately 120 kb that was successfully transferred to E. coli J53 Az^r by plate mating. This was determined to be Inc A/C by plasmid replicon typing (5). The only other β -lactamase gene identified was *bla*_{TEM-1}. However, the high imipenem MIC relative to those of the other carbapenems is difficult to explain and it is possible that an undetected mechanism may contribute to the final resistance phenotype. Multilocus sequence typing showed that the isolate belonged to ST410 (ST23 complex). This sequence type has been associated with spread of carbapenem-nonsusceptible, KPC-2-producing E. coli strains in Greece (6).

IMP-8 was first described in *Klebsiella pneumoniae* from Taiwan (7), where it has established itself as the dominant MBL among the *Enterobacteriaceae*. It is a variant of IMP-2, from which it differs by 2 amino acids. Until recently, the distribution of bla_{IMP-8} was restricted to Taiwan and China. However, recently it has been reported in *Pseudomonas mendocina* in Portugual (8), *Enterobacter cloacae* in Argentina (9), *K. oxytoca* in Spain (10), and *K. pneumoniae* in Tunisia (11).

Given the close links between Taiwan, China, and Singapore, it is surprising that this is the only IMP-8 producer found so far in Singapore. However, these strains may be difficult to detect because of their low ertapenem and meropenem MICs. Taiwanese researchers have reported that only a third of IMP-8 producers had a positive modified Hodge test result. Furthermore, meropenem combined disk testing detected only 40% in combination with EDTA and 2% in combination with dipicolinic acid (12). The phenotype of our isolate was similarly difficult to recognize because the modified Hodge test result was weak, like that of an NDM-1 producer, and could easily have been dismissed as negative.

Nucleotide sequence accession number. The sequence determined in this work is available in GenBank under accession number KF534724.

REFERENCES

- Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. 2001. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. Clin. Microbiol. Infect. 7:88– 91. http://dx.doi.org/10.1046/j.1469-0691.2001.00204.x.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis. 70:119–123. http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.002.
- Rosser SJ, Young HK. 1999. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J. Antimicrob. Chemother. 44:11–18. http://dx.doi.org/10.1093/jac/44.1.11.
- Li B, Sun JY, Liu QZ, Han LZ, Huang XH, Ni YX. 2011. First report of *Klebsiella oxytoca* strain coproducing KPC-2 and IMP-8 carbapenemases. Antimicrob. Agents Chemother. 55:2937–2941. http://dx.doi.org/10.1128 /AAC.01670-10.
- Johnson TJ, Nolan LK. 2009. Plasmid replicon typing. Methods Mol. Biol. 551:27–35. http://dx.doi.org/10.1007/978-1-60327-999-4_3.
- Mavroidi A, Miriagou V, Malli E, Stefos A, Dalekos GN, Tzouvelekis LS, Petinaki E. 2012. Emergence of *Escherichia coli* sequence type 410 (ST410) with KPC-2 β-lactamase. Int. J. Antimicrob. Agents 39:247–250. http://dx.doi.org/10.1016/j.ijantimicag.2011.11.003.
- Yan JJ, Ko WC, Wu JJ. 2001. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-β-lactamase, IMP-8, from a clinical isolate of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 45: 2368–2371. http://dx.doi.org/10.1128/AAC.45.8.2368-2371.2001.
- Santos C, Caetano T, Ferreira S, Mendo S. 2010. First description of bla_{IMP-8} in a Pseudomonas mendocina isolated at the Hospital Infante D. Pedro, Aveiro, Portugal. Res. Microbiol. 161:305–307. http://dx.doi.org /10.1016/j.resmic.2010.03.004.
- Gomez S, Rapoport M, Togneri A, Viegas-Caetano J, Faccone D, Corso A, Petroni A, Pasteran F. 2011. Emergence of metallo-β-lactamases in *Enterobacteriaceae* from Argentina. Diagn. Microbiol. Infect. Dis. 69:94– 97. http://dx.doi.org/10.1016/j.diagmicrobio.2010.08.025.
- Vergara-López S, Domínguez MC, Conejo MC, Pascual A, Rodríguez-Baño J. 5 July 2013. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*. Clin. Microbiol. Infect. http://dx.doi.org/10.1111/1469-0691.12288.
- Chouchani C, Marrakchi R, Henriques I, Correia A. 2013. Occurrence of IMP-8, IMP-10, and IMP-13 metallo-β-lactamases located on class 1 integrons and other extended-spectrum β-lactamases in bacterial isolates from Tunisian rivers. Scand. J. Infect. Dis. 45:95–103. http://dx.doi.org/10 .3109/00365548.2012.717712.
- 12. Liao IC, Chen HM, Wu JJ, Tsai PF, Wang LR, Yan JJ. 2011. Metalloβ-lactamase-producing *Enterobacteriaceae* isolates at a Taiwanese hospital: lack of distinctive phenotypes for screening. APMIS 119:543–550. http://dx.doi.org/10.1111/j.1600-0463.2011.02772.x.

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