AAC Journals.ASM.org

Dominance of IMP-4-Producing *Enterobacter cloacae* among Carbapenemase-Producing *Enterobacteriaceae* in Australia

Hanna E. Sidjabat,[a] Nicola Townell,[b] Graeme R. Nimmo,[b] Narelle M. George,[b] Jennifer Robson,[c] Renu Vohra,[d] Louise Davis,[e] Claire Heney,[b] David L. Paterson[a,b]

University of Queensland, UQ Centre for Clinical Research, Brisbane, Queensland, Australia[a]; Pathology Queensland, Brisbane, Queensland, Australia[b]; Sullivan Nicolaides Pathology, Brisbane, Queensland, Australia[c]; QML Pathology, Brisbane, Queensland, Australia[d]; Communicable Diseases Unit, Department of Health, Brisbane, Queensland, Australia[e]

The prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) has been increasing worldwide. bla_{IMP} has been reported to be the predominant carbapenemase-encoding gene within *Enterobacteriaceae* in Australia. However, there are limited data currently available on CPE from Queensland, Australia. A total of 58 CPE isolates were isolated between July 2009 and March 2014 from Queensland hospitals. The clonality of isolates was determined by Diversilab repetitive sequence-based PCR. The isolates were investigated for the resistance mechanisms carbapenemase, extended-spectrum β-lactamase, and AmpC β-lactamase and for aminoglycoside resistance and plasmid-mediated quinolone resistance genes by PCR. The plasmid types associated with carbapenemase-encoding genes were characterized. The majority of the CPE were *Enterobacter cloacae* ($n = 29$). The majority of Queensland CPE isolates were IMP producers and comprised 11 species ($n = 48$). Nine NDM-producing *Enterobacteriaceae* were identified. One NDM-producing *Klebsiella pneumoniae* isolate coproduced OXA-48. One *K. pneumoniae* isolate was an OXA-181 producer. The incidence of IMP producers increased significantly in 2013. bla_{IMP-4} was found in all IMP-producing isolates. bla_{TEM}, *qnrB*, and *aacA4* were common among IMP-4 producers. The HI2 (67%) and L/M (21%) replicons were associated with bla_{IMP-4}. All HI2 plasmids were of sequence type 1 (ST1). All but one of the NDM producers possessed $bla_{CTX-M-15}$. The 16S rRNA methylase genes found among NDM producers were *armA*, *rmtB*, *rmtC*, and *rmtF*. The substantial increase in the prevalence of CPE in Queensland has been associated mainly with the emergence *E. cloacae* strains possessing HI2 plasmids carrying bla_{IMP-4} over the past 2 years. The importation of NDM producers and/or OXA-48-like producers in patients also contributed to the increased emergence of CPE.

The most substantial threat to antibiotic susceptibility in Gram-negative bacteria is the emergence of carbapenemase-producing *Enterobacteriaceae* (CPE). Some carbapenemases tend to dominate in certain countries of the world. For example, *Klebsiella pneumoniae* carbapenemase (KPC) is the carbapenemase found most frequently in *Enterobacteriaceae* in the United States (1), while NDM is frequently found in the Indian subcontinent (2) and SPM is found in Brazil (3). Despite the fact that there have been reports of KPC (4), NDM (5, 6), OXA-48 (7) and even a unique carbapenemase, AIM-1 (8), in Australia, none have become dominant there. In Australia, IMP-producing *Enterobacteriaceae*, particularly *Enterobacter cloacae*, have been reported to be the predominant form of CPE (9–11). Antibiotic-resistant *E. cloacae* not only is a common nosocomial pathogen but also has been reported to cause bloodstream infections (10, 12). The mortality rate for infections by IMP-producing *E. cloacae* causing bacteremia can be as high as 40% (12).

Carbapenemase-encoding genes are usually located on large plasmids that play an important part in their extensive spread worldwide. Plasmids cause the dissemination of antimicrobial resistance, including carbapenemase-encoding genes, through horizontal transfer among bacterial species of the *Enterobacteriaceae* family. Plasmids of different replicon types or incompatibility types (Inc), such as IncA/C, FII, L/M, and HI2, have been commonly associated with the carriage and transmission of carbapenemase-encoding genes (13).

In Australia, hospital-based health care is regulated by the state government. A state-owned central microbiology laboratory located in Brisbane, the capital city of Queensland, provides reference service to the majority of public hospitals in Queensland. Our aim was to characterize the mechanisms responsible for carbapenem resistance in CPE isolates in the state of Queensland, Australia. These isolates were referred by hospitals and pathology laboratories to a state reference laboratory between June 2009 and March 2014.

(Part of this study was presented as a poster presentation at the Antimicrobials 2014 Conference of the Australian Society for Antimicrobials [February 2014] [46].)

MATERIALS AND METHODS

Bacterial isolates and susceptibility testing. We investigated *Enterobacteriaceae* isolates with meropenem resistance determined by broth microdilution Vitek2 (bioMérieux) methods, using EUCAST breakpoints of >4 μg/ml (14). A total of 165 *Enterobacteriaceae* isolates with reduced susceptibility to meropenem, as determined by the Vitek2 system using Vitek card AST-N246 (bioMérieux), were referred to our laboratory at the

Received 23 September 2014 Returned for modification 30 October 2014
Accepted 19 April 2015

Accepted manuscript posted online 27 April 2015

Citation Sidjabat HE, Townell N, Nimmo GR, George NM, Robson J, Vohra R, Davis L, Heney C, Paterson DL. 2015. Dominance of IMP-4-producing *Enterobacter cloacae* among carbapenemase-producing *Enterobacteriaceae* in Australia. Antimicrob Agents Chemother 59:4059–4066. doi:10.1128/AAC.04378-14.

Address correspondence to Hanna E. Sidjabat, h.sidjabat@uq.edu.au.

doi:10.1128/AAC.04378-14

University of Queensland Centre for Clinical Research from seven major hospitals in Queensland between June 2009 and March 2014. Of note, the total numbers of nonrepetitive *Enterobacteriaceae* isolates tested and of *Enterobacteriaceae* isolates phenotypically determined to be resistant to meropenem by disk susceptibility testing from the same period were 196,282 and 153 isolates, respectively. The isolates were received for confirmation of the presence of carbapenemase-encoding genes. The specimen types and the geographical locations of the hospitals were recorded. In addition, wherever possible, the patient's travel history was recorded. Only one isolate per species from each patient was included, unless multiple species were isolated. Isolate species identification was performed biochemically by using Vitek2 GN-ID panels and confirmed with protein profiling using Vitek matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (bioMérieux).

Antimicrobial susceptibility testing, MIC determinations, and phenotypic tests for carbapenemase. All isolates were tested for antibiotic susceptibility to ceftazidime, ceftazidime-clavulanic acid, cefotaxime, cefotaxime-clavulanic acid, cefoxitin, cefepime, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, and amikacin by using the Vitek2 system (bioMérieux, VIC, Australia), and results were interpreted according to EUCAST breakpoints (14). MICs of ertapenem, meropenem, and imipenem were determined by using the Etest (bioMérieux, VIC, Australia). All isolates were phenotypically tested for the production of carbapenemase by using the modified Hodge test (MHT) and the Carba NP test (15). The phenotypic detection of metallo-β-lactamase (MBL) was performed by using EDTA as an inhibitor, and the detection of KPC was performed by using boronic acid as an inhibitor, as previously described (6, 16).

Genotypic characterization of antibiotic resistance mechanisms. PCR for bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{KPC} was performed on all suspect isolates by using previously described methods, and sequencing was performed to confirm the carbapenemase genes and the variants of these carbapenemase genes (6, 16, 17). PCR and sequencing to capture the entire integron harboring bla_{IMP} were performed to identify the genetic environment of bla_{IMP} and other antibiotic resistance genes located in the class 1 integron. The class 1 integron containing bla_{IMP-4} underwent PCR and sequencing using primers HS317 and the HS320, as previously described (18). PCR and sequencing of $bla_{OXA-48-like}$ and bla_{NDM} were performed by using previously described primers (19, 20). All isolates were tested by PCR for the presence of bla_{TEM}, bla_{CTX-M}, bla_{CMY}, bla_{SHV}, *aacA4*, and 16S rRNA methylase genes (*armA* and *rmtA* to *rmtF*) (21, 22). The variants of β-lactamase genes were determined by sequencing of the PCR products. Plasmid-mediated quinolone resistance (PMQR) was determined by PCR for *qnrA*, *qnrB*, and *qnrS* (23). In addition, PCR to detect the IS*CR* element was performed as previously described (24).

Clonal analysis of *Enterobacter* spp., *K. pneumoniae*, and *Escherichia* spp. The clonal relationships of isolates of the same species were characterized by a semiautomated method, repetitive sequence-based PCR (rep-PCR) typing. The clonal relatedness of *Enterobacter cloacae* and *Enterobacter asburiae*, *K. pneumoniae*, and *Escherichia coli* isolates was determined by Diversilab rep-PCR using appropriate kits according to the manufacturer's instructions (bioMérieux, VIC, Australia) (25). Phylogenetic groups of *E. coli* were determined as previously described (26). Multilocus sequence typing (MLST) was performed for *E. cloacae*, *K. pneumoniae*, and *E. coli* according to methods described on relevant websites (http://pubmlst.org/ecloacae/, http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html, and http://mlst.warwick.ac.uk/mlst/, respectively).

Genetic features of bla_{IMP}-carrying plasmids and bla_{NDM}-carrying plasmids. All isolates were characterized for common replicon types associated with carbapenemase-encoding genes, such as types A/C, L/M, HI2, FII, FIA, FIB, X, I1, and N, by using previously described methods (27). A specific set of primers was designed to target the HI2 replicon in this study, HI2F (5′-CTGGTGGGCATAACTCACCT-3′) and HI2R (5′-TCACCAGGGCTT TCTCTGTT-3′), which gave 942-bp amplicons. pMLST was performed to determine the sequence type (ST) of HI2 plasmids as described on the PubMLST website (http://pubmlst.org/plasmid/primers/incHI2.shtml). Plasmid transfer by liquid mating experiments was performed on 12 representative isolates of IMP producers that were selected based on bacterial species and plasmid types. An *E. coli* K-12 nalidixic acid-resistant non-lactose fermenter was used as the recipient in the conjugation experiments, as previously described (28). *E. coli* K-12 isolates acquiring plasmids carrying carbapenemase genes by conjugation were selected on MacConkey agar supplemented with 150 μg/ml ampicillin and 0.1 μg/ml meropenem. The recipients containing IMP plasmids were selected based on the colony morphology of non-lactose fermentation of *E. coli* K-12. Plasmid transfer by electroporation was performed for NDM and/or OXA-48 producers. Plasmids extracted by alkaline lysis were electroporated into *E. coli* TOP10 cells (Invitrogen, VIC, Australia). The *E. coli* TOP10 isolates acquiring plasmids carrying carbapenemase genes were selected on Luria-Bertani agar supplemented with 150 μg/ml ampicillin and 0.1 μg/ml meropenem. The plasmid replicon types were determined for the *E. coli* recipients acquiring plasmids carrying carbapenemase-encoding genes by PCR-based plasmid replicon typing (29). Other resistance genes carried on the carbapenemase plasmids were also determined.

This work was approved by the Royal Brisbane and Women's Hospital Human Research Ethics Committee (approval number HREC/13/QRBW/391 [Epidemiology, clinical significance, treatment, and outcome of infections by carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter* spp. in Queensland]).

RESULTS

Bacterial isolates and antibiotic resistance mechanisms. A total of 58 carbapenemase-producing *Enterobacteriaceae* isolates were included in this study. Two previously reported NDM producers from this region were not included in this study (6, 19). The non-CPE producers ($n = 105$) had reduced susceptibility to ertapenem but remained susceptible to meropenem and imipenem. The non-CPE producers were negative by MHT and Carba NP testing. Although the confirmed CPE isolates were mainly from southeast Queensland, the region which has the majority of the population in Queensland, a total of 6 CPE isolates were from hospitals further north, including hospitals in Townsville and Rockhampton, which are 600 to 1,300 km away from southeast Queensland.

The majority of cases of CPE infection were caused by carbapenemase-producing *E. cloacae* ($n = 32$). Two patients possessed pairs of isolates of different species, *K. pneumoniae* and *E. cloacae* as well as *Raoultella planticola* and *Citrobacter freundii*. One patient harboring *E. cloacae* and *E. coli* was previously described (30). These previously reported pairs of IMP-4-producing *E. cloacae* and *E. coli* isolates were included in the molecular characterization and the dendrogram for clonal analysis for comparison with other CPE.

The majority of CPE were isolated in 2013 ($n = 27$). Seven CPE were isolated in 2012, and only <5 CPE were isolated per annum prior to 2012 (Fig. 1). The majority of isolates were obtained from urine (53%). The other isolates were obtained from blood (14%), respiratory samples (12%), wounds (8%), and rectal swabs (13%). Isolates were initially determined to be meropenem resistant by the Vitek2 system. However, the isolates were not always resistant to meropenem by disk susceptibility testing using EUCAST breakpoints (<22-mm diameter for ertapenem and <16-mm diameter for meropenem disks for carbapenem-resistant isolates) (14). All isolates showed production of carbapenemase by MHT using ertapenem, meropenem, and imipenem disks (10 μg). All isolates showed MBL phenotypes by using EDTA. No KPC phenotype was found. The MIC_{90}s of ertapenem and meropenem for CPE were

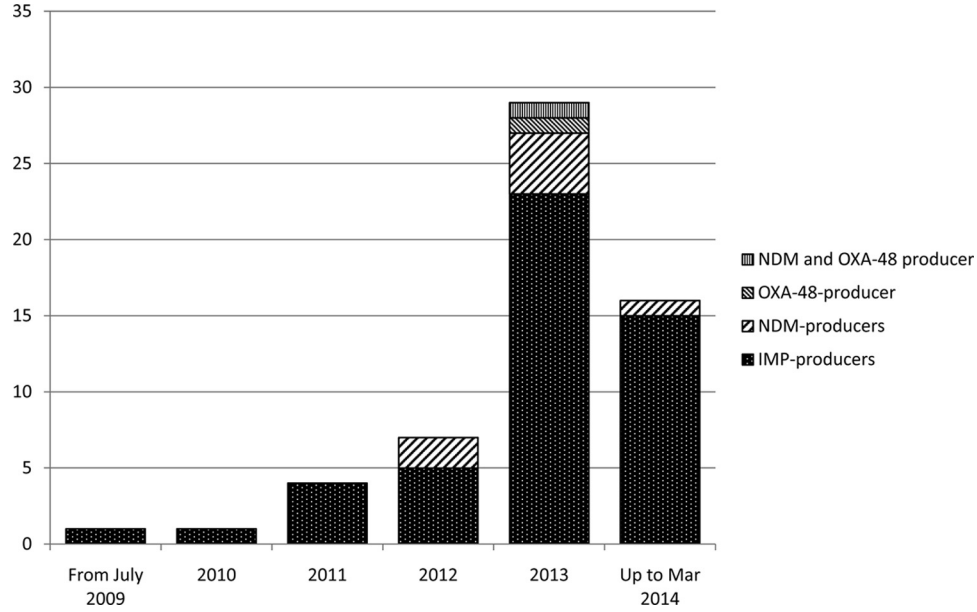

FIG 1 Incidence of carbapenemase-producing *Enterobacteriaceae* in Queensland, Australia, from July 2009 to March 2014. The *y* axis indicates number of CPE cases.

both >32 μg/ml, and that of imipenem was 24 μg/ml. The MIC_{50}s of ertapenem, meropenem, and imipenem were 16, 4, and 3 μg/ml, respectively.

bla_{IMP} and bla_{NDM} were detected in 48 and 7 isolates, respectively. No isolate harbored bla_{KPC}. The IMP-producing *Enterobacteriaceae* comprised *E. cloacae* ($n = 29$), *E. asburiae* ($n = 2$), *Enterobacter aerogenes* ($n = 1$), *K. pneumoniae* ($n = 4$), *E. coli* ($n = 4$), *Escherichia hermannii* ($n = 2$), *Serratia marcescens* ($n = 1$), *C. freundii* ($n = 2$), *Citrobacter koseri* ($n = 1$), *Proteus mirabilis* ($n = 1$), and *R. planticola* ($n = 1$) (Table 1). One NDM-producing *K. pneumoniae* isolate coproduced OXA-48. One *K. pneumoniae* isolate produced OXA-181 (Table 2). The NDM producers were probably acquired by patients during overseas travel. In three NDM cases, the patients had a history of travel to India (Table 2). Of the remaining patients, the travel history of one patient could not be retrieved, one patient had a history of travel to Myanmar, and one patient was a migrant from Pakistan. The patient with an NDM isolate coproducing OXA-48 had been transferred from Romania.

The bla_{IMP} variants in all isolates were bla_{IMP-4}. The IMP producers were distinct from the NDM producers in the composition of other resistance genes present. These isolates usually carried bla_{TEM} (90%) and the aminoglycoside resistance gene *aacA4* (90%). The PMQR gene *qnrB* was commonly found in IMP pro-

TABLE 1 Characteristics of IMP-4-producing *Enterobacteriaceae* from Queensland

Species	No. of isolates	bla_{IMP} variant	No. (%) of isolates carrying gene								No. (%) of isolates with replicon type	
			bla_{TEM-1}	bla_{SHV}	bla_{CTX-M}	$bla_{CMY-2-like}$	*qnrB*	*qnrA*	*qnrS*	*aac(6′)-Ib*	HI2	L/M
E. cloacae	29	bla_{IMP-4}	27	7[a]	2[c]	1	27	8	2	26	22	7
E. asburiae	2	bla_{IMP-4}	2	2						2	2	
E. aerogenes	1	bla_{IMP-4}	1				1			1		1
E. coli	4	bla_{IMP-4}	2				1	1		3	2	
E. hermannii	2	bla_{IMP-4}	2	2[a]			3			2	2	
K. pneumoniae	4	bla_{IMP-4}	4	4[b]			4	1		4	1	2
C. freundii	1	bla_{IMP-4}	1	1	1[c]	1[d]	1	1		1	1	
C. koseri	1	bla_{IMP-4}	1				1		1	1	1	
S. marcescens	1	bla_{IMP-4}	1				1			1		
P. mirabilis	1	bla_{IMP-4}	1	1[a]			1			1	1	
R. planticola	1	bla_{IMP-4}	1				1			1		
Total	48		43 (90)	17 (35)	3 (6.4)	2 (4.3)	41 (85)	11 (23)	3 (6.25)	43 (90)	32 (67)	10 (21)

[a] bla_{SHV-12}.
[b] bla_{SHV-1} ($n = 2$), bla_{SHV-28} ($n = 1$), and bla_{SHV-12} ($n = 1$).
[c] $bla_{CTX-M-15}$.
[d] bla_{CMY-48}.

TABLE 2 Characteristics of NDM- and OXA-48-like-producing *Enterobacteriaceae*

Isolate	Yr of isolation	Patient travel history	Specimen type	Sequence type	Gene(s) detected								Replicon type(s) detected
					bla_{NDM} variant	$bla_{OXA-48-like}$ variant	bla_{CTX-M} variant[a]	bla_{CMY} variant	bla_{SHV} variant	bla_{TEM} variant	16S rRNA methylase[b]	PMQR	
E. coli CR7	2012	India	Urine	410	bla_{NDM-1}		$bla_{CTX-M-15}$	bla_{CMY-42}			*armA*	*aac(6′)-Ib*	*qnrA*	A/C, FII, I1, X, FIA, FIB
E. coli CR15	2013	Myanmar	Wound	101	bla_{NDM-4}		$bla_{CTX-M-15}$	bla_{CMY-42}		bla_{TEM-1}	*rmtB*	*aac(6′)-Ib*		I1, FIA, FIB, FII, X
E. coli CR53	2014	Pakistan	Blood	4450	bla_{NDM-4}			bla_{CMY-42}		bla_{TEM-1}	*rmtB*			FII, I1
E. cloacae CR16	2012	India	Urine	265	bla_{NDM-1}		$bla_{CTX-M-15}$		bla_{SHV-12}		*armA*, *rmtC*	*aac(6′)-Ib*		HI2, HI2A, FII, FIB
E. cloacae CR37	2013	Not available	Blood	127	bla_{NDM-7}		$bla_{CTX-M-15}$			bla_{TEM-1}		*aac(6′)-Ib*	*qnrB*	FII, X
K. pneumoniae CR36	2013	India	Urine	147	bla_{NDM-1}		$bla_{CTX-M-15}$		bla_{SHV-11}	bla_{TEM-1}	*rmtF*	*aac(6′)Ib*	*qnrB*	ColV
K. pneumoniae CR38	2013	Romania	Urine	15	bla_{NDM-1}	bla_{OXA-48}	$bla_{CTX-M-15}$		bla_{SHV-28}	bla_{TEM-1}	*rmtC*			L/M, FII
K. pneumoniae CR39	2013	Thailand	Rectal	231		$bla_{OXA-181}$	$bla_{CTX-M-15}$	bla_{CMY-4}	bla_{SHV-1}	bla_{TEM-1}	*armA*, *rmtF*	*aac-(6′)Ib*	*qnrS1*	A/C, FII, FIA, FIB

[a] All $bla_{CTX-M-15}$ genes were on separate plasmids carrying bla_{NDM}.
[b] All 16S rRNA methylase genes were on the plasmids carrying bla_{NDM}, except for *E. coli* CR37.

ducers (85%), while *qnrA* and *qnrS* were found in only 23% and 6.3% of IMP producers, respectively (Table 1). bla_{SHV}, bla_{CTX-M}, and bla_{CMY} were less frequently found (Table 1). Other than bla_{NDM-1}, the bla_{NDM-4} and bla_{NDM-7} variants were detected (Table 2). PMQR genes (*qnrA* and *qnrB*) were less common among NDM producers. The OXA-181-producing *K. pneumoniae* isolate possessed *qnrS*. All except one of the NDM producers coproduced CTX-M-15. In addition, bla_{TEM}, bla_{CMY}, and bla_{SHV} were found in 71%, 42%, and 42% of NDM producers, respectively. bla_{CMY-4}, bla_{CMY-42}, bla_{SHV-1}, bla_{SHV-11}, bla_{SHV-12}, and bla_{SHV-28} were identified among NDM and OXA-48-like producers (Table 2). The NDM producers were usually resistant to amikacin (6 out of 7 isolates), which coincided with the possession of the 16S rRNA methylase genes *armA*, *rmtB*, *rmtC*, and/or *rmtF* (Table 2). In contrast, none of IMP producers possessed a 16S rRNA methylase gene. All of these IMP producers were susceptible to amikacin.

Clonal analysis of *Enterobacter* spp., *K. pneumoniae*, and *E. coli*. There were no predominant clones observed among *Enterobacter* spp., using 95% similarity as the cutoff. Several small clusters comprising 2 to 4 isolates with identical rep-PCR patterns were observed (clusters A to G) (Fig. 2). In this study, isolates were considered nonepidemiologically related when they were isolated from different hospitals or isolated >1 year apart. Several *E. cloacae* isolates were clonal although not epidemiologically related. Examples of these isolates were isolates in clusters B (ST45), C (ST133), and E (ST65). Isolates within these clusters were isolated from different hospitals or in different years. Several identical strains that were closely temporally related potentially indicated person-to-person transmission, such as in cluster A and cluster D. Several new *E. cloacae* STs were detected in our study isolates, i.e., ST263 and ST265. Interestingly, the two NDM-producing *E. cloacae* isolates had 94% similarity to each other (ST127 and ST265) and had 90% similarity to IMP-4-producing *E. cloacae* ST263 isolate CR22 (Fig. 2). Of note, these two NDM producers had only one allele difference. Furthermore, the NDM-producing *E. cloacae* isolate (CR16) was identical or clonally related to a previously reported NDM-producing *E. cloacae* isolate from Tasmania (data not shown) (19).

No clonality was observed among IMP- and NDM-producing *E. coli* and *K. pneumoniae* isolates (Fig. 3). Three novel STs were detected among IMP-4-producing *K. pneumoniae* isolates in our study, ST1727, ST1728, and ST1729 (Fig. 3A). ST101 and ST410 NDM-producing *E. coli* isolates that were found in this study (Table 2) were previously reported (31, 32). Also, we found NDM-producing *E. coli* ST4450, which had never been reported to produce NDM previously. Interestingly, the OXA-181-producing *K. pneumoniae* isolate was of ST231, which was previously reported to produce NDM (33). It is noteworthy that one IMP-4-producing *E. coli* isolate belonged to the pandemic sequence type ST131 (34). Interestingly, of the four IMP-4-producing *E. coli* isolates, three belonged to typical virulent extraintestinal pathogenic *E. coli* phylogenetic groups B2 ($n = 2$) and D ($n = 1$) (Fig. 3B). In contrast, all NDM-producing *E. coli* isolates belonged to commensal groups A and B1.

Genetic features of bla_{IMP-4}-carrying plasmids and bla_{NDM}-carrying plasmids. The majority of clinical IMP-4-producing isolates had HI2 replicon plasmids (67%). Remarkably, the STs of the HI2 plasmids carrying bla_{IMP-4} were all ST1 (Fig. 2). L/M plasmids were less common (21%). The IS*CR1* element was present in all IMP-4 producers. Both HI2 and L/M plasmids were transferred by

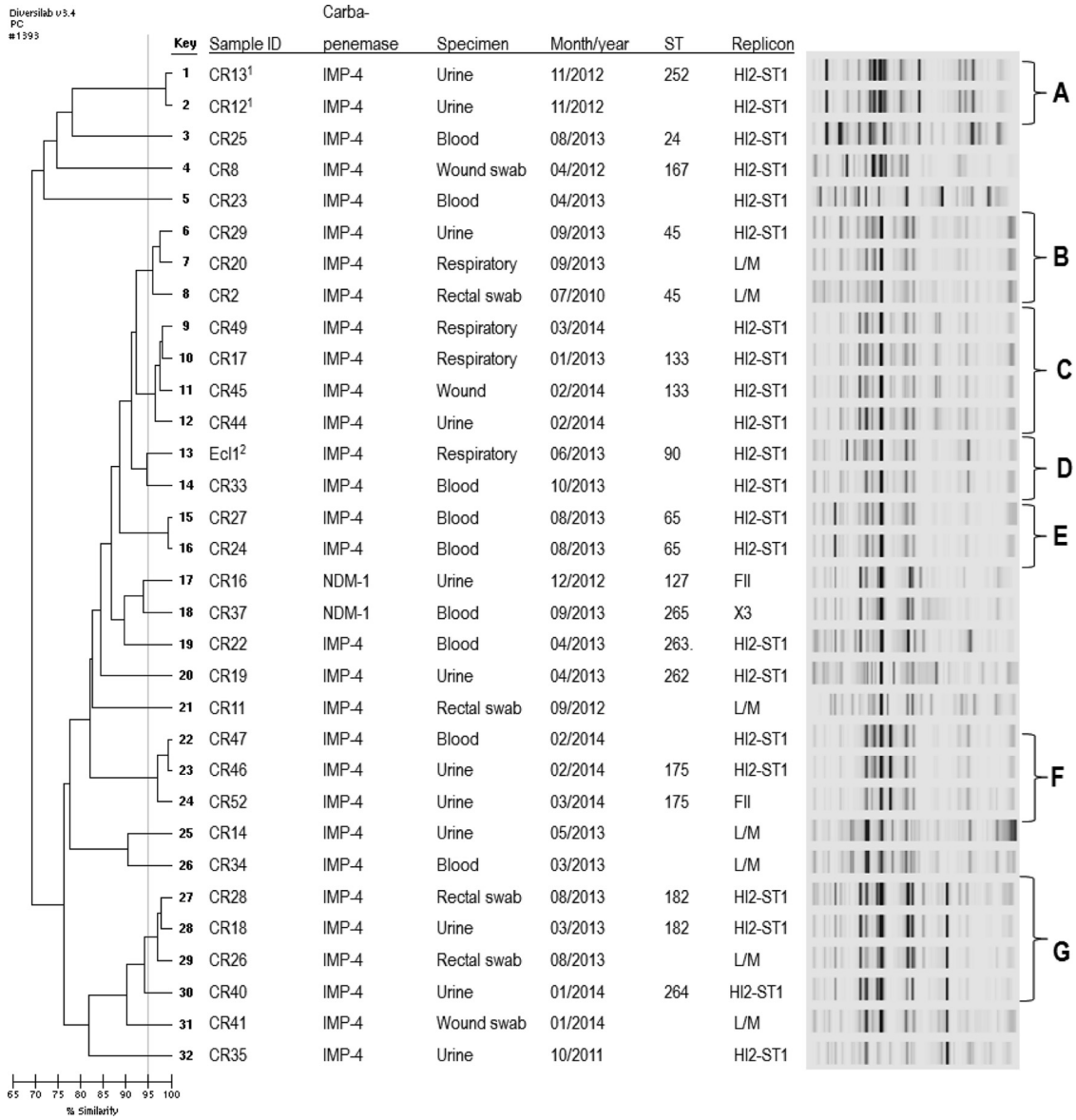

FIG 2 Dendrogram of carbapenemase-producing *Enterobacter* spp. Note that all isolates are *E. cloacae*, except for isolates CR12 and CR13. [1], *E. asburiae*; [2], Ecl1 was described previously (30).

conjugation. However, the plasmid transformation experiment showed that only L/M plasmids but not HI2 plasmids were transferred (data not shown). In this study, we proved that the $bla_{\text{TEM-1}}$, *qnrB2*, and *aacA4* genes were located in the bla_{IMP}-carrying plasmids. Twelve *E. coli* K-12 transconjugants acquiring plasmids carrying $bla_{\text{IMP-4}}$ caused an increase of meropenem MICs. The MICs of meropenem for these *E. coli* K-12 transconjugants ranged from 1 to 4 μg/ml, with an MIC_{50} of 2 μg/ml. Of note, the meropenem MIC for the *E. coli* K-12 recipient was 0.012 μg/ml. All the $bla_{\text{IMP-4}}$ and *aacA4* genes were located in the class 1 integron. IS*CR1* was located downstream of the class 1 integron.

Of the NDM producers, bla_{NDM}-carrying plasmids were transferred successfully into *E. coli* TOP10 cells. The $bla_{\text{CTX-M-15}}$ genes were carried by plasmids separate from those carrying bla_{NDM}. However, except for one *E. coli* isolate where *rmtB* was in a separate plasmid, the 16S rRNA methylase-encoding genes were located on the bla_{NDM}-carrying plasmids.

DISCUSSION

Having lagged behind other countries, Australia is now seeing an increasing incidence of CPE, with the majority being IMP producers. The original IMP producer was initially described in $bla_{\text{IMP-1}}$-producing *Serratia marcescens* in Japan in 1991 (35). $bla_{\text{IMP-4}}$ was first reported in *Acinetobacter baumannii* in the 1990s in Hong Kong (36). In Australia, $bla_{\text{IMP-4}}$ was first detected in Melbourne and Sydney in 2002 and has remained the most commonly reported variant of bla_{IMP} (9–11). Here, we describe 11 different species of IMP-4-producing *Enterobacteriaceae*, including an uncommon species, *Raoultella planticola*, which has recently been reported to produce IMP-8 in catheter-related cases in Taiwan

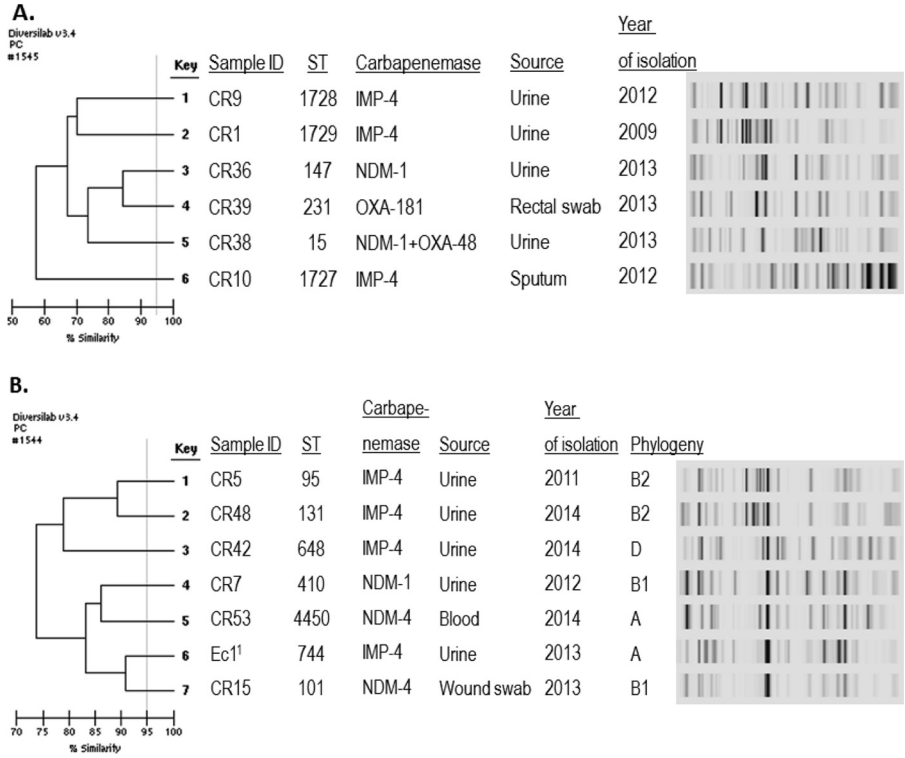

FIG 3 Dendrograms of carbapenemase-producing *K. pneumoniae* (A) and *E. coli* (B). [1], *E. coli* Ec1 was described previously (30).

(37). We also report the first occurrence of IMP-4-producing *P. mirabilis*. The presence of an endemic carbapenemase-encoding gene, bla_{IMP-4}, in a pandemic strain of *E. coli* ST131, which is known for its association with CTX-M-15 production and hypervirulence, raises concerns about the potential clonal dissemination of IMP-producing *E. coli* ST131. An example of spread by clonal expansion is KPC-producing *E. coli* ST131. KPC producers were initially prevalent among *K. pneumoniae* isolates. However, since the acquisition of KPC plasmids by *E. coli* ST131, further extensive spread with a predominance of KPC-producing *E. coli* ST131 has occurred (38, 39).

Previously, bla_{IMP-4} was detected in a class 1 integron containing *intI1*, bla_{IMP-4}, *qacG*, *aacA4*, and *catB3* (3 to 4 kb in size) (9). The size of the integron in our study was 1.2 kb and consisted of bla_{IMP-4} and *aacA4*. A previous study in Australia showed that bla_{IMP-4} was carried on A/C and L/M plasmids (9), with only one isolate from China carrying an HI2 plasmid (9). Here, we reveal the high incidence of HI2 plasmids carrying bla_{IMP-4} (67%) in Queensland (Table 1). HI2 plasmids carrying bla_{IMP-8} were reported in Taiwan (40). It is interesting that these Taiwanese HI2 plasmids carrying bla_{IMP-4} also carried bla_{TEM-1}, *qnrB2*, and *aacA4*. The two plasmid replicon types HI2 and L/M have now shown a strong association with bla_{IMP}. Due to the high incidence of HI2 and L/M plasmids carrying carbapenemase genes in *Enterobacteriaceae* in Australia, surveillance for *Enterobacteriaceae* possessing these plasmids in addition to commonly reported carbapenemases is recommended.

It is noteworthy that the variant IMP-4 has also been the predominant MBL reported elsewhere in Australia (9–11). The mechanism of spread of the IMP-4 producers within Queensland is unknown. In other hospitals in Australia, environmental contamination in burn and intensive care units was often identified as the source of the outbreak (10, 41). Our study isolates were referred from a wide geographic coastal area of Queensland from Townsville to Brisbane (1,300 km apart). The facts that the *E. cloacae* isolates were diverse and that 11 different species were identified in this study showed the capability of bla_{IMP-4}-carrying plasmids to spread horizontally among members of the *Enterobacteriaceae*. The presence of HI2 plasmid with ST1 harboring bla_{IMP-4} across different strains of *E. cloacae* and 7 other species of *Enterobacteriaceae* in our region has contributed to the increasing number of cases caused by IMP-4 producers. The diverse range of IMP-4-producing *Enterobacteriaceae* has also been reported in Sydney and Melbourne (9, 11, 42). Initially, *Serratia marcescens* was the predominant IMP-4 producer in Australia (11); however, *E. cloacae* has now emerged as the predominant species (10). Our study also describes several novel STs of *E. cloacae* and the first description of an *E. cloacae* ST isolated from Australia. The MLST scheme for *E. cloacae* was relatively recently developed (43). Interestingly, NDM-producing *E. cloacae* ST265 and ST127 had 4 alleles identical to those of the ST66 and ST114, which were considered high-risk international clones (44). In addition, we reveal three novel STs of *K. pneumoniae*. There are very scarce data on STs of *K. pneumoniae* in Australia apart from STs of imported carbapenemase-producing *K. pneumoniae* isolates (6, 7).

The limitation of this study is that the potential reservoirs and sources of infection by IMP producers as well as clinical outcomes were not investigated, and this warrants further study. bla_{IMP} is locally endemic and is now geographically widespread throughout Australia. Infection control and prevention strategies are an important means of reducing transmission and the overall burden of CPE (45). In conclusion, to control the spread of the CPE in Aus-

tralia, isolates with reduced susceptibility to carbapenem should undergo molecular confirmation.

ACKNOWLEDGMENTS

We thank all microbiology laboratory staff in Queensland who referred the isolates. We thank the curators of the Institut Pasteur MLST system (Paris, France) for importing novel alleles, profiles, and/or isolates (http://bigsdb.web.pasteur.fr) and Toru Akiyama for the new *Enterobacter cloacae* alleles and ST (http://pubmlst.org/ecloacae/).

This study is partially funded by the Pathology Queensland Study, Education and Research Trust Fund (grant 4177).

REFERENCES

1. **Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG.** 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. http://dx.doi.org/10.1128/AAC.00126-09.
2. **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N.** 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis **10:**597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2.
3. **Martins AF, Zavascki AP, Gaspareto PB, Barth AL.** 2007. Dissemination of Pseudomonas aeruginosa producing SPM-1-like and IMP-1-like metallo-beta-lactamases in hospitals from southern Brazil. Infection **35:**457–460. http://dx.doi.org/10.1007/s15010-007-6289-3.
4. **Partridge SR, Ginn AN, Wiklendt AM, Ellem J, Wong JS, Ingram P, Guy S, Garner S, Iredell JR.** 2015. Emergence of bla_{KPC} carbapenemase genes in Australia. Int J Antimicrob Agents **45:**130–136. http://dx.doi.org/10.1016/j.ijantimicag.2014.10.006.
5. **Shoma S, Kamruzzaman M, Ginn AN, Iredell JR, Partridge SR.** 2014. Characterization of multidrug-resistant Klebsiella pneumoniae from Australia carrying blaNDM-1. Diagn Microbiol Infect Dis **78:**93–97. http://dx.doi.org/10.1016/j.diagmicrobio.2013.08.001.
6. **Sidjabat H, Nimmo GR, Walsh TR, Binotto E, Htin A, Hayashi Y, Li J, Nation RL, George N, Paterson DL.** 2011. Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi metallo-beta-lactamase. Clin Infect Dis **52:**481–484. http://dx.doi.org/10.1093/cid/ciq178.
7. **Espedido BA, Steen JA, Ziochos H, Grimmond SM, Cooper MA, Gosbell IB, van Hal SJ, Jensen SO.** 2013. Whole genome sequence analysis of the first Australian OXA-48-producing outbreak-associated Klebsiella pneumoniae isolates: the resistome and in vivo evolution. PLoS One **8:**e59920. http://dx.doi.org/10.1371/journal.pone.0059920.
8. **Yong D, Toleman MA, Bell J, Ritchie B, Pratt R, Ryley H, Walsh TR.** 2012. Genetic and biochemical characterization of an acquired subgroup B3 metallo-beta-lactamase gene, blaAIM-1, and its unique genetic context in Pseudomonas aeruginosa from Australia. Antimicrob Agents Chemother **56:**6154–6159. http://dx.doi.org/10.1128/AAC.05654-11.
9. **Espedido BA, Partridge SR, Iredell JR.** 2008. bla(IMP-4) in different genetic contexts in Enterobacteriaceae isolates from Australia. Antimicrob Agents Chemother **52:**2984–2987. http://dx.doi.org/10.1128/AAC.01634-07.
10. **Leung GH, Gray TJ, Cheong EY, Haertsch P, Gottlieb T.** 2013. Persistence of related bla-IMP-4 metallo-beta-lactamase producing Enterobacteriaceae from clinical and environmental specimens within a burns unit in Australia—a six-year retrospective study. Antimicrob Resist Infect Control **2:**35. http://dx.doi.org/10.1186/2047-2994-2-35.
11. **Peleg AY, Franklin C, Bell JM, Spelman DW.** 2005. Dissemination of the metallo-beta-lactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. Clin Infect Dis **41:**1549–1556. http://dx.doi.org/10.1086/497831.
12. **Hayakawa K, Miyoshi-Akiyama T, Kirikae T, Nagamatsu M, Shimada K, Mezaki K, Sugiki Y, Kuroda E, Kubota S, Takeshita N, Kutsuna S, Tojo M, Ohmagari N.** 2014. Molecular and epidemiological characterization of IMP-type metallo-beta-lactamase-producing Enterobacter cloacae in a large tertiary care hospital in Japan. Antimicrob Agents Chemother **58:**3441–3450. http://dx.doi.org/10.1128/AAC.02652-13.
13. **Carattoli A, Aschbacher R, March A, Larcher C, Livermore DM, Woodford N.** 2010. Complete nucleotide sequence of the IncN plasmid pKOX105 encoding VIM-1, QnrS1 and SHV-12 proteins in Enterobacteriaceae from Bolzano, Italy compared with IncN plasmids encoding KPC enzymes in the USA. J Antimicrob Chemother **65:**2070–2075. http://dx.doi.org/10.1093/jac/dkq269.
14. **EUCAST.** 2013. Breakpoint tables for interpretation of MICs and zone diameters. EUCAST, Vaxjo, Sweden. http://www.eucast.org/clinical_breakpoints/.
15. **Dortet L, Poirel L, Nordmann P.** 2012. Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test. Antimicrob Agents Chemother **56:**6437–6440. http://dx.doi.org/10.1128/AAC.01395-12.
16. **Sidjabat HE, Silveira FP, Potoski BA, Abu-Elmagd KM, Adams-Haduch JM, Paterson DL, Doi Y.** 2009. Interspecies spread of Klebsiella pneumoniae carbapenemase gene in a single patient. Clin Infect Dis **49:**1736–1738. http://dx.doi.org/10.1086/648077.
17. **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.
18. **Sidjabat HE, Townsend KM, Hanson ND, Bell JM, Stokes HW, Gobius KS, Moss SM, Trott DJ.** 2006. Identification of bla(CMY-7) and associated plasmid-mediated resistance genes in multidrug-resistant Escherichia coli isolated from dogs at a veterinary teaching hospital in Australia. J Antimicrob Chemother **57:**840–848. http://dx.doi.org/10.1093/jac/dkl057.
19. **Rogers BA, Sidjabat HE, Silvey A, Anderson TL, Perera S, Li J, Paterson DL.** 2013. Treatment options for New Delhi metallo-beta-lactamase-harboring enterobacteriaceae. Microb Drug Resist **19:**100–103. http://dx.doi.org/10.1089/mdr.2012.0063.
20. **Zarakolu P, Day KM, Sidjabat HE, Kamolvit W, Lanyon CV, Cummings SP, Paterson DL, Akova M, Perry JD.** 2015. Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey. Eur J Clin Microbiol Infect Dis **34:**519–525. http://dx.doi.org/10.1007/s10096-014-2255-z.
21. **Sidjabat HE, Paterson DL, Adams-Haduch JM, Ewan L, Pasculle AW, Muto CA, Tian GB, Doi Y.** 2009. Molecular epidemiology of CTX-M-producing Escherichia coli isolates at a tertiary medical center in western Pennsylvania. Antimicrob Agents Chemother **53:**4733–4739. http://dx.doi.org/10.1128/AAC.00533-09.
22. **Sidjabat HE, Paterson DL, Qureshi ZA, Adams-Haduch JM, O'Keefe A, Pascual A, Rodriguez-Bano J, Doi Y.** 2009. Clinical features and molecular epidemiology of CMY-type beta-lactamase-producing Escherichia coli. Clin Infect Dis **48:**739–744. http://dx.doi.org/10.1086/597037.
23. **Gibson JS, Cobbold RN, Heisig P, Sidjabat HE, Kyaw-Tanner MT, Trott DJ.** 2010. Identification of Qnr and AAC(6′)-1b-cr plasmid-mediated fluoroquinolone resistance determinants in multidrug-resistant Enterobacter spp. isolated from extraintestinal infections in companion animals. Vet Microbiol **143:**329–336. http://dx.doi.org/10.1016/j.vetmic.2009.11.031.
24. **Toleman MA, Bennett PM, Walsh TR.** 2006. ISCR elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev **70:**296–316. http://dx.doi.org/10.1128/MMBR.00048-05.
25. **Sidjabat HE, Derrington P, Nimmo GR, Paterson DL.** 2010. Escherichia coli ST131 producing CTX-M-15 in Australia. J Antimicrob Chemother **65:**1301–1303. http://dx.doi.org/10.1093/jac/dkq098.
26. **Clermont O, Bonacorsi S, Bingen E.** 2000. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol **66:**4555–4558. http://dx.doi.org/10.1128/AEM.66.10.4555-4558.2000.
27. **Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, Whichard JM, Rossolini GM.** 2006. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerg Infect Dis **12:**1145–1148. http://dx.doi.org/10.3201/eid1207.051555.
28. **Sidjabat HE, Hanson ND, Smith-Moland E, Bell JM, Gibson JS, Filippich LJ, Trott DJ.** 2007. Identification of plasmid-mediated extended-spectrum and AmpC beta-lactamases in Enterobacter spp. isolated from dogs. J Med Microbiol **56:**426–434. http://dx.doi.org/10.1099/jmm.0.46888-0.
29. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005.

Identification of plasmids by PCR-based replicon typing. J Microbiol Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
30. **Sidjabat HE, Heney C, George NM, Nimmo GR, Paterson DL.** 2014. Interspecies transfer of blaIMP-4 in a patient with prolonged colonization by IMP-4-producing Enterobacteriaceae. J Clin Microbiol **52:**3816–3818. http://dx.doi.org/10.1128/JCM.01491-14.
31. **Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J, Livermore DM, Woodford N.** 2011. Phylogenetic diversity of Escherichia coli strains producing NDM-type carbapenemases. J Antimicrob Chemother **66:** 2002–2005. http://dx.doi.org/10.1093/jac/dkr226.
32. **Sartor AL, Raza MW, Abbasi SA, Day KM, Perry JD, Paterson DL, Sidjabat HE.** 2014. Molecular epidemiology of NDM-1-producing Enterobacteriaceae and Acinetobacter baumannii isolates from Pakistan. Antimicrob Agents Chemother **58:**5589–5593. http://dx.doi.org/10.1128/AAC.02425-14.
33. **Giske CG, Froding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore D, Woodford N, Walsh TR.** 2012. Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of blaNDM-1 in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother **56:**2735–2738. http://dx.doi.org/10.1128/AAC.06142-11.
34. **Rogers BA, Sidjabat HE, Paterson DL.** 2011. Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother **66:**1–14. http://dx.doi.org/10.1093/jac/dkq415.
35. **Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, Yoshimura F, Kato N.** 1994. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of Serratia marcescens that shows imipenem resistance. Antimicrob Agents Chemother **38:**71–78. http://dx.doi.org/10.1128/AAC.38.1.71.
36. **Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, Woodford N, Livermore DM.** 2001. IMP-4, a novel metallo-beta-lactamase from nosocomial Acinetobacter spp. collected in Hong Kong between 1994 and 1998. Antimicrob Agents Chemother **45:**710–714. http://dx.doi.org/10.1128/AAC.45.3.710-714.2001.
37. **Tseng SP, Wang JT, Liang CY, Lee PS, Chen YC, Lu PL.** 2014. First report of bla(IMP-8) in Raoultella planticola. Antimicrob Agents Chemother **58:**593–595. http://dx.doi.org/10.1128/AAC.00231-13.
38. **O'Hara JA, Hu F, Ahn C, Nelson J, Rivera JI, Pasculle AW, Doi Y.** 2014. Molecular epidemiology of KPC-producing Escherichia coli: occurrence of ST131-fimH30 subclone harboring pKpQIL-like IncFIIk plasmid. Antimicrob Agents Chemother **58:**4234–4237. http://dx.doi.org/10.1128/AAC.02182-13.
39. **Naas T, Cuzon G, Gaillot O, Courcol R, Nordmann P.** 2011. When carbapenem-hydrolyzing beta-lactamase Kpc meets Escherichia coli ST131 in France. Antimicrob Agents Chemother **55:**4933–4934. http://dx.doi.org/10.1128/AAC.00719-11.
40. **Chen YT, Lauderdale TL, Liao TL, Shiau YR, Shu HY, Wu KM, Yan JJ, Su IJ, Tsai SF.** 2007. Sequencing and comparative genomic analysis of pK29, a 269-kilobase conjugative plasmid encoding CMY-8 and CTX-M-3 beta-lactamases in Klebsiella pneumoniae. Antimicrob Agents Chemother **51:**3004–3007. http://dx.doi.org/10.1128/AAC.00167-07.
41. **Kotsanas D, Wijesooriya WR, Korman TM, Gillespie EE, Wright L, Snook K, Williams N, Bell JM, Li HY, Stuart RL.** 2013. "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. Med J Aust **198:**267–269. http://dx.doi.org/10.5694/mja12.11757.
42. **Espedido B, Iredell J, Thomas L, Zelynski A.** 2005. Wide dissemination of a carbapenemase plasmid among Gram-negative bacteria: implications of the variable phenotype. J Clin Microbiol **43:**4918–4919. http://dx.doi.org/10.1128/JCM.43.9.4918-4919.2005.
43. **Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T.** 2013. Multilocus sequence typing (MLST) for characterization of Enterobacter cloacae. PLoS One **8:**e66358. http://dx.doi.org/10.1371/journal.pone.0066358.
44. **Izdebski R, Baraniak A, Herda M, Fiett J, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, MOSAR WP2, WP3, and WP5 Study Groups.** 2015. MLST reveals potentially high-risk international clones of Enterobacter cloacae. J Antimicrob Chemother **70:**48–56. http://dx.doi.org/10.1093/jac/dku359.
45. **Australian Commission on Safety and Quality in Health Care.** 2013. Recommendations for the control of multi-drug resistant Gram-negatives: carbapenem resistant Enterobacteriaceae. Commonwealth of Australia, Sydney, Australia.
46. **Sidjabat HE, Townell N, George N, Caffery M, Douglas J, Nimmo GR, Vaska V, Paterson DL.** 2014. Increasing emergence of IMP-4 producing *Enterobacteriaceae* throughout Queensland hospitals, poster P01.18. Antimicrobials 2014 Conference of the Australian Society for Antimicrobials.