Enterobacterales with acquired carbapenemases, 2021

Background

The acquired or transferable (as opposed to chromosomally encoded) carbapenemases found in Enterobacterales belong to three of the four major classes of β-lactamases: classes A, B and D.[1] Class A acquired carbapenemases include the *Klebsiella pneumoniae* carbapenemases, the so-called KPCs, as well as the IMI (imipenem-hydrolyzing β-lactamase) and GES (Guiana extended-spectrum β-lactamase) carbapenemases. Class B metallo-β-lactamases (MBLs) include several types of acquired carbapenemases, the most common being the New Delhi metallo-β-lactamases (NDMs), and the IMP and VIM metallo-β-lactamases. Class D acquired carbapenemases in Enterobacterales normally belong to the OXA-48 group of β-lactamases although genes from other OXA groups have also been reported. DNA mutations resulting in changes in the amino acid sequence of the carbapenemase have produced an ever-increasing range of subtypes or variants of each type of carbapenemase. For example, since the first NDM (NDM-1) was described in 2009, a further 30 subtypes (designated NDM-2 to NDM-31) have been described, with each subtype differing by at least one amino acid from any other subtype.

In New Zealand, diagnostic microbiology laboratories are requested to refer all suspected carbapenemase-producing Enterobacterales (CPE) isolates to ESR for confirmation and further investigation. They are also asked to provide susceptibility testing results and information on risk factors, such as recent travel history. This report summarises the characteristics of CPE isolates received by ESR in 2021. Reports on CPE confirmed between 2009, when the first isolate was identified in New Zealand, and 2020 are available on the ESR website at https://surv.esr.cri.nz/antimicrobial/AccqEnterobacteriaceae.php.

Methods

Isolates with a carbapenemase gene detected by PCR by the referring laboratory underwent Illumina-based whole genome sequencing (WGS). Select isolates were characterised using Nanopore-based long-read sequencing. Genomic DNA was extracted using the Roche High Pure PCR template preparation kit. DNA libraries were created using the Nextera XT DNA preparation kit (Illumina) or the gDNA Rapid Barcoding kit (Oxford Nanopore Technologies), and sequencing was performed using Illumina or Nanopore technology respectively. Illumina-based data were analysed using an in-house

developed pipeline linking together open-source packages and in-house scripts, which enables the carbapenemase gene subtype, the acquired resistome and the multi-locus sequence type to be determined. Open-source packages used included the Nullarbor2: 'Reads to report' for public health and clinical microbiology pipeline,[2] SKESA,[3] MLST[4], ABRicate[5] using ResFinder[6] and PlasmidFinder databases.[7] Nanopore sequencing data were assembled using Flye.[8] Hybrid Illumina and long-read assembly was performed using Pilon.[9]

Submitted isolates that were carbapenemase PCR negative, or not tested using PCR by the referring laboratory, underwent inhibitor-based phenotypic tests, the modified carbapenemase inactivation method (mCIM) and a selection of the following PCRs based on phenotypic screening results: KPC (*bla*$_{KPC}$), IMI (*bla*$_{IMI}$), GES (*bla*$_{GES}$), NDM (*bla*$_{NDM}$), IMP (*bla*$_{IMP}$), VIM (*bla*$_{VIM}$), GIM (*bla*$_{GIM}$), SIM (*bla*$_{SIM}$), SPM (*bla*$_{SPM}$) and OXA (*bla*$_{OXA}$).[10,11,12,13,14,15,16] Isolates that were positive in any of these tests underwent Illumina-based WGS.

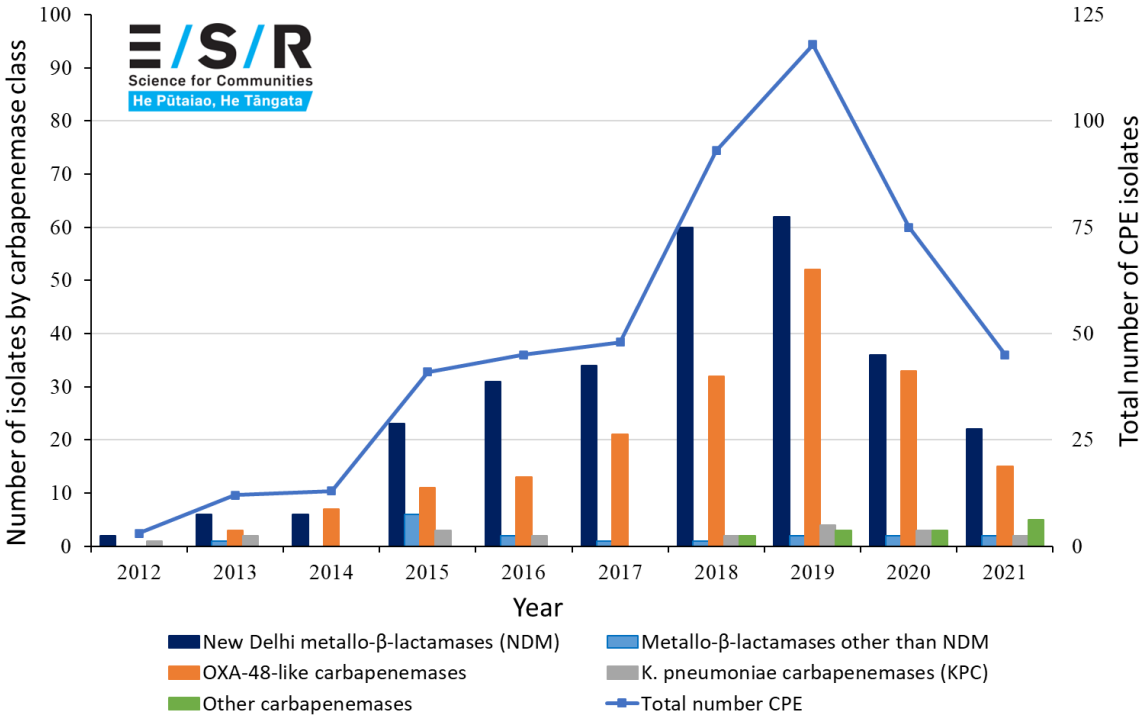
Results

Forty-six distinct CPE were isolated from 40 patients in 2021 (Figure 1 and Table 1). Three patients had two distinct CPE isolates, and one patient had four distinct CPE (see Table 1, footnote 1). Compared to data in 2020, both the number of CPE and the number of patients CPE were isolated from was reduced. This decrease likely reflects the interruption to international travel experienced during the ongoing SARS-CoV-2 pandemic.

Of the CPE confirmed in 2021, 60.9% (28/46) were isolated from screening specimens. Among the 18 CPE from clinical specimens 12 (66.7%) were from urinary sources, three (16.7%) were from sterile sites, one from an ear (5.6%), one from sputum (5.6%) and one was from a skin and soft tissue infection (5.6%).

Laboratories from the Auckland region referred the majority of confirmed CPE isolates (33, 71.7%), followed by the Wellington region (8, 17.4%). Most patients were ≥65 years of age (24, 52.2%), with 26.1% of cases among 45-64 year olds, 17.4% of cases among 15-44 year olds, and 4.4% under 15 years of age.

Figure 1. Number of carbapenemase-producing Enterobacterales (CPE) isolates identified in New Zealand, by carbapenemase class, each year from 2012 to 2021



Note: Multiple, distinct CPE isolates from the same patient are included, but duplicate isolates of the same species with the same type(s) of carbapenemase(s) from the same patient are excluded.

Types of carbapenemases identified

As observed in previous years, the most frequently identified carbapenemases were MBL, with various subtypes of NDM accounting for 47.8% (22/46) of all carbapenemase genes identified in CPE in 2021 (Figure 1 and Table 1) and 54.7% (287/525) of all carbapenemases in New Zealand to date. IMP comprised 4.3% (2/46) of carbapenemases identified in 2021, while IMP and VIM MBLs together account for 3.2% (17/525) of all carbapenemases identified to date.

In 2021, the second most common carbapenemases identified were OXA-48-like carbapenemases, which accounted for a 32.6% (15/46) of the carbapenemases identified (Table 1). They have accounted for 31.5% (188/525) of all carbapenemases identified in CPE in New Zealand.

A total of seven other acquired carbapenemase genes were found in New Zealand CPE in 2021. Three isolates contained plasmid-associated IMI genes, with the gene location confirmed using data from both Illumina and Nanopore-based long-read sequencing. Two isolates contained OXA-23, which is a gene more commonly found in *Acinetobacter baumannii* but has been found in six New Zealand CPE to date. Two isolates contained a

Table 1. Types of carbapenemases identified among carbapenemase-producing Enterobacterales by species, 2021

Carbapenemase type and subtype	Number of isolates per species					All species
	Escherichia coli	*Klebsiella pneumoniae*	*Enterobacter cloacae complex*	*Citrobacter spp.*	*Klebsiella aerogenes*	
NDM	**13**	**5**	**2**	**2**	**0**	**22**
NDM-1	1	5	2	1	0	9
NDM-5	11	0	0	1	0	12
NDM-19	1	0	0	0	0	1
OXA-48-like	**9**	**5**	**0**	**0**	**1**	**15**
OXA-48	6	2	0	0	1	9
OXA-181	3	3	0	0	0	6
Other carbapenemase genes	**3**	**3**	**3**	**0**	**0**	**9**
IMI-2	0	0	2	0	0	2
IMI-3	0	0	1	0	0	1
IMP-4	1	1	0	0	0	2
KPC-2	0	1	0	0	0	1
KPC-3	0	1	0	0	0	1
OXA-23	2	0	0	0	0	2
Total	**25**	**13**	**5**	**2**	**1**	**46**[1]

1 The 46 isolates include multiple, distinct CPE from four patients:

- *E. coli* and *K. aerogenes* with OXA-48;
- *E. coli* with NDM-5 and *K. pneumoniae* with NDM-1;
- Two *E. coli* with OXA-48, with multilocus sequence type 69 and 1485.
- *E. coli* with NDM-19, *E. coli* with OXA-181, *K. pneumoniae* with NDM-1 and *K. pneumoniae* with OXA-181

KPC gene, which have accounted for 3.8% (20/525) of all CPE in New Zealand, of which nineteen were in *Klebsiella pneumoniae* and one was in an isolate belonging to the *Enterobacter cloacae* complex.

No isolates were identified in 2021 that contained more than one carbapenemase gene.

Probable place of acquisition of carbapenemase–producing Enterobacterales

Travel history was available for 36 of the 40 patients, of which 47.2% (17/36) had been overseas. Six patients were thought to have acquired their carbapenemase genes in the Indian subcontinent, four in other parts of Asia, three in the Eastern Mediterranean, two in Europe, and one in both Africa and the Western Pacific. However as two patients with multiple CPE isolates had visited the Eastern Mediterranean, the highest number of carbapenemase genes were likely to have been acquired in the Eastern Mediterranean (Table 2).

Of the CPE likely to have been acquired overseas, 42.9% (9/17) were from patients who had been hospitalised.

Transmission of carbapenemase–producing Enterobacterales in New Zealand

Nineteen patients had no history of recent overseas travel and for 12 of these the likely source could not be identified. The other seven isolates were associated with three CPE cross-transmission events within New Zealand. Two *E. coli* containing OXA-48 were linked to a community cluster in the Wellington region that started in August 2018 (related to a food outlet). Four *E. coli* with NDM-5 were associated with a transmission event in a hospital in the Wellington region, although the index case was not identified. One *K. pneumoniae* with KPC-3 may have been acquired from a household contact who reported recent travel.

Table 2. Probable place of acquisition of carbapenemase-producing Enterobacterales, 2021

Carbapenemase type and subtype	Number of isolates[1] Probable region of acquisition							
	Indian subcontinent	Eastern Mediterranean	New Zealand[2]	Other parts of Asia[3]	Europe	Overseas[4]	Not known[5]	Total
NDM	**4**	**5**	**8**	**1**	**1**	**1**	**2**	**22**
NDM-1	1	3	2	1	1	1	0	9
NDM-5	3	1	6	0	0	0	2	12
NDM-19	0	1	0	0	0	0	0	1
OXA-48-like	**2**	**2**	**4**	**2**	**2**	**1**	**2**	**15**
OXA-48	0	0	4	1	2	0	2	9
OXA-181	2	2	0	1	0	1	0	6
Other carbapenemases	**0**	**0**	**7**	**1**	**0**	**0**	**1**	**9**
IMI-2	0	0	1	1	0	0	0	2
IMI-3	0	0	1	0	0	0	0	1
IMP-4	0	0	2	0	0	0	0	2
KPC-2	0	0	1	0	0	0	0	1
KPC-3	0	0	1	0	0	0	0	1
OXA-23	0	0	1	0	0	0	1	2
Total	**6**	**7**	**19**	**4**	**3**	**2**	**5**	**46**

1 Includes multiple isolates from three patients who had two distinct CPE and one patient who had four distinct CPE (see Table 1, footnote 1). Two of these patients had reported recent travel to the Eastern Mediterranean (with one hospitalised while there), one patient had recent travel to Europe, and the travel history of one patient was not known.

2 Includes 7 isolates from probable CPE cross-transmission events in New Zealand: two *E. coli* isolates with OXA-48, four *E. coli* with NDM-5, one *K. pneumoniae* with KPC-3. The likely source of the other 12 CPE was not determined.

3 All Asia other than the Indian subcontinent.

4 One isolate with NDM-1 from the Western Pacific and one with OXA-181 from Africa.

5 Isolates from five patients where the travel history was not reported.

Antimicrobial susceptibility testing results

Referring laboratories were asked to provide ESR with their susceptibility results, which are summarised in Table 3. The 17 meropenem-susceptible isolates with a carbapenemase gene contained either OXA-48 (9), OXA-181 (5), OXA-23 (2) or IMI-3 (1). Multi-resistance, defined as resistance to three or more classes of antimicrobials, was common. However, there were exceptions, including one isolate with IMI-3 that was susceptible to all reported antimicrobials, which were amikacin, ceftazidime-avibactam, ciprofloxacin, cotrimoxazole, gentamicin, ertapenem and meropenem.

Table 3. Susceptibility results generated by diagnostic laboratories for carbapenemase producing Enterobacterales, 2021

	Percent (number)			
Antimicrobial	**Susceptible**	**Non-susceptible**	**Resistant**	**Total number of isolates tested**
Amoxicillin-clavulanate	0.0 (0)	100.0 (36)	100.0 (36)	36
Cefoxitin	25.0 (8)	75.0 (24)	75.0 (24)	32
Ciprofloxacin	21.7 (10)	80.4 (36)	76.1 (35)	46
Co-trimoxazole	39.2 (20)	58.1 (25)	58.1 (25)	43
Ertapenem	11.1 (3)	88.9 (24)	88.9 (24)	27
Fosfomycin	71.4 (10)	28.6 (4)	28.6 (4)	14
Gentamicin	62.2 (28)	37.8 (17)	35.6 (16)	45
Imipenem	15.4 (2)	84.6 (11)	53.9 (7)	13
Meropenem	37.0 (17[1])	63.0 (29)	50.0 (23)	46
Norfloxacin	31.6 (8)	68.4 (13)	68.4 (13)	33
Piperacillin/ tazobactam	6.1 (2[2])	93.9 (31)	93.9 (31)	33
Trimethoprim	40.0 (12)	60.0 (18)	60.0 (18)	30

1 The 17 meropenem-susceptible isolates with a carbapenemase gene contained either OXA-48 (9), OXA-181 (5), OXA-23 (2) or IMI-3 (1).
2 Both piperacillin-tazobactam susceptible isolates contained IMI-2

Resistome

Most isolates with a carbapenemase gene also had a number of other resistance genes present, including genes conferring resistance to aminoglycosides (34, 73.9%), sulphonamides (30, 65.2%), fluoroquinolones (28, 60.6%), trimethoprim (26, 56.5%) and tetracycline (23, 56.0%). Three isolates contained 16S ribosomal methyl transferases, which were found in isolates with NDM. Three isolates with *mcr* all had *mcr*-9 together with either IMP-4 or NDM-1.

Multi-locus sequence types identified

The multi-locus sequence type (MLST) was available for 24 of the 25 *E. coli* with acquired carbapenemase genes. The only sequence types found in more than one isolate were ST-405 (5 isolates) and ST-131 (3 isolates). All five isolates with ST-405 contained NDM-5, and four were associated with a cluster in the Wellington region. Two of the ST-131 isolates contained OXA-48 and were associated with a community cluster in the Wellington region. The third isolate ST-131 isolate contained NDM-5.

Multi-locus sequence types were available for 12 of the 13 *K. pneumoniae* isolates. Nine distinct sequence types were found, and no sequence type was found in more than two isolates.

Conclusion

Carbapenem resistance continues to be of concern to New Zealand. The interruption in travel due to the SARS-CoV-2 pandemic has slowed the rate of increase, however the diverse range of multi-locus sequence types identified and the number of patents reporting recent overseas travel supports the theory that most of the CPE identified in New Zealand have originated overseas. The number of isolates likely to have been acquired locally continued to increase (34.9% in 2020 (22/63), compared to 46.3% in 2021 (19/41)), although the figure for 2021 is likely to have been affected by a reduced number of isolates being introduced across New Zealand's border. NDM remains the dominant carbapenemase type, followed by OXA-48. As expected, CPE isolates described in this report are highly multi-drug resistant, across multiple antimicrobial classes, with limited treatment options available. Vigilance must be maintained to detect isolates early, to limit further spread and prevent outbreaks within healthcare, residential facilities and communities. ESR must continue to receive confirmed or suspected CPE isolates from diagnostic laboratories for further molecular characterisation and to help identify any linkages with cross transmission events.

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