Carbapenemase-producing Enterobacterales (CPE) in New Zealand, 2022

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 (KPCs), the IMI (imipenem-hydrolyzing β-lactamase) as well as the 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 60 subtypes (designated NDM-2 to NDM-61) 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 2022. Reports on CPE confirmed between 2009, when the first isolate was identified in New Zealand, and 2021 are available on the ESR website at https://www.esr.cri.nz/expertise/public-health/antimicrobial-resistance.

Methods

Isolates with a carbapenemase gene detected by PCR by the referring laboratory underwent Illumina-based whole genome sequencing (WGS). Select isolates, including all isolates with an IMI carbapenemase, were also characterised using Nanopore-based long-read sequencing to determine if the carbapenemase gene was located on the bacterial chromosome or plasmid. Isolates with chromosomally-located carbapenemase genes were excluded from this report.

Genomic DNA was extracted using the Roche High Pure PCR template preparation kit or the Chemagic 360 (Perkin Elmer). DNA libraries were created using the Nextera XT DNA preparation kit (Illumina), the plexWell Library Preparation kit (SeqWell), or the gDNA Rapid Barcoding kit (Oxford Nanopore Technologies), and sequencing was performed using Illumina or Nanopore technology. 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]

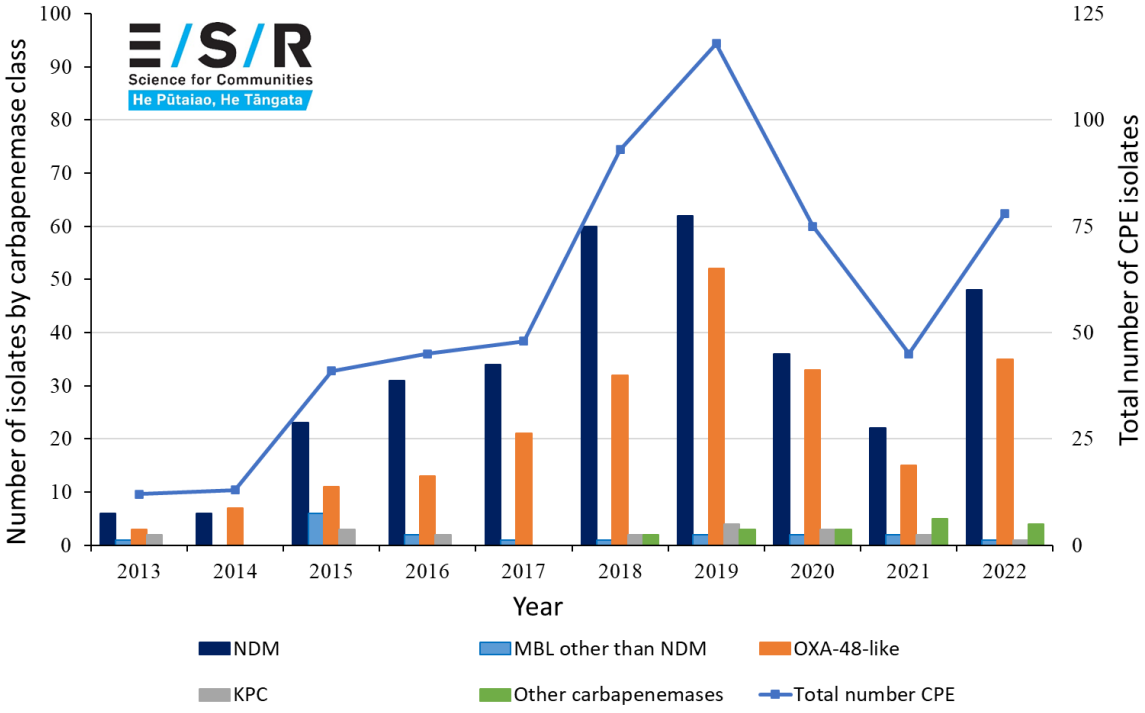
Isolates referred to ESR that were carbapenemase PCR negative, or not tested using PCR by the referring laboratory, underwent inhibitor-based phenotypic tests, the modified carbapenemase inactivation method (mCIM) and a selection of the following PCRs based on phenotypic screening results: KPC (*bla*$_{KPC}$), 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 negative in these tests were not included in this report as they were determined not to contain a carbapenemase gene. Isolates positive in any of these tests underwent Illumina-based sequencing to confirm the presence of the carbapenemase gene and its subtype or detect any carbapenemase genes not found by PCR.

Results

Seventy-eight distinct CPE were isolated from 53 patients in 2022 (Figure 1 and Table 1). Species were predominantly *Escherichia coli*, followed by *Klebsiella pneumonie*. Eleven patients had two distinct CPE isolates, four patients had three distinct CPE, and one patient had seven distinct CPE (see Table 1, footnote 1). Compared to data in 2021, both the number of CPE and the number of patients CPE were isolated from increased. This increase likely reflects the re-opening of the New Zealand border during 2022, following the easing of SARS-CoV-2 pandemic travel restrictions.

Of the CPE confirmed in 2022 the sample source was provided for 98.7% (77/78) of the isolates. Of those with known source, 72.7% (56/77) were isolated from screening specimens. Among the 21 CPE from clinical specimens 16 (76.2%) were from urinary sources, three (14.3%) were from blood, one (4.8%) was from a tissue sample, and one was from a respiratory specimen (4.8%).

Figure 1. Number of carbapenemase-producing Enterobacterales (CPE) isolates identified in New Zealand, by carbapenemase class, each year from 2013 to 2022



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.

Laboratories from the Auckland region referred the majority of confirmed CPE isolates (63, 80.8%). All other regions referred less than five CPE during 2022. 39.7% of CPE were

Table 1. Types of carbapenemases identified among carbapenemase-producing Enterobacterales by species, 2022

Carbapenemase type and subtype	Number of isolates per species				All species
	Escherichia coli	*Klebsiella pneumoniae*	*Citrobacter spp.*	Other species	
NDM	**21**	**12**	**2**	**2**	**37**
NDM-1	0	5	0	1[2]	6
NDM-5	20	6	2[1]	1[3]	29
NDM-6	0	1	0	0	1
NDM-7	1	0	0	0	1
OXA-48-like	**20**	**3**	**1**	**0**	**24**
OXA-48	13	1	1	0	15
OXA-181	0	2	0	0	2
OXA-232	1	0	0	0	1
OXA-244	4	0	0	0	4
OXA-484	2	0	0	0	2
NDM and OXA-48-like	**4**	**6**	**0**	**1**	**11**
NDM-1 and OXA-181	0	0	0	1[4]	1
NDM-5 and OXA-232	0	2	0	0	2
NDM-5 and OXA-181	4	4	0	0	8
Other carbapenemase genes	**5**	**1**	**0**	**0**	**6**
IMP-4	1	0	0	0	1
KPC-3	0	1	0	0	1
OXA-23	4	0	0	0	4
Total	**50**	**22**	**3**	**3**	**78[5]**

1. One *Citrobacter farmeri* and one *Citrobacter freudii*
2. *Enterobacter cloacae* complex
3. *Klebsiella oxytoca*
4. *Providencia rettgeri*
5. The 78 isolates include multiple, distinct CPE from 16 patients:
    - Four patients with *E. coli* with NDM-5 and *E. coli* with OXA-181;

- Three patients with *E. coli* with NDM-5 and *K. pneumoniae* with NDM-5
- One patient with *E. coli* with NDM-5 and *E. coli* with NDM-5 & OXA-181;
- One patient with *E. coli* with OXA-181 and *E. coli* with NDM-5 & OXA-181;
- One patient with *E. coli* with OXA-181 and *Citrobacter spp.* with OXA-181;
- One patient with *K. pneumoniae* with NDM-5 and *K. oxytoca* with NDM-5;
- One patient with *E. coli* with NDM-5, *E. coli* with OXA-181 and *E. coli* with NDM-5 & OXA-181;
- One patient with *E. coli* with NDM-5, *K. pneumoniae* with NDM-5, and *C. farmeri* with NDM-5;
- One patient with *E. coli* with NDM-5, *K. pneumoniae* with NDM-5 and *K. pneumoniae* with NDM-5 & OXA-181;
- One patient with *E. coli* ST410 with OXA-181; *E. coli* ST448 with OXA-181, and *E. coli* ST448 with NDM-5 & OXA-181;
- One patient with *E. coli* with NDM-5, *E. coli* with OXA-181, *K. pneumoniae* with NDM-5 & OXA-181, *K. pneumoniae* with NDM-1, *K. pneumoniae* with OXA-232, *C. freundii* with NDM-5, and *P, rettgeri* with NDM-1 and OXA-181.

from patients ≥65 years of age, 25.6% of cases among 45-64 year olds, 30.8% of cases among 15-44 year olds, and 3.9% under 15 years of age.

Types of carbapenemases identified

As observed in previous years, the most frequently identified carbapenemase genes were MBL, with various subtypes of NDM accounting for 53.9% (48/89) of all carbapenemase genes in 2022 (Figure 1 and Table 1) and 54.6% (335/614) of all carbapenemase genes in New Zealand to date. There was only one IMP carbapenemase gene found in 2022 (1.1%, 1/89), with IMP and VIM MBLs together accounting for 2.9% (18/614) of all carbapenemase genes identified in New Zealand to date.

OXA-48-like genes were the second most common carbapenemase gene identified in New Zealand in 2022, accounting for 39.3% (35/89) of all carbapenemase genes (Table 1) and 36.3% (223/614) of all carbapenemase genes in CPE in New Zealand to date.

A total of five other acquired carbapenemase genes were found in New Zealand CPE in 2022. Four isolates contained OXA-23, which is a gene more commonly found in *Acinetobacter baumannii* complex, but has been found in 12 CPE isolated in New Zealand to date. One further isolate contained a KPC gene, which have accounted for 3.4% (21/614) 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.

Eleven isolates were identified in 2022 that contained more than one carbapenemase gene. Eight contained NDM-5 & OXA-181, two isolates contained NDM-1 & OXA-232, and one isolate contained NDM-1 & OXA-181.

Probable place of acquisition of carbapenemase–producing Enterobacterales

Travel history was available for 45 of the 53 patients, of which 68.9% (31/45) had been overseas. Twenty-one patients were thought to have acquired their carbapenemase genes in the Indian subcontinent, four in the Western Pacific, two in other parts of Asia, one in the Eastern Mediterranean, one in Europe, one in Africa, and one in either the Indian subcontinent or other parts of Asia. (Table 2). Of the CPE likely to have been acquired overseas, 71.0% (22/31) were from patients who had been hospitalised. CPE isolated from patients that had been overseas but had not been hospitalised, had travelled to the Indian subcontinent (8) or the Eastern Mediterranean (1).

Table 2. Probable place of acquisition of carbapenemase-producing Enterobacterales, 2022

Carbapenemase type and subtype	Number of isolates[1] Probable region of acquisition						
	Indian subcontinent	New Zealand[2]	Western Pacific	Other parts of Asia[3]	Overseas[4]	Not known[5]	Total
NDM	**18**	**8**	**4**	**1**	**3**	**3**	**37**
NDM-1	2	0	3	0	1	0	6
NDM-5	16	8	0	0	2	3	29
NDM-6	0	0	0	1	0	0	1
NDM-7	0	0	1	0	0	0	1
OXA-48-like	**11**	**8**	**0**	**1**	**1**	**3**	**24**
OXA-48	0	2	0	0	0	0	2
OXA-181	9	3	0	0	0	3	15
OXA-232	1	0	0	1	0	0	2
OXA-244	0	0	0	0	1	0	1
OXA-484	1	3	0	0	0	0	4
NDM & OXA-48-like	**9**	**2**	**0**	**0**	**0**	**0**	**11**
NDM-1 & OXA-48	1	0	0	0	0	0	1
NDM-1 & OXA-232	2	0	0	0	0	0	2
NDM-5 & OXA-181	6	2	0	0	0	0	8
Other carbapenemases	**0**	**2**	**0**	**0**	**1**	**3**	**6**
IMP-4	0	0	0	0	0	1	1
KPC-3	0	0	0	0	1	0	1
OXA-23	0	2	0	0	0	2	4
Total	**38**	**20**	**4**	**2**	**5**	**9**	**78**

1 Includes multiple isolates from 16 patients who had multiple distinct CPE (see Table 1, footnote 1). Nine of these patients reported recent travel to the Indian subcontinent (with five hospitalised while there), five patients had no recent travel history, one patient had recent travel to both the Indian subcontinent and other parts of Asia, and the travel history of one patient was not known.

2 Includes 11 isolates from four probable CPE cross-transmission events in New Zealand: three *E. coli* isolates with OXA-484, one *E. coli* with OXA-48, and six CPE with NDM-5 (three *E. coli*, two *K. pneumoniae* and one *C. farmeri*). The likely source of the other nine CPE was not determined.

3 All Asia other than the Indian subcontinent.

4 One isolate with NDM-1 from a patient that travelled to Africa, two isolates with NDM-5 from a patient that was hospitalised in the Indian subcontinent and in Singapore, and one isolate with OXA-244 from a patient that travelled to the Eastern Mediterranean, and one isolate with KPC-3 from a patient that travelled to Europe.

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

Transmission of carbapenemase–producing Enterobacterales in New Zealand

Fourteen patients, with a total of 20 CPE, had no history of recent overseas travel. For seven patients, with a total of nine CPE, the likely source could not be identified. The other seven patients were associated with four CPE cross-transmission events within New Zealand, which involved a total of 11 CPE.

The first probable transmission event involved plasmids found in seven CPE containing NDM-5 (three *E. coli*, two *K. pneumoniae* and one *C. farmeri*). These seven CPE were isolated from three epidemiologically linked patients from a hospital in the Auckland region. None of the patients had recent international travel and Nanopore-based long read sequencing determined that all contained the same plasmid.

There were two probable cross-transmission events resulting from transmission of CPE within members of two different families. The first family transmission event involved three patients belonging one family. Two family members are thought to have acquired their CPE in New Zealand with the suspected index case receiving healthcare in India. No isolate was cultured from the suspected index case. The second cluster involved one person who probably acquired their CPE in New Zealand, from a family member who had recently received healthcare in India. Both family clusters involved sequence type 410 *E. coli* with OXA-484. The strains were similar although there were differences between isolates from each family group. These differences suggested that CPE isolated in New Zealand were likely to have resulted from two separate introductions of an *E. coli* lineage circulating in India, rather than a direct epidemiological link between all cases.

The fourth probable transmission event involved an *E. coli* containing OXA-48 linked to a community cluster in the Wellington region that started in August 2018 (related to a food outlet).[17] To the end of 2022, a total of 28 genetically related isolates have been isolated from patients from the Wellington region, with one new case identified in 2022.

Antimicrobial susceptibility testing results

Referring laboratories were asked to provide ESR with their susceptibility results, which are summarised in Table 3. The 21 meropenem-susceptible CPE contained an OXA carbapenemase gene, with the genes found being OXA 181 (12), OXA-23 (4), OXA-484 (3) or OXA-48 (2). Multi-resistance, defined as resistance to three or more classes of antimicrobials, was common. However, there were exceptions, including one isolate with OXA-23 that was reported as resistant to amoxicillin-clavulanate but susceptible to all other antimicrobials tested, which were

Table 3. Susceptibility results generated by diagnostic laboratories for carbapenemase-producing Enterobacterales, 2022

	Percentage of isolates for each carbapenemase group											
	Total CPE (n=78)				MBL (n=49)				OXA-48-like (n = 22)			
Antimicrobial	S[1]	NS[1]	R[1]	No. Tested	S[1]	NS	R[1]	No. Tested	S[1]	NS[1]	R[1]	No. Tested
Amoxicillin-Clavulanate	0	100.0	100.0	60	0	100.0	100.0	35	0	100.00	100.0	19
Cefoxitin	7.8	92.2	86.3	51	0	100	100	31	23.5	76.5	58.8	17
Ciprofloxacin	23.0	77.0	75.7	74	24.4	75.6	73.3	45	9.1	90.9	90.9	22
Co-trimoxazole	25.7	74.3	74.3	70	11.6	88.4	88.4	43	47.6	52.4	52.4	21
Ertapenem	2.9	97.1	97.1	34	0.0	100	100	23	0.0	100	100	7
Fosfomycin	90.9	9.1	9.1	22	86.7	13.3	13.3	15	100	0.0	0.0	6
Gentamicin	50.0	50.0	48.7	74	32.6	67.4	67.4	46	72.7	27.3	22.7	22
Imipenem	15.0	85.0	70.0	20	0.0	100	92.3	13	40	60	20	5
Meropenem[2]	29.2	70.8	66.7	72	0.0	100	97.7	44	81.0	19.1	9.5	21
Norfloxacin	27.9	72.1	72.1	43	33.3	66.7	66.7	24	6.3	93.8	93.8	16
Piperacillin-Tazobactam	0.0	100.0	100.0	56	0.0	100.0	100.0	34	0.0	100.0	100.0	18
Trimethoprim	23.1	76.9	76.9	52	6.7	93.3	93.3	30	38.9	61.1	61.1	18

1 S = susceptible, NS = non-susceptible, R = resistant,

≥ 90% Susceptible | 70 - 89% Susceptible | < 70% Susceptible

2 The 21 meropenem-susceptible isolates with a carbapenemase gene contained either OXA-181 (12), OXA-23 (4), OXA-484 (3) or OXA-48 (2).

ciprofloxacin, co-trimoxazole, ertapenem, fosfomycin, gentamicin, meropenem, norfloxacin and trimethoprim.

Resistome

Most isolates with a carbapenemase gene also had a number of other resistance genes present, including genes conferring resistance to aminoglycosides (62, 79.5%), sulphonamides (60, 76.9%), fluoroquinolones (54, 69.2%), trimethoprim (59, 75.6%) and tetracycline (41, 52.6%). Twenty-seven isolates contained a 16S ribosomal methyl transferase gene, which confers high-level resistance to all clinically relevant aminoglycosides. The 16S ribosomal methyl transferases genes were found with NDM-5 (13), NDM-5 & OXA-181 (6), NDM-1 (2), OXA-181 (2), NDM-1 & OXA-232 (2), NDM-1 & OXA-181 (1), and OXA-232 (1). Isolates containing 16S ribosomal methyl transferases with known travel history, came from patients with either recent travel to the Indian subcontinent (15/26, 57.7%) or no recent travel history (11/26, 42.3%). One isolate with a mobile colistin resistance gene was identified. This isolate contained the carbapenemase IMP-4 and the *mcr*9 gene. No other *mcr* genes were identified in any of the isolates.

Multi-locus sequence types identified

The multi-locus sequence type (MLST) was available for 47 of the 50 *E. coli* with acquired carbapenemase genes. A diverse range of sequence types were found, with only ST-410 and ST-448 found in more than five isolates. Four sequence types were found in three isolates each (ST-38, ST-44, ST-167 and ST-648) and four sequence types were found in two isolates each (ST-10, ST-131, ST-156, ST-361). All other sequence types were only found once.

There were eight ST-410 isolates, with four containing OXA-484 associated with the family cross-transmission events as well as isolates with NDM-5 (2 isolates), NDM-7 (1 isolate) and OXA-181 (1 isolate). There were six isolates with ST-448, all isolated from patients with travel to the Indian subcontinent. These isolates had NDM-5 (2 isolates), OXA-181 (2 isolates), or NDM-5 & OXA-181 (2 isolates).

Multi-locus sequence types were available for 21 of the 22 *K. pneumoniae* isolates. Thirteen distinct sequence types were found, with the most common sequence type, ST-147, found in found in four isolates. Three ST-13 isolates, three ST-437, and two ST-280 isolates were also found. All other sequence types were only found once. The three ST-13 isolates were associated with the Auckland hospital cluster, but no other clusters were identified.

Conclusion

Carbapenem resistance continues to be of concern to New Zealand. The numbers of CPE found in 2022 increased compared to numbers of CPE found in 2020 and 2021. This increase is likely to be attributable to the re-opening of the New Zealand border following restrictions imposed to control the SARS-CoV-2 pandemic, and supports the theory that most of the CPE identified in New Zealand have originated overseas. The increase in the number of cases likely to have originated overseas has resulted in an overall decrease in the proportion of cases thought to have been acquired locally, although the number of locally acquired cases has remained reasonable consistent between 2019-2022 fluctuating between 20-22 cases. 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 and we thank the diagnostic laboratories for their ongoing contribution to this important surveillance.

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