Science for Communities

Enterobacterales with acquired carbapenemases, 2020

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). 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. This report summarises the characteristics of CPE isolates received by ESR in 2020. Reports on CPE confirmed between 2009, when the first isolate was identified in New Zealand, and 2019 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). WGS 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 v.2.3.0,[3] MLST[4] and ABRicate[5] using ResFinder[6] and PlasmidFinder databases.[7]

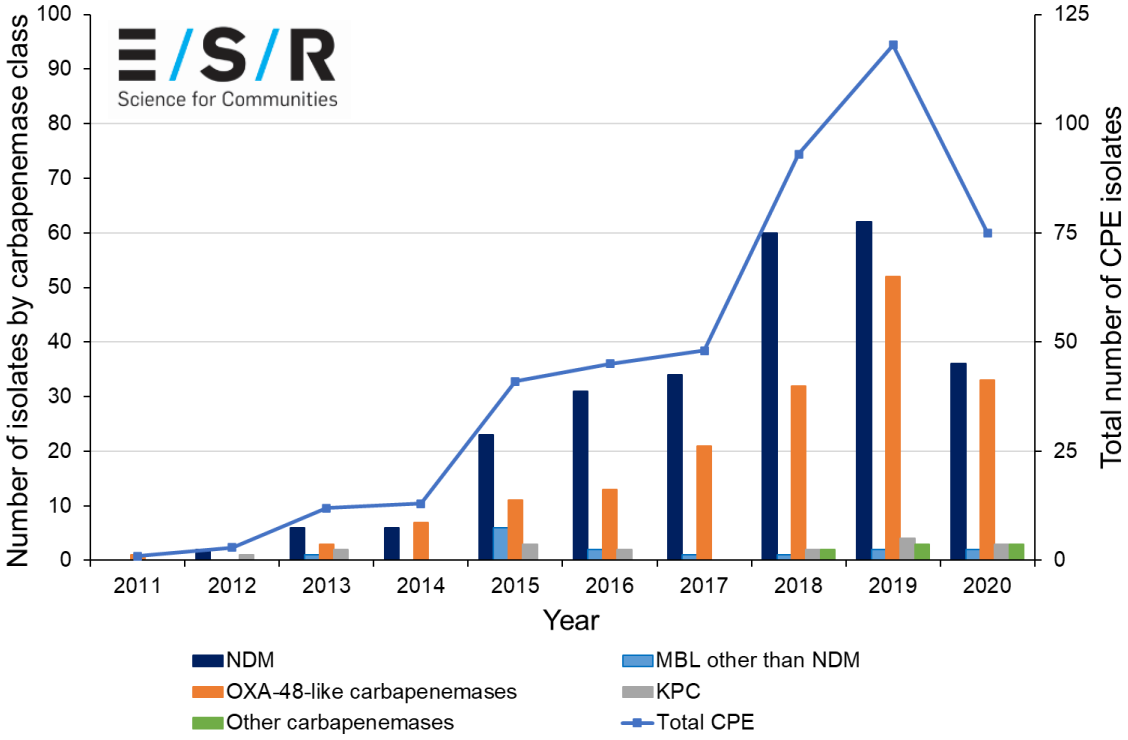
Submitted isolates that were carbapenemase PCR negative, or not tested by the referring laboratory, underwent inhibitor-based phenotypic tests, the modified carbapenemase inactivation method (mCIM) and PCRs for the following carbapenemases: KPC (*bla*$_{KPC}$),

IMI (*bla*$_{IMI}$),GES (*bla*$_{GES}$), NDM (*bla*$_{NDM}$), IMP (*bla*$_{IMP}$), VIM (*bla*$_{VIM}$), SIM (*bla*$_{SIM}$) and OXA (*bla*$_{OXA}$). Isolates that were positive in any of these tests underwent WGS. Basic epidemiological data, including overseas travel and hospitalisation history, was collected for patients with confirmed CPE.

Results

Seventy-five distinct CPE were isolated from 70 patients in 2020 (Figure 1 and Table 1). Five patients had two distinct CPE isolates (see Table 1, footnote 1). Compared to data in 2019, both the number of CPE (Figure 1) and the number of patients was reduced. This decrease likely reflects the interruption to international travel experienced during the ongoing SARS-CoV-2 pandemic.

Figure 1. Number of carbapenemase-producing Enterobacterales (CPE) isolates identified in New Zealand, by carbapenemase class, each year from 2011 to 2020



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. In 2020, there were two CPE isolates that carried the genes encoding for both NDM and OXA-48-like carbapenemases. These two isolates are counted in the number of isolates for both these carbapenemase classes.

Table 1. Types of carbapenemases identified among carbapenemase-producing Enterobacterales by species, 2020

Carbapenemase type and subtype	Number of isolates per species					All species
	Escherichia coli	*Klebsiella pneumoniae*	*Enterobacter cloacae complex*	*Citrobacter spp.*	*Proteus mirabilis*	
NDM	**25**	**6**	**0**	**2**	**13**	**34**
NDM-1	2	3	0	1	0	6
NDM-4	0	0	0	1	0	1
NDM-5	19	3	0	0	1	23
NDM-6	2	0	0	0	0	2
NDM-7	2	0	0	0	0	2
OXA-48-like	**28**	**3**	**0**	**0**	**0**	**31**
OXA-48	11	2	0	0	0	13
OXA-181	10	1	0	0	0	11
OXA-244	5	0	0	0	0	5
OXA-484	2	0	0	0	0	2
NDM and OXA-48-like	**1**	**1**	**0**	**0**	**0**	**2**
NDM-5 and OXA-232	0	1	0	0	0	1
NDM-5 and OXA-484	1	0	0	0	0	1
IMP	**0**	**1**	**1**	**0**	**0**	**2**
IMP-4	0	1	1	0	0	2
KPC	**0**	**2**	**1**	**0**	**0**	**3**
KPC-2	0	2	1	0	0	3
OXA-23	**3**	**0**	**0**	**0**	**0**	**3**
Total	**57**	**13**	**2**	**2**	**1**	**75**[1]

1 The 75 isolates include multiple, distinct CPE from five patients:

- *E. coli* with NDM-5 and *E. coli* with OXA-484;
- *E. coli* with OXA-181 and *E. coli* with NDM-5 and OXA-484;
- *K. pneumoniae* with NDM-1 and *P. mirabilis* with NDM-5;
- *K. pneumoniae* with NDM-5 and *K. pneumoniae* with NDM-5 and OXA-232;
- *K. pneumoniae* with KPC-2 and *Enterobacter cloacae complex* with KPC-2.

Of the CPE confirmed in 2020, 65.3% (49/75) were isolated from screening specimens. Among the 26 CPE from clinical specimens, 23 (79.3%) were from urinary sources, two were from skin and soft tissue infections (7.7%), and one (3.8%) was from an ear swab.

Laboratories from the Auckland region referred the majority of confirmed CPE isolates (39, 52.0%), followed by the Wellington region (17, 22.7%). Most patients were ≥65 years of age (34, 48.0%), with 25.3% of cases among 45-64 year olds, 16.0% 15-44 year olds, and 10.7% under 15 years of age.

Types of carbapenemases identified

As observed in previous years, the most frequently identified carbapenemases were MBL, with various subtypes of NDM accounting for 46.8% (36/77) of all carbapenemase genes identified in CPE in 2020 (Figure 1 and Table 1) and 55.7% (265/479) of all carbapenemases in New Zealand to date. IMP comprised 2.6% (2/77) of carbapenemases identified in 2020, while IMP and VIM MBLs together account for 3.1% (15/479) of all carbapenemases identified to date.

OXA-48-like carbapenemases were next most common, comprising 42.9% (33/77). Compared to previous years, the proportion of OXA-48-like carbapenemases has increased (Figure 1) and account for 34.8% (173/479) of the overall total.

Three of the acquired carbapenemase genes identified in 2020 were KPC types. To date, KPCs have accounted for 3.8% (18/479) of all CPE in New Zealand, of which seventeen were identified in *Klebsiella pneumoniae* and one in an isolate belonging to the *Enterobacter cloacae* complex.

More than one class of carbapenemase was identified in two CPE isolates in 2020 (see Table 1, footnote 1): an *Escherichia. coli* with NDM-5 and OXA-484, and a *K. pneumoniae* with NDM-5 and OXA-232.

Probable place of acquisition of carbapenemase–producing Enterobacterales

Travel history was available for 58 of the 70 patients, and 65.1% of the isolates (41/63) from these patients had been overseas; the Indian subcontinent was the most frequent probable place of acquisition (Table 2).

Of CPE acquired overseas, 41.5% (17/41) were from patients who had been hospitalised, with 16/24 cases not associated with hospitalisation likely acquired on the Indian subcontinent.

Table 2. Probable place of acquisition of carbapenemase-producing Enterobacterales, 2020

Carbapenemase type and subtype	Number of isolates[1]								
	Probable region of acquisition								
	Indian subcontinent	New Zealand[2]	Other parts of Asia[3]	Europe	Overseas[4]	Africa[5]	Eastern Mediterranean	Not known	Total
NDM	**17**	**6**	**1**	**1**	**2**	**0**	**0**	**7**	**34**
NDM-1	1	2	0	1	0	0	0	2	6
NDM-4	1	0	0	0	0	0	0	0	1
NDM-5	12	4	1	0	1	0	0	5	23
NDM-6	1	0	0	0	1	0	0	0	2
NDM-7	2	0	0	0	0	0	0	0	2
OXA-48-like	**10**	**10**	**0**	**2**	**1**	**2**	**1**	**5**	**31**
OXA-48	0	8	0	1	1	0	0	3	13
OXA-181	8	0	0	0	0	2	0	1	11
OXA-244	0	2	0	1	0	0	1	1	5
OXA-484	2	0	0	0	0	0	0	0	2
NDM and OXA-48-like	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**
NDM-5 and OXA-232	1	0	0	0	0	0	0	0	1
NDM-5 and OXA-484	1	0	0	0	0	0	0	0	1
Other carbapenemase	**0**	**6**	**2**	**0**	**0**	**0**	**0**	**0**	**8**
IMP-4	0	2	0	0	0	0	0	0	2
KPC-3	0	1	2	0	0	0	0	0	2
OXA-23	0	3	0	0	0	0	0	0	3
Total	**29**	**22**	**3**	**3**	**3**	**2**	**1**	**12**	**75**

1 Includes multiple isolates from five patients who had two distinct CPE (see Table 1, footnote 1). Four had recent travel to India, with two being hospitalised there. One patient was hospitalised in South East Asia.

2 Includes 11 isolates from five probable CPE cross-transmission events in New Zealand: six *E. coli* isolates with OXA-48, three *E. coli* with NDM-5, one *E. coli* with NDM-1, one *K. pneumoniae* with KPC-2, and one *K. pneumoniae* with NDM-1. The likely source of the other 11 CPE was not determined.

3 All Asia other than the Indian subcontinent.

4 Includes patients that had been to an unknown overseas country (NDM-6), multiple countries in Asia (NDM-5) or multiple continents (OXA-48).

Of the five patients with more than one distinct CPE, two had been in India but were not reported to have been hospitalised there, two were hospitalised in India, and one was hospitalised in South East Asia.

Transmission of carbapenemase–producing Enterobacterales in New Zealand

Twenty-two patients had no history of recent overseas travel, and for 11 of these the source could not be identified. The other 11 isolates were associated with five distinct CPE cross-transmission events, including six isolates containing OXA-48 from a known community cluster that started in August 2018 in the Wellington region (related to a food outlet), and three separate transmission events occurring within households following likely initial introduction from overseas.

In addition, one probable NDM-1 plasmid transmission event occurred between an *E. coli* and a *K. pneumoniae* isolate at North Shore Hospital, as plasmids from the two isolates were indistinguishable when characterised using Illumina and long-read nanopore sequencing.

Resistome

Most isolates with a carbapenemase gene also had a number of other resistance genes present, including genes conferring resistance to aminoglycosides (62, 82.7%), sulphonamides (59, 78.7%), fluoroquinolones (45, 60.0%) and tetracycline (42, 56.0%). Of note, three isolates with *mcr* were identified, all *mcr*-9, present alongside either IMP-4 or KPC-2. Ten isolates contained 16S ribosomal methyl transferases.

Multi-locus sequence types identified

The multi-locus sequence type (MLST) was available for 52 of the 57 *E. coli* with acquired carbapenemase genes. Thirty distinct sequence types were identified. The most common sequence type was ST-131 (8 isolates) and ST-405 (7 isolates). The remaining sequence types had three or less isolates each. All ST-131 isolates contained the OXA-48 carbapenemase gene and included the six isolates associated with the Wellington cluster. All ST-405 isolates contained the NDM-5 carbapenemase gene.

Multi-locus sequence types were available for 13 *K. pneumoniae* isolates, of which 11 different sequence types were represented.

Conclusion

Carbapenem resistance continues to be of concern to New Zealand but the interruption in travel due to the SARS-CoV-2 pandemic has slowed the rate of increase. NDM remains the dominant carbapenemase, followed by OXA-48. Whilst the overall number of CPE isolates has reduced, the proportion of isolates locally acquired within New Zealand has increased as compared to 2019 (34.9% (22/63) compared to 20.6% (20/97)). 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. Future ESR reports should include a summary of molecular relatedness of isolates for better visualisation of clusters and transmission events occurring within New Zealand.

---

[1] Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev 2007; 20: 440-58.
[2] Available at https://github.com/tseemann/nullarbor.
3 Souvorov, A., Agarwala, R. & Lipman, D. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 19, 153 (2018). https://github.com/ncbi/SKESA
4 Available at https://github.com/tseemann/mlst.
5 Available at https://github.com/tseemann/abricate.
6 Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020 Dec 1;75(12):3491-3500.
7 Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014 Jul;58(7):3895-903.