New Zealand Institute
For Public Health and
Forensic Science

# *Pseudomonas* spp. with acquired carbapenemase genes in New Zealand, 2009-2022

This paper summarises the results of the laboratory surveillance undertaken by PHF Science of *Pseudomonas* spp. with acquired carbapenemase genes in Aotearoa New Zealand (NZ) between 2009 and 2022.

**Summary**

- Carbapenem-resistant *Pseudomonas aeruginosa* is a major global health concern and remains a WHO priority pathogen.
- Whilst the burden of carbapenemase-producing *Pseudomonas* (CPP) is lower than carbapenemase-producing Enterobacterales (CPE), this is likely to be an under-estimate and numbers are increasing.
- The metallo-β-lactamase's VIM and NDM are the predominant carbapenemases found in CPP. Their presence significantly limits treatment options due to activity against many β-lactam antibiotics.
- Genomic analyses indicate that whilst there is significant variation and diversity in the CPP identified, many of the globally recognised "high-risk clones" are present in NZ.
- Clustering analyses show there may have been previously unidentified transmission events, that are not able to be linked epidemiologically. This highlights the importance of ongoing genomic surveillance of this hospital-associated pathogen, which can persist in environmental reservoirs.
- Prospective, continuous surveillance must continue in order to promptly identify future transmission events, to initiate prompt intervention to prevent CPP becoming endemic in NZ hospitals.
- Diagnostic laboratories must be supported to continue to refer suspected CPP to PHF Science for characterisation.

**Background**

Acquired carbapenem resistance in gram-negative pathogens is a major, on-going public health concern due to the high morbidity and mortality associated with these infections. Carbapenemase genes confer resistance to carbapenems, that are often reserved for treating severe bacterial infections, and many other β-lactams antibiotics. Carbapenemase genes are commonly found on plasmids, along with genes conferring resistance to multiple other classes of antimicrobials, leading to multidrug-resistance. The presence of multiple resistance genes on the same plasmid, coupled with the plasmid's ability to transfer between bacteria, plays a critical role in the rapid spread of both carbapenem resistance and multidrug resistance.[1]

*Pseudomonas aeruginosa* is an opportunistic pathogen and a leading cause of healthcare-associated infections such as surgical site infections, ventilator-associated pneumonia, catheter-associated urinary tract infections and bloodstream infections.[1] Infections can be difficult to treat because of intrinsic resistance to many commonly used antimicrobial agents as well as acquired resistance through both chromosomal mutations and acquisition of mobile genetic elements (such as plasmids), which can develop quickly. Spread within the hospital setting can occur following breaches in infection prevention practices and because the hospital environment provides a reservoir for persistence, particularly around water-sources such as sinks and other equipment.

The acquired or transferable carbapenemases found in *Pseudomonas* spp. belong to three of the four major classes of β-lactamases.[2]

- Ambler class A includes KPC and GES enzymes.
- Ambler class B are the metallo-β-lactamases (MBLs), which include NDM, VIM and IMP.
- Ambler class D includes the OXA-23, OXA-40 and OXA-51 groups of β-lactamases.

This report summarises the characteristics of *Pseudomonas* spp. with acquired carbapenemase genes, found in New Zealand up to 31 December 2022.

**Methods**

In New Zealand, diagnostic microbiology laboratories are requested to refer all suspected CPP to PHF Science for confirmation and further characterisation. Multiple, distinct CPP 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. CPP referral to PHF Science began in 2009, and this report covers the period from 1 January 2009 to 31 December 2022.

Isolates with a carbapenemase gene detected by PCR by the referring laboratory underwent Illumina-based whole genome sequencing (WGS). Genomic DNA was extracted using the Roche High Pure PCR template preparation kit or the Chemagic 360 (Perkin Elmer), DNA libraries were prepared using the Nextera XT DNA preparation kit (Illumina) or the plexWell Library Preparation kit (SeqWell), and sequencing was performed using Illumina technology.

CPP referred to PHF Science that were carbapenemase PCR negative, or not tested using PCR by the referring laboratory, underwent inhibitor-based phenotypic tests using at least one of the following tests: the modified Hodge test, the 2-mercaptopropionic acid (MPA) inhibitor-based phenotypic test, the carbapenem inactivation test and/or the modified carbapenem inactivation test.[3] If needed, a selection of PCRs were available at PHF Science for the following acquired carbapenemase genes: GES, NDM, IMP, VIM, GIM, SIM, SPM and OXA.[4,5,6,7] A PCR for the chromosomally-located POM gene was also available.[8] Isolates that were positive in at least one carbapenemase screening test or in an acquired carbapenemase PCR were characterised using Illumina-based WGS. Since 2017, isolates that were positive in at least one of the carbapenemase screening tests, but PCR negative, were also characterised using WGS to identify potential carbapenemase genes.

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 SKESA,[9] 7-gene MLST[10] and AMRFinderPlus[11]. Core genome MLST (cgMLST) using Chewbacca[12] was

performed to assess genomic relatedness, and a minimum-spanning tree based on cgMLST allele profiles was built using GrapeTree.[13] The cgMLST schema for *P. aeruginosa* assesses variation in 2419 gene targets[14] and a cgMLST pairwise allelic difference threshold of ≤12 was used to investigate possible clusters.[15]

Basic epidemiological data were requested from referring laboratories for patients with confirmed CPP, including risk factors such as overseas travel and hospitalisation history.

**Limitations**

CPP are not notifiable in New Zealand, and isolates are referred to PHF Science on a voluntary basis. Consequently, there is likely to be an under ascertainment of cases in this cohort and epidemiological data is more limited compared with notifiable diseases. The analysis of genomic relatedness using WGS data was not done in real time until approximately 2019, limiting our ability to identify epidemiological linkages between cases.

**Results**

A total of 52 carbapenemase-producing *Pseudomonas* spp. were received at PHF Science between 2009 and 2022. Of these, 45 were non-duplicate isolates (Table 1) from 44 patients. A further ten isolates contained a carbapenemase gene naturally found in *Pseudomonas* spp, including seven isolates with POM-1, two isolates with PAM-1 and one isolate likely to contain the chromosomally-encoded GES-20.[a] The seven isolates with POM-1 were either confirmed or suspected as being *Pseudomonas otitidis*. All 45 non-duplicate *Pseudomonas* spp. with an acquired carbapenemase gene were *Pseudomonas aeruginosa*, except for one *Pseudomonas putida* isolate with DIM-1. All carbapenemases identified were MBLs, except for one GES-5 carbapenemase found in 2017 (Table 1 and Figure 1).

[a] Sequencing data from the GES PCR amplicon showed single-nucleotide polymorphisms at two sites. The ambiguity at these two sites is fully explained by the presence of both the ESBL GES-19 and the carbapenemase GES-20. Isolates with these genes have been reported in Mexico, which had chromosomally-located copies of GES-19 and GES-20.[15]

**Table 1. Carbapenemase types and probable place of acquisition of carbapenemase-producing *Pseudomonas* spp., 2009-2022**

| Carbapenemase type & subtype | Probable region of acquisition | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Indian subcontinent | New Zealand | Other parts of Asia[1] | Western Pacific | Europe | Africa | Unknown[2] | Americas | Eastern Mediterranean | Total |
| **VIM** | **7** | **8** | **2** | **0** | **3** | **1** | **1** | **0** | **1** | **23** |
| VIM-2 | 5 | 7 | 2 | 0 | 3 | 1 | 1 | 0 | 1 | 20 |
| VIM-4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| VIM-5 | 2 | 0 | 0 | **0** | **0** | 0 | 0 | 0 | 0 | 2 |
| **NDM** | **5** | **1** | **1** | **2** | **0** | **1** | **0** | **0** | **0** | **10** |
| NDM-1 | 5 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 10 |
| **IMP** | **0** | **1** | **4** | **0** | **0** | **0** | **1** | **0** | **0** | **6** |
| IMP-1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| IMP-7 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| IMP-13 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| IMP-14 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| IMP-26 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Other genes** | **0** | **0** | **1** | **2** | **0** | **0** | **0** | **0** | **0** | **3** |
| DIM-1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| GES-5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Multiple genes** | **2** | **0** | **0** | **0** | **0** | **0** | **0** | **1** | **0** | **3** |
| NDM-1 & DIM-1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NDM-1 & VIM-2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| IMP-18 & VIM-2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| **Total** | **14[3]** | **10** | **8** | **4** | **3** | **2** | **2** | **1** | **1** | **45** |

1 All Asia other than the Indian subcontinent.
2 Isolates from two patients where the travel history was not reported.
3 Includes one patient with a two CPP, one with NDM-1 and one with VIM-2.

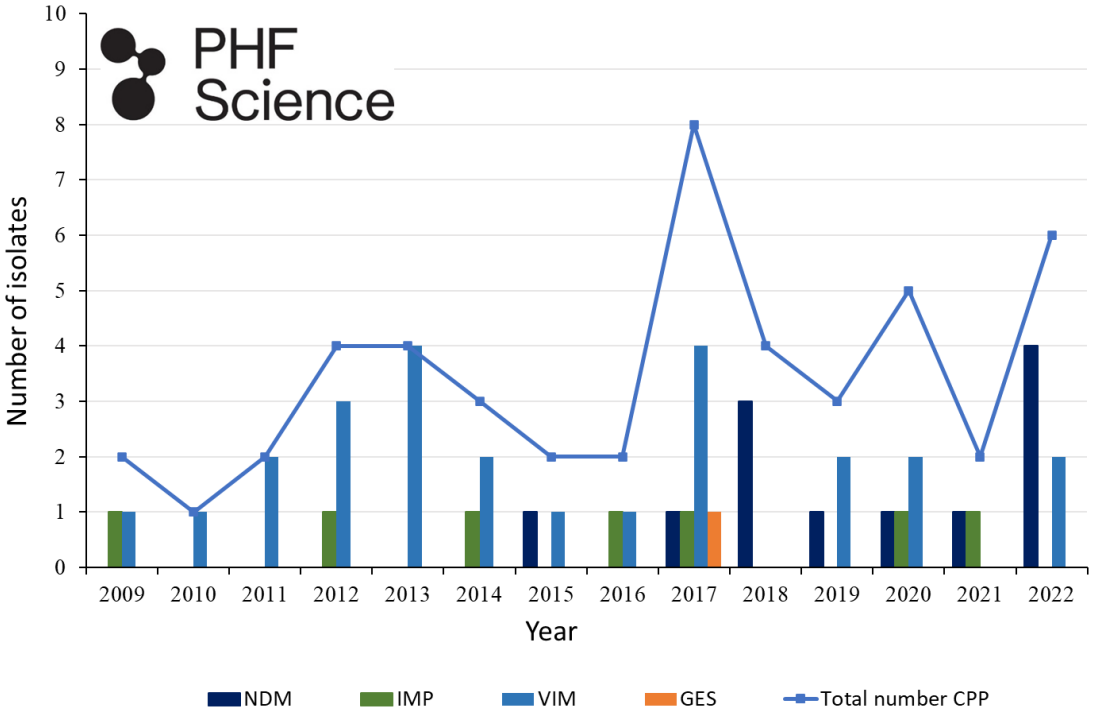


**Figure 1. Number of carbapenemase-producing *Pseudomonas* spp. identified in New Zealand, by carbapenemase class, from 2009 to 2022**

Laboratories from the Auckland region referred the majority of CPP (28, 62.2%), followed by Canterbury region (6, 13.3%) and the Bay of Plenty (3, 6.7%). Of the 45 isolates, 35 (77.8%) were from clinical samples with 17 (37.8%) from urine, seven (15.6%) from sterile sites, six (13.3%) from skin and soft tissue infections, and five (11.1%) from sputum. Ten isolates (22.2%) were from screening specimens.

Most (48.9%, 22/45) isolates were from people 65 years or over, with 12 (26.7%) from patients aged 45-64, nine (20.0%) from patients 15-44 years and two (4.4%) from people under 15 years. Most isolates were found in males (33/45, 73.3%).

The most frequently identified carbapenemase genes have been various subtypes of VIM. VIM-2 is predominant, although NDM-1 has become more prominent since 2015, and was the most common carbapenemase gene found in 2022. VIM carbapenemases were found in 55.5% of isolates (25/45: 23 isolates with VIM only and two isolates with two multiple MBL genes) (Table 1). The NDM-1 carbapenemase was the next most frequent, accounting for 26.7%

(12/45) of carbapenemase genes in CPP, followed by IMP carbapenemases that accounted for 15.6% (7/45) of carbapenemase genes in CPP (Table 1).

Travel history was available for 42 of the 44 patients, and of these 76.2% (32/42) had recent overseas travel history (Table 1). The greatest percentage of patients who had travelled overseas had recent travel to the Indian subcontinent (13/32, 40.6%), followed by other parts of Asia (8/32, 25.0%). Of the patients who had travelled overseas, 62.5% (20/32) had been hospitalised while out of New Zealand, most commonly on the Indian subcontinent (9/20, 45.0%) and other parts of Asia (5/20, 25.0%).

Ten CPP isolates were obtained from patients with no recent international travel, with seven of these isolates containing VIM-2. No epidemiological linkages between any patients with CPP were reported, including those with no recent international travel. However, as CPP are not notifiable, there is likely to be missing epidemiological information. Furthermore, much of this work was carried out retrospectively, which increases the difficulty of epidemiological investigations. Ongoing prospective cluster analysis is important for timely identification of transmission events and hidden environmental reservoirs.

CPP were often isolated from patients along with other carbapenemase-producing organisms (CPO), including CPE and carbapenem resistant *Acinetobacter baumannii*. Twelve patients with a CPP had least one other CPO. Five patients had two distinct CPO and seven patients had between three and seven[16] different CPO. All patients with multiple CPO had recent overseas travel history, and all except two patents were hospitalised while overseas. The two patients who had not been hospitalised travelled to the Indian subcontinent. The regions where patients with multiple CPO travelled were: India (7), Other parts of Asia (2), Africa (1), Europe (1), and the Western Pacific (1).

***Resistome***

All CPP were likely multidrug resistant, as they contained resistance genes that confer resistance to three or more antimicrobial classes (Table 2). The genes found included those conferring resistance to: fosfomycin (42, 93.3%), phenicols (41, 91.1%), aminoglycosides (34, 75.6%), tetracycline (26, 57.8%), trimethoprim (25, 55.6%), sulphonamides (19, 68.9%) and fluoroquinolones (9, 20.0%). Three isolates (6.7%) had an ESBL gene and 42 (93.3%) had an AmpC. No *mcr* genes, conferring resistance to colistin, were identified but five isolates (11.1%) contained a 16S ribosomal methyl transferase gene. All isolates contained *mex* gene(s) that are required for Mex-Opr multidrug efflux pumps. These pumps are prevalent in *P. aeruginosa* and contribute to efflux-mediated antibiotic resistance.

**Table 2: Multi-resistance found in carbapenemase-producing *Pseudomonas* spp. found in New Zealand 2009-2022**

| Isolates with resistance genes that confer resistance to:[1] | | | | | No. of isolates | Percent (%) |
|---|---|---|---|---|---|---|
| Amino-glycosides | Fluoro-quinolones | Fosfomycin | Phenicols | Sulphonamides | | |
| Yes | No | Yes | Yes | Yes | 19 | 42.2 |
| Yes | No | Yes | Yes | No | 9 | 20.0 |
| Yes | Yes | Yes | Yes | Yes | 5 | 11.1 |
| No | No | Yes | Yes | No | 3 | 6.7 |
| No | No | Yes | Yes | Yes | 2 | 4.4 |
| Yes | Yes | Yes | No | Yes | 1 | 2.2 |
| Yes | Yes | No | No | Yes | 1 | 2.2 |
| Yes | No | No | No | Yes | 1 | 2.2 |
| No | Yes | Yes | Yes | No | 1 | 2.2 |
| Yes[2] | No | Yes | Yes | Yes | 1 | 2.2 |
| Yes* | Yes | Yes | Yes | No | 1 | 2.2 |
| No | No | No | No | Yes | 1 | 2.2 |

1 Data presented for a subset of antimicrobial classes.

2 Includes isolates with 16S ribosomal methyl transferase genes

***Genetic relatedness of carbapenemase-producing Pseudomonas spp.***

The 7-gene multi-locus sequence type (MLST) was available for 34 of the 45 *Pseudomonas* spp. with acquired carbapenemase genes. Data for the other isolates failed QC or had missing data for at least one of the MLST loci. For isolates with MLST data, 12 different sequence types were identified. The most common sequence types were ST233 (10 isolates), ST235 (5 isolates) and ST357 (4 isolates), all recognised global "high-risk clones" associated with drug resistance and worldwide dissemination.[17] The remaining sequence types were found in less than four isolates each. All ten ST233 isolates contained VIM-2, all three isolates with ST773 contained NDM-1, both ST316 isolates contained NDM-1, and both ST823 isolates contained VIM-2. The other sequence types with multiple isolates contained different carbapenemase genes.

WGS data was used to investigate the genomic relatedness of isolates (Figure 2). Three clusters were identified when a threshold of 12 cgMLST alleles was used:

- One cluster involved four isolates, found between 2009-2013. The isolates were from cases from the Auckland region with no recent international travel reported. All isolates were ST233 with VIM-2, but differed at four to seven cgMLST loci. The genetic similarity between these isolates suggests the cases may have been exposed to a common source in New Zealand. However, identification of this cluster was not in real time and a detailed epidemiological investigation has not been able to be carried out.
- The second cluster involved two isolates that were ST233, contained VIM-2, and differed from each other at nine cgMLST loci. The isolates were found in 2012 and 2013 and referred to PHF Science from MedLab Central, in the North Island of New Zealand, and Canterbury Southern Community Laboratories, located in the South Island. Both isolates were from patients who reported recent travel to the UK.
- The third cluster involved two ST773 isolates with NDM-1 that differed at nine cgMLST loci. One isolate was cultured from a patient in 2018 who had been hospitalised in the Indian subcontinent and the second was cultured from a patient in 2020 who had no recent travel history. Both isolates were from cases in the Auckland region.

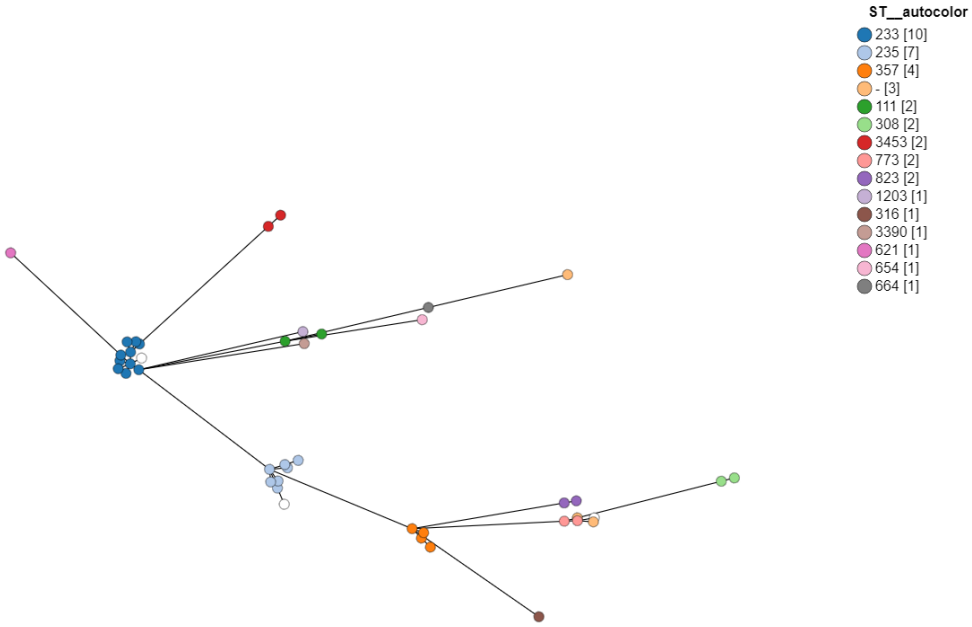


**Figure 2. Genomic relatedness of carbapenemase-producing *Pseudomonas* spp. identified in New Zealand, by carbapenemase class, from 2009 to 2022**

The 12 cgMLST threshold is likely to be conservative, and isolates that differ by less than 12 alleles may not have been transmitted directly between patients. There was no epidemiological evidence provided with the samples to indicate that transmission of CPP had occurred in New Zealand. Cluster analysis has been performed retrospectively and therefore potentially linked cases were not able to be followed up with referring laboratories and health care facilities in real time, so there may be missing epidemiological data.

## Conclusion

Carbapenem resistance continues to be of concern to New Zealand. Whilst the majority of CPO in New Zealand to date have been Enterobacterales (CPE), with over 550 isolates found between 2009 and 2022, the numbers of CPP have increased over time. The burden of CPP is also likely to be an under-estimate since routine screening for multidrug resistant organisms in hospitals may not universally include testing for CPP. This is reflected in the fact that CPP are more likely than CPE to be found in clinical specimens, rather than screening specimens.

The majority of acquired carbapenemase genes found in *Pseudomonas* spp. have been various subtypes of VIM. However, the number of isolates with NDM

genes has increased in recent years, and in 2022 the number of NDM was greater than the number of isolates with VIM. An increase in NDM genes in CPP has also been observed in Australia.[18]

The resistome from the CPP isolates described in this report suggests that isolates are highly multidrug resistant, across multiple antimicrobial classes, with limited treatment options available. This is of major concern since treatment of CPP infections with VIM and NDM rely on access to antibiotics such as cefiderocol, which remain unfunded, not readily available and difficult to access in New Zealand.

Genomic analysis confirms that the internationally recognised high-risk clones, responsible for successful global dissemination of this highly drug-resistant nosocomial pathogen, are already present in NZ.

Most CPP identified in New Zealand, are thought to be acquired overseas, however transmission of CPP in New Zealand cannot be discounted. Genomic analysis contained in this report has identified three possible, previously unidentified, clusters. One of these clusters involved patients that had recent international travel to the same region, and it is possible that they acquired their genetically similar CPP independently while overseas. A further two clusters involve at least one patient without reported international travel, and the source of these CPP in these patients is not known. A detailed epidemiological investigation was outside the scope of this study, although there is no epidemiological evidence of transmission for any of the clusters.

The possibility that transmission of CPP has occurred in New Zealand highlights the important role of ongoing genomic surveillance of CPP to identify clusters and potential nosocomial acquisition in real-time. If left unrecognised, such transmission events could potentially lead to larger transmission chains and outbreaks with significant adverse consequences. Sporadic and low-frequency transmissions from the environment may be difficult to link epidemiologically due to hidden reservoirs or complex epidemiological links from unidentified colonised source patients.[15]

In conclusion, ongoing vigilance and early detection, plus prospective

surveillance and epidemiological study of CPP is critical to guide appropriate infection prevention strategies for control of this important pathogen. Diagnostic laboratories must be supported to maintain methods for early detection of CPP, and for referral of suspected CPP isolates to PHF SCIENCE for molecular characterisation. This will aid the timely identification of transmission events and help prevent CPP becoming endemic in NZ healthcare environments.

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