CASE STUDY

# An isolate of ST235 Pseudomonas aeruginosa harbouring IMP-26 in New Zealand

*Sean Munroe, Hermes Pérez Cardona , Kristin Dyet and Julia Howard*

## ABSTRACT

*Pseudomonas aeruginosa* is an important cause of nosocomial infections worldwide. Worryingly, many *P. aeruginosa* are multidrug -resistant, and the carbapenem-resistant *P. aeruginosa* have been recognised as a global threat by the World Health Organisation (WHO).A prevalent international clone of *P. aeruginosa* is ST235, which is associated with high-level antibiotic resistance and poor clinical outcomes. We report an isolate of ST235 *P. aeruginosa* harbouring IMP-26, which to the best of our knowledge is the first case of in New Zealand, in a patient with no history of overseas travel.

**Keywords:** *Pseudomonas aeruginosa*, Carbapenemase, IMP-26.

***N Z J Med Lab Sci 2022; 76(2): 84-85.***

## INTRODUCTION

*Pseudomonas aeruginosa* is an important cause of nosocomial infections worldwide (1). These infections can be difficult to treat due to isolates of *P. aeruginosa* possessing intrinsic resistance, and the ability of the organism to acquire further resistance to multiple classes of antibiotic agents (2).The most prevalent and widespread clone of *P. aeruginosa* worldwide is ST235, and this clone has the potential to become readily resistant to aminoglycosides, beta-lactams and carbapenems (3).Carbapenem-resistant *P. aeruginosa* is recognised as a global threat by the World Health Organisation (WHO). It is listed as one of three bacteria with critical priority for antibiotic research therapy due to the increased spread of these strains (4).

## CASE REPORT

In September 2020 in Hamilton, NZ, we isolated a *P. aeruginosa*, identified by the Vitek MALDI-TOF (BioMérieux), from the abdominal drain fluid of an inpatient who had no history of overseas travel. Disc (Oxoid) susceptibility testing showed the isolate was resistant to ceftazidime, cefepime, piperacillin-tazobactam, meropenem, gentamicin, tobramycin and ciprofloxacin but susceptible to aztreonam and amikacin (Table 1.) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. The isolate was resistant to ceftazidime-avibactam and ceftolozane-tazobactam (Liofilchem MIC Strip) and susceptible to colistin (broth microdilution) (Table 1.) Phenotypic screening for carbapenemase activity was performed by CARBA PAcE (MAST Group) and was positive within 10 minutes. Based on this a GeneXpert Carba R (Cepheid) was performed, which revealed the isolate possessed the $bla_{IMP}$ gene.

Genomic DNA was extracted using the Roche High Pure PCR template preparation kit, the DNA library was created using the Nextera XT DNA preparation kit (Illumina), and sequencing was performed using Illumina technology. Whole Genome Sequencing (WGS) data was analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. Open-source packages used included the Nullarbor (https://github.com/tseemann/nullarbor): 'Reads to report' for public health and clinical microbiology pipeline. WGS data was analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. Open-source packages used included the Nullarbor2: 'Reads to report' for public health and clinical microbiology pipeline SKESA v.2.3.0 (https://github.com/ncbi/SKESA), MLST (https://github.com/tseemann/mlst) and ABRicate (https://github.com/tseemann/abricate) using ResFinder and PlasmidFinder databases. The isolate was sequenced with a depth of 93x. Analysis of the *de novo* assembly of the WGS reads identified the *bla*IMP-26 gene and showed that the isolate was ST235.

**Table 1.** Susceptibility profile.

| Antibiotic | Interpretation (Zone size/MIC) |
|---|---|
| Amikacin | Susceptible (19mm) |
| Aztreonam | Susceptible (20mm) |
| Cefepime | Resistant (6mm) |
| Ceftazidime | Resistant (6mm) |
| Ceftazidime-avibactam | Resistant (>256 mg/L) |
| Ceftolozane-tazobactam | Resistant (>256 mg/L) |
| Ciprofloxacin | Resistant (6mm) |
| Colistin | Susceptible (1.0 mg/L) |
| Gentamicin | Resistant (6mm) |
| Meropenem | Resistant (>32 mg/L) |
| Piperacillin-tazobactam | Resistant (19mm) |
| Tobramycin | Resistant (6mm) |

## DISCUSSION

IMP-26 producing *P. aeruginosa* was first described in Singapore in 2009, (5) and then subsequently described from retrospective analysis of clinical samples from Malaysia and the Philippines (6,7), as well as in Vietnam, where isolates had spread in a medical setting (8). It has also been found in clinical samples from Uganda (9). IMP-26 has also been found in other Gram-negative bacteria, such as in *Enterobacter cloacae* in China (10).

Carbapenemase producing *P.aeruginosa* are infrequently isolated in New Zealand. The first *P. aeruginosa* harbouring a carbapenemase gene in New Zealand was isolated in 2009. Between 2009 and December 2021 39 isolates of *P. aeruginosa* harbouring acquired carbapenemase genes have been submitted to ESR. Metallo-beta lactamases have been the most common gene type identified, with VIM the most predominant, at the time of writing (ESR data).

This is the first known case of the IMP-26-producing *P. aeruginosa* in New Zealand. To date, most isolates with acquired carbapenemase genes in New Zealand are thought to have been acquired overseas, so it is of concern that this isolate with IMP-26 was found in a patient with no history of overseas travel and no history of travel from family in the same household. Only six other *P. aeruginosa* isolates possessing

IMP beta-lactamase genes have been reported in New Zealand (unpublished data). Travel history was reported for five of the cases, indicating they were likely to have been acquired in an overseas hospital (China, Thailand (three cases) and Peru).

Patient screening for carbapenemase-producing *P. aeruginosa* can be challenging for laboratories, as highlighted by the WHO Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *P. aeruginosa* in health care facilities (11). These guidelines stopped short of recommending active surveillance for carbapenem-resistant *P. aeruginosa* as whilst it may be beneficial, the potential benefit is dependent on clinical setting, epidemiological stage and body sites sampled. Unlike carbapenemase-producing Enterobacterales (CPE) where faecal material or rectal swabs were considered the best methods for surveillance, detection of carbapenem-resistant *P. aeruginosa* carriage may be improved by the addition of urine and pharyngeal swabs as well (12).

Interestingly, our patient had a rectal screening swab collected during his hospital stay and P. aeruginosa was not isolated, but subsequent abdominal drains continued to grow the IMP-producing *P. aeruginosa*.

In conclusion, to the best of our knowledge this the first case of IMP-26 producing *P. aeruginosa* in New Zealand, from a patient with no overseas travel history. Active surveillance and outbreak screening for organisms such as these may prove difficult for laboratories in the absence of official recommendations of appropriate sites and methods.

## AUTHOR INFORMATION

Sean Munroe, BMLSc, Technical Specialist[1]
Hermes Pérez Cardona, MEng, Bioinformatician[3]
Kristin Dyet, PhD, Senior Scientist[2]
Julia Howard, FRCPA FRCPath, Clinical Microbiologist[1,4]

[1]Microbiology Department, Waikato Hospital, Hamilton, New Zealand
[2]Antibiotic Reference Laboratory, Institute of Environmental Science and Research (ESR) Wellington, New Zealand
[3]Pathogen Genomics, ESR Wellington, New Zealand
4Microbiology Department, Canterbury Health Laboratories, Canterbury District Health Board, Christchurch, NZ

**Corresponding Author:** Sean Munroe
Email: SeanEdward.munroe@waikatodhb.health.nz

## REFERENCES

1. Blanc DP, Petignat C, Janin, B, et al. Frequency and molecular diversity of Pseudomonas aeruginosa upon admission and during hospitalization: a prospective epidemiologic study. *Clin Microbiol Infec*; 1998; 4: 242-247.
2. Strateva T, Yordanov D. Pseudomonas aeruginosa - a phenomenon of bacterial resistance. *J Med Microbiol*. 2009; 58: 1133-1148.
3. Treepong P, Kos VN, Guyeux, C, et al. Global Emergence of widespread Pseudomonas aeruginosa ST235 clone. Clin Microbiol Infect 2018; 24: 258–266 .
4. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organisation* 2017; *https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf*
5. Koh TH, Khoo CT, Tan TT, et al. Multilocus sequence types of carbapenem-resistant Pseudomonas aeruginosa in Singapore carrying metallo-beta lactamase genes, including the novel bla(IMP-26) gene. *J Clin Microbiol* 2010; 48: 2563-2564.
6. Kim MJ, Bae IK, Jeong SH, et al. Dissemination of metallo-β-lactamase producing Pseudomonas aeruginosa of sequence type 235 in Asian countries, *J Antimicrob Chemother* 2013; 68: 2820-2824.
7. Kazmlerczak KM, Rabine S, Hackel M, et al. Multiyear, multinational survey of the incidence and global distribution of metallo-β-lactamase-producing Enterobacteriaceae and Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2015; 60: 1067-1078.
8. Tada T, Nhung PH, Miyoashi-Aklyama T, et al. Multidrug-resistant sequence type 235 Pseudomonas aeruginosa clinical isolates producing IMP-26 with increased carbapenem-hydrolyzing activities in Vietnam. *Antimicrob Agents Chemother* 2016; 60: 6853-6858.
9. Kateete DP, Nakanjako R, Namugenyi J, et al. Carbapenem resistant Pseudomonas aeruginosa and Acinetobacter *baumannii* at Mulago Hospital in Kampala, Uganda (2007-2009). *SpringerPlus* 2016; 5:1308.
10. Wang S, Zhou K, Xiao S, et al. A multidrug resistance plasmid pIMP26, carrying blaIMP-26, fosA5,blaDHA-1, and qnrB4in Enterobacter cloacae. *Sci Rep* 2019; 9(1): 10212.
11. Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities. Geneva: *World Health Organization*; 2017. Licence: CC BY-NC-SA 3.0 IGO.
12. Araoka H, Kimura M, Abe M, et al. Appropriate sampling sites for the surveillance of multidrug-resistant Pseudomonas aeruginosa colonisation. *Jpn J Infect Dis* 2014; 67: 118-119.