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Dual VIM-Producing *Proteus mirabilis*, Including a Novel VIM-75, Among Elderly Patients in a Medical Center from Hungary: Report from the 2020 SENTRY Antimicrobial Surveillance Program

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Introduction

- *Proteus mirabilis* is often involved in urinary tract infections and is naturally resistant to several antimicrobial agents, including colistin.
- Decreased susceptibility to imipenem commonly occurs in *P. mirabilis* isolates due to the acquisition of carbapenemase genes as well as the loss of porins and the reduced expression of penicillin-binding proteins (PBPs) PBP1a and PBP2; however, resistance to other carbapenems can occur due to acquisition of carbapenemases.
- Carbapenemases of the Verona integron-borne metallo-β-lactamase (VIM) family are among the most widespread globally, with >75 VIM variants reported so far.
- We identified an outbreak of *P. mirabilis* isolates carrying 2 distinct bla_{VIM} genes each, among elderly patients from a medical center in Hungary.

Materials and Methods

- A total of 16 *P. mirabilis* isolates were received from Hungary during 2020 as part of the SENTRY Antimicrobial Surveillance Program.
- Isolates were susceptibility tested by reference broth microdilution as described by the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) and M100 (2022) documents.
  - Quality control (QC) was performed according to the CLSI M100 (2022) criteria.
- Carbapenem-nonsusceptible isolates were submitted to whole genome sequencing and analysis.
  - Total genomic DNA was used as input material for library construction.
  - DNA libraries were prepared using the Nextera XT™ library construction protocol and index kit (Illumina, San Diego, CA, USA).
  - DNA libraries were sequenced on a MiSeq Sequencer using MiSeq Reagent Kit v3 (600 cycle; Illumina).
  - FASTQ format sequencing files for each sample were assembled independently using *de novo* assembler SPAdes 3.13.0.
- An in-house designed software was used for *in silico* screening of resistance genes from the assembled contigs.
- Isolates were evaluated for core genome multi-locus sequence typing (cgMLST) on the 1928 Diagnostics (https://www.1928diagnostics.com/) platform to decipher epidemiologic relationships.
  - cgMLST phylogenetic tree was generated using the UPGMA method that uses a reference genome and genes that are found in at least 95% of the sample genomes. The analysis considered how many of the core genes differ between samples.

Results

- Among the 16 *P. mirabilis* isolates from Hungary, 5 carbapenem-nonsusceptible, multidrug-resistant isolates were identified.
  - 3 isolates were isolated from urinary tract infections and 1 each from bloodstream infection and pneumonia.
  - Carbapenem-nonsusceptible *P. mirabilis* isolates were recovered from elderly patients (range 66–92 years old, median 85 years old) between February and March 2020.
- All isolates were resistant to ceftriaxone, cefepime, imipenem, gentamicin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, and plazomicin (Table 1).
  - These isolates were susceptible to meropenem (0.25–0.5 mg/L) and ertapenem (0.03–0.25 mg/L).
- Sequencing analysis revealed that these isolates carried bla_{VIM-4} and bla_{VIM-75} on a class 1 integron within *IS26* and were separated by *aac(6')-IIc* (Figure 1).
  - *IS26* was located on a compound plasmid carrying other resistance-encoding genes, including *armA*.
  - The integron structure showed 99% identity to the bla_{VIM-1} and bla_{VIM-4}–carrying integron located on an IncA/C plasmid described from a *Vibrio cholerae* isolated from a seagull in France in 2015 (GenBank accession number KR262557).
    - bla_{VIM-75} is a single amino acid variant (Q60R) of bla_{VIM-1}.
- These isolates also carried $bla_{CTX-M-15}$ and many other resistance-encoding genes, including *aac(3)-IId*, *aac(6`)-Iic*, *ant(3``)-Ia*, *aph(3`)-Ia*, *aph(6)-Ia*, *aph(6)-Id*, *catA1*, *dfrA1*, *mph(E)*, *msr(E)*, *sul1*, *sul2*, and bla_{TEM-1}.
- Mutations in the quinolone resistance-determining regions of the DNA gyrase (GyrA S83I) and Topoisomerase IV (ParC S84I) genes were present in all isolates.
  - Additionally, alterations in GyrB (E466D) and ParE (K84E, S459A) were identified.
- Based on cgMLST analysis, these 5 *P. mirabilis* isolates were considered highly similar genetically.
  - Only 7–19 SNPs were detected among the core genome sequences evaluated (Figure 2).

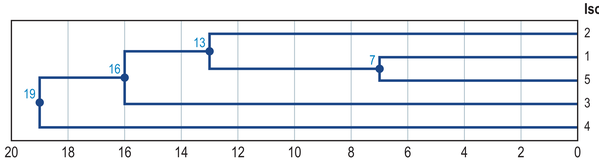
Table 1. Antimicrobial susceptibility of *Proteus mirabilis* isolates

Isolate #	MIC (mg/L)															
	CAZ	CRO	CPM	AZT	CAZ-AVI	IPM	ERT	MEM	MEM-VAB	LEV	NIT	PIP-TAZ	AMK	TOB	TIG	SXT
1	>32	>8	16	2	>32	>8	0.12	0.25	0.25	16	>64	64	>32	>16	2	>4
2	>32	>8	>32	8	>32	>8	0.06	0.5	0.5	>32	>64	>128	>32	>16	4	>4
3	>32	>8	32	8	32	>8	0.25	0.25	0.25	>32	>64	64	>32	>16	2	>4
4	32	>8	32	4	16	>8	0.03	0.5	0.5	>32	>64	32	>32	>16	4	>4
5	>32	>8	32	4	>32	>8	0.03	0.5	0.5	32	>64	>128	>32	>16	1	>4

CAZ, ceftazidime; CRO, ceftriaxone; CPM, cefepime; AZT, aztreonam; CAZ-AVI, ceftazidime-avibactam; IPM, imipenem; ERT, ertapenem; MEM, meropenem; MEM-VAB, meropenem-vaborbactam; LEV, levofloxacin; NIT, nitrofurantoin; PIP-TAZ, piperacillin-tazobactam; AMK, amikacin; TOB, tobramycin; TIG, tigecycline; SXT, trimethoprim-sulfamethoxazole.

Figure 1. Genetic context of bla_{VIM-4} and bla_{VIM-75}–carrying integron in *P. mirabilis* isolates

Figure 2. cgMLST analysis of VIM-4 and VIM-75–producing *P. mirabilis* isolates



Isolate Sr #	Medical service	Infection type	Culture date
2	Emergency	UTI	Feb 2020
1	Intensive care unit	PHIP	Feb 2020
5	Emergency	BSI	Feb 2020
3	Emergency	UTI	Feb 2020
4	Long-term care	UTI	Mar 2020

Abbreviations: PHIP pneumonia in hospitalized patients; UTI, urinary tract infection; BSI, bloodstream infection

Conclusions

- The outbreak of multidrug-resistant *P. mirabilis* isolates carrying bla_{VIM-4} and bla_{VIM-75} in a Hungarian medical center is concerning.
- Four out of 5 isolates were listed as non-nosocomial, indicating presence of a community reservoir.
- Despite carrying two VIM carbapenemases the *P. mirabilis* isolates remained susceptible to meropenem and ertapenem.
- Surveillance should continue for imipenem-resistant *P. mirabilis* to understand the spread of and treatment options for infections caused by these pathogens.

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