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Novel reservoirs of metallo-β-lactamases in soil bacteria

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BACKGROUND

- Metallo-beta-lactamases (MBLs) confer resistance to last resort beta-lactam antibiotics such as imipenem and meropenem used to treat life-threatening infections by Gram-negative bacteria [1].
- New classes of MBLs are continuously emerging in clinical bacteria from unknown sources [2].
- Soil bacteria are considered as an important sources of antibiotic resistance genes [3,4].

OBJECTIVE

The aim of this study was to explore the occurrence of MBLs in soil bacteria and to elucidate the evolutionary relationships between MBLs occurring in soil and in clinical bacteria.

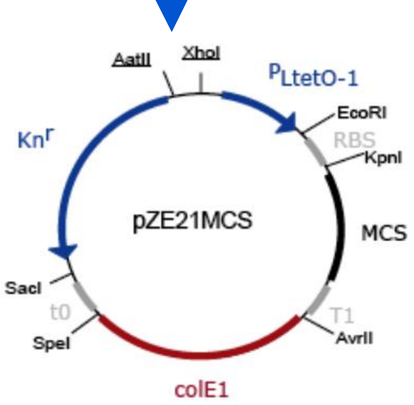
METHODS

Bacteria were isolated from 30 different soil types obtained from different geographical origin.

Selective agents (µg/ml)
- Meropenem (4)
- Cycloheximide (100)
- Vancomycin (8)

16S rDNA sequencing was used for taxonomic characterization.

- Carbapenemase production was tested by carbapenem hydrolysis test (CarbaNP test).
- Minimum inhibitory concentration (MIC) of meropenem and MBL activity were determined by E-test.



- MBL-encoding genes were detected by cloning and whole genome sequencing, followed by bioinformatics analysis.

- Reference strains with > 95% 16S rDNA similarity to the MBL-producing bacteria from soil were tested for carbapenemase production.

RESULTS

Isolation of meropenem-resistant bacteria and identification of new MBLs

Meropenem-resistant bacteria (n=152) were isolated from all soil samples

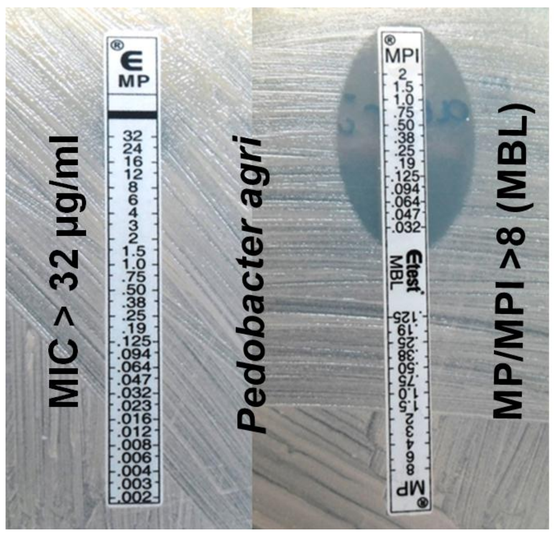


Fig. 1. MBL confirmatory test

- 20% of the soil isolates were confirmed to produce MBLs **(Fig. 1)**.
- Novel MBL-encoding genes were detected in:
  - *Pedobacter agri* (bla_{PGR-1})
  - *Pedobacter roseus* (bla_{PEDO-1})
  - *Chryseobacterium scophthalmum* (bla_{CSP-1})
  - *Epilithonimonas tenax* (bla_{TEN-1})
  - *Sphingomonas* spp. strain SH (bla_{SPG-1})
  - *Massilia timonae* (bla_{MSI-1})

- These new MBL-encoding genes were distantly related to the ones encountered in clinically-relevant Gram-negative spp. **(Fig. 2)**.
- The MIC of meropenem increased 3-fold (0.094 µg/ml) by bla_{PEDO-1} and bla_{CSP-1} expression and 15-fold (0.5 µg/ml) by bla_{TEN-1} expression in the TOP10 *Escherichia coli* used for cloning.

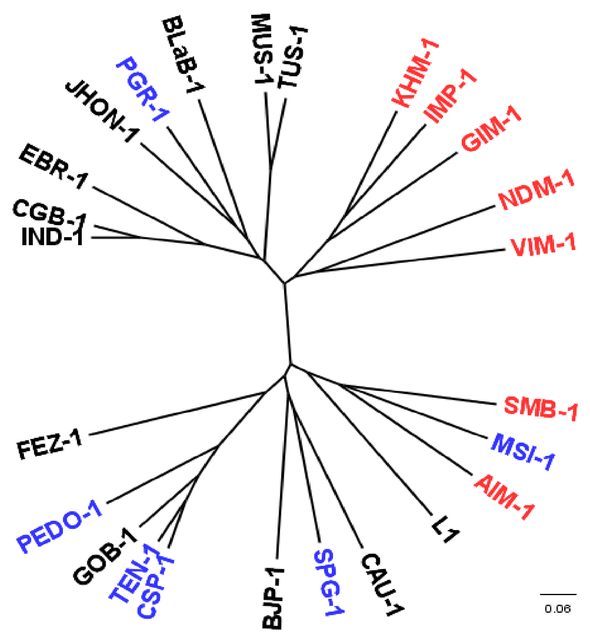


Fig. 2. Phylogenetic tree of constitutive (black), acquired (red) and new MBLs (blue)

Genetic organization of selected MBLs

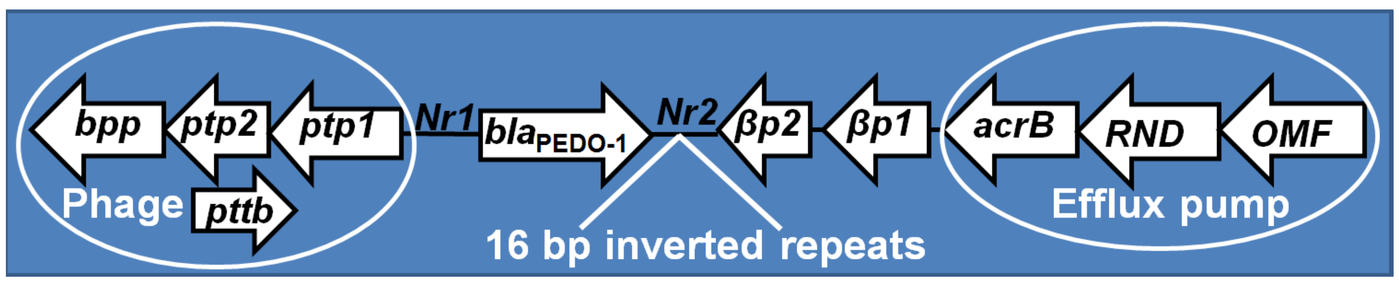


Fig. 3. bla_{PEDO-1} is associated with putative phage protein- and efflux pump protein-encoding genes suggesting that it is an acquired MBL.

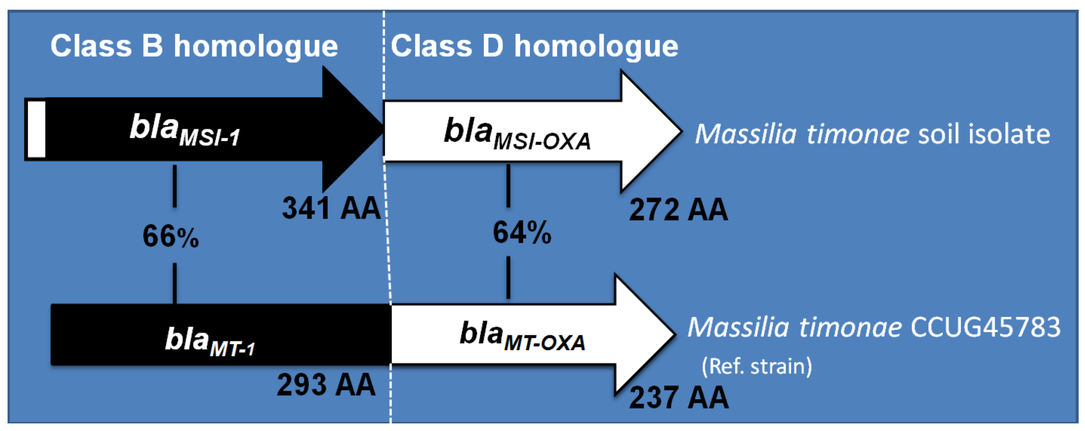


Fig. 4. *Massilia timonae* soil isolate harbors overlapping β-lactamases.

CONCLUSIONS

- **MBL producers are widespread in soil.**
- **The novel MBL-encoding genes in soil bacteria are mainly harbored on the chromosome and distantly related to those occurring in clinically-relevant Gram-negative spp.**
- **The MBLs from soil bacteria confer reduced susceptibility when expressed in *E. coli*, thus constituting potential sources of carbapenem resistance in clinical strains.**

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