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Preferential Selection of IMP and VIM Metallo-β-Lactamases by Imipenem in *Pseudomonas aeruginosa*

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Key Words
Competition studies · Carbapenems · Metallo-β-lactamase

Abstract
Mixed populations of *Pseudomonas aeruginosa,* comprising a carbapenem-susceptible and a carbapenem-resistant strain, containing either the IMP or VIM β-lactamases, were exposed to imipenem. They developed resistance to both imipenem and meropenem. When the same mixed populations were exposed to meropenem, only the mixture containing the VIM β-lactamases became resistant to both carbapenems.

Introduction

Pseudomonas aeruginosa is capable of causing many types of infection and is a potent source of hospital-acquired infection. In many cases the causative organism is resistant to a wide range of antimicrobial agents, which reduces the number of therapeutic options. *P. aeruginosa* also has the ability to develop resistance simultaneously to more than 1 class of antibiotics, such as the fluoroquinolones and carbapenems [1]. The carbapenems imipenem and meropenem have excellent activity against this pathogen; however, acquisition of the transferable metallo-β-lactamase groups VIM and IMP can render these bacteria carbapenem resistant. The β-lactamase genes bla_{IMP} and bla_{VIM} were first described in 1991 and 1999, respectively [2, 3]. Since then they have been identified in several Asian and European countries and more recently in the Americas, including the USA. The mobile elements harbouring these resistance genes not only confer resistance to the carbapenems but usually also to other β-lactams, aminoglycosides and fluoroquinolones. The dissemination of these metallo-β-lactamases is an increasing global threat to the effective treatment of *P. aeruginosa* infections; however, our understanding of these resistance mechanisms has been restricted to the genotypic classification of the different genes and the phenotypic expression of their resistance profiles. The aim of this study was to understand the dynamics of the selection of strains harbouring the resistance genes bla_{VIM} and bla_{IMP}, and the ability of these carbapenemases to confer resistance to the carbapenems imipenem and meropenem in *P. aeruginosa*.

Materials and Methods

Bacterial Isolates

P. aeruginosa strains, containing bla_{VIM-2} and bla_{IMP-1}, were kindly provided by Dr. N. Woodford. The susceptible *P. aeruginosa* strain ATCC 27853 was used as the susceptible comparator strain. The antimicrobial agents were obtained from their respec-

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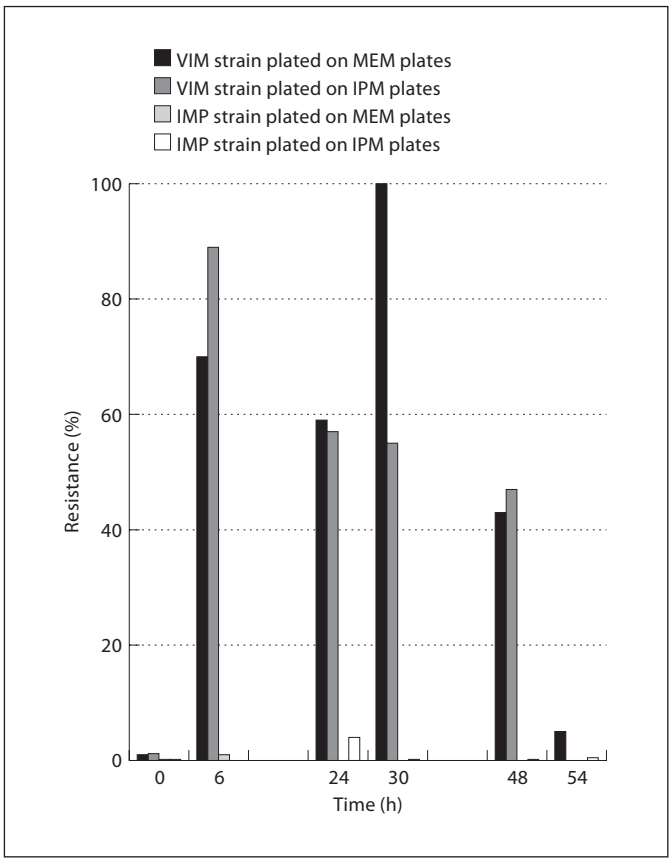

Fig. 1. Meropenem competition studies. *P. aeruginosa* containing either the VIM or the IMP β-lactamase strains were mixed with the sensitive strain and then challenged with meropenem. The mixture was serially diluted at the time intervals shown and plated onto agar plates containing meropenem (MEM) or imipenem (IPM).

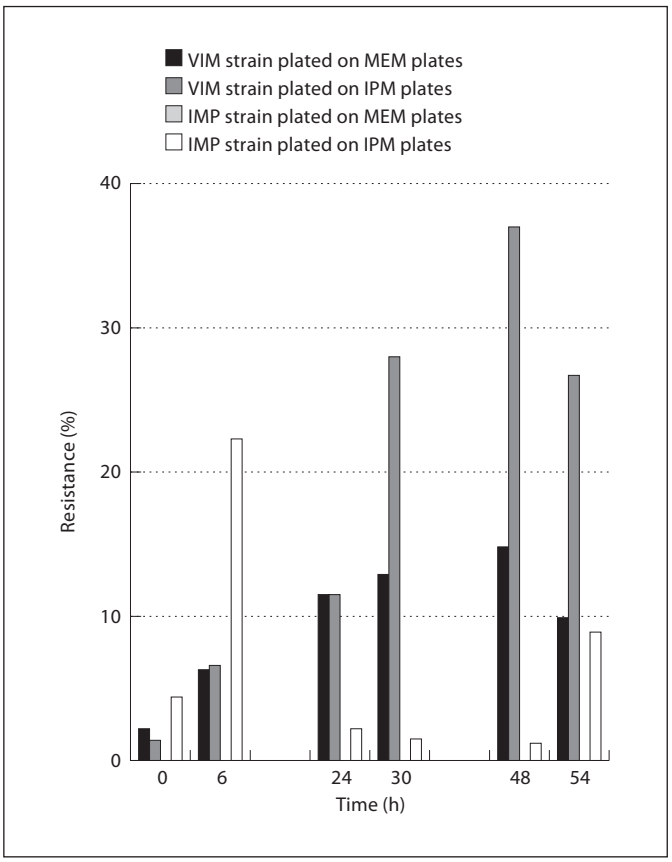

Fig. 2. Imipenem competition studies. *P. aeruginosa* containing either the VIM or IMP β-lactamase strains were mixed with the sensitive strain and then challenged with imipenem. The mixture was serially diluted at the time intervals shown and plated onto agar plates containing meropenem (MEM) or imipenem (IPM).

tive manufacturers and stored and prepared according to the manufacturer's guidelines. The susceptibilities of imipenem, meropenem and ertapenem to the 3 isolates were performed according to the guidelines of the British Society for Antimicrobial Chemotherapy [4].

Competition Studies

The ability of a bacteria population to develop resistance is usually measured by the ability to mutate. This may not be an accurate representation of the rise of resistance within a sensitive population. Instead, we have devised the following competition studies to establish the proliferation and dominance of the strains containing $bla_{\text{VIM-2}}$ and $bla_{\text{IMP-1}}$ over sensitive strains under antibiotic challenge. These competition studies enable the analysis of a continuous mixed culture of bacteria and the differential selection of resistant isolates over susceptible isolates and are designed to provide a detailed picture of how metallo-β-lactamase-producing, carbapenem-resistant isolates emerged from the population.

Table 1. Minimum inhibitory concentrations of carbapenems for the 3 test strains

	Imipenem	Meropenem	Ertapenem
VIM, mg/l	128	64	>128
IMP, mg/l	128	>128	>128
ATCC 27853, mg/l	1	0.25	4

Both the $bla_{\text{VIM-2}}$- and $bla_{\text{IMP-1}}$-containing isolates and the sensitive *P. aeruginosa* isolate were grown overnight in iso-sensitest broth at 37°C in an orbital shaking incubator. Each resistant strain culture (0.1 ml) was mixed with 9.9 ml of the sensitive strain in separate sterile universal containers. Imipenem or meropenem was added to the broth mixture at time 0 at a concentra-

tion of 1 mg/l. The broth was serially diluted and spread on plates with imipenem and meropenem at concentrations of 8 mg/l and on plates with no antibiotic at regular time intervals. The colonies were counted and the percentage resistance development calculated for each strain and antibiotic:

$$\% \text{ resistance} = \frac{\text{cfu/ml antibiotic plate}}{\text{total cfu/ml}} \times 100.$$

Results

The minimum inhibitory concentrations of imipenem, meropenem and ertapenem for the 3 strains used in this study are shown in table 1. When meropenem was added to the broth containing the $bla_{\text{VIM-2}}$-resistant and sensitive strains, the percentage resistance to meropenem increased from approximately 3 to 100% within 30 h (fig. 1). The percentage resistance to imipenem increased to approximately 90% within 6 h; the proportion of the $bla_{\text{VIM-2}}$-resistant strain reduced after these peaks. This result was in stark contrast to the result obtained when meropenem was added to the broth containing the $bla_{\text{IMP-1}}$-resistant and sensitive strains; in this case the percentage resistance to meropenem and imipenem remained very low, the $bla_{\text{IMP-1}}$-resistant strain never rose higher than 1%.

When imipenem was added to the broth containing the $bla_{\text{VIM-2}}$-resistant and sensitive strains, the percentage of meropenem resistance caused by the VIM strain remained below 15% (fig. 2), actually only representing a gradual rise from 2.2% in 48 h. On the other hand, the percentage resistance to imipenem increased to 37% after 48 h. When the experiment was repeated with the IMP strain in the mixture, meropenem resistance remained at <1% throughout the experiment, whereas imipenem resistance increased to 22% after only 6 h.

Exposure of the mixture of $bla_{\text{VIM-2}}$-resistant and sensitive strains to meropenem allowed the $bla_{\text{VIM-2}}$ strain to proliferate and dominate the culture, conferring resistance to both imipenem and meropenem. Challenging the same mixture with imipenem resulted in a lower proportion of resistance to meropenem and imipenem. Exposure of the mixture containing the $bla_{\text{IMP-1}}$ strain to meropenem resulted in almost no selection of meropenem or imipenem resistance, but exposure of the $bla_{\text{IMP-1}}$ strain mixture to imipenem resulted only in the development of imipenem resistance.

Discussion

This study has shown that challenge with imipenem promotes the selection of strains carrying either metallo-β-lactamase, whereas meropenem only promotes the selection of strains carrying the VIM metallo-β-lactamase and hinders the selection of the strain harbouring the IMP metallo-β-lactamase in vitro. It is interesting to note that the IMP metallo-β-lactamase was the first of this family of β-lactamases to emerge and spread worldwide and it is tempting to link this with the earlier release of imipenem. In countries where there has been a more focussed use of meropenem, strains carrying the VIM β-lactamase have slowly emerged. It would be noteworthy to investigate the prescribing practices of the hospitals in which isolates containing the VIM and IMP β-lactamases have occurred and to compare this with the practices of hospitals which do not currently have a problem, to determine whether exposure to meropenem prevented the emergence of *P. aeruginosa* containing the IMP β-lactamase.

Acknowledgement

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