Frequency and predictors of colonization of the respiratory tract by VIM-2-producing *Pseudomonas aeruginosa* in patients of a newly established intensive care unit

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The aim of this study was to examine the frequency and predictors of colonization of the respiratory tract by metallo-β-lactamase (MBL)-producing Gram-negative bacteria in patients admitted to a newly established intensive care unit (ICU) of a tertiary care hospital. Specimens of tracheobronchial aspirates for microbiological studies were obtained every day for the first 3 days of the ICU stay and subsequently every third day for the rest of the ICU stay. PCR analysis and nucleotide sequencing were performed to identify bacteria that had MBL genes. Thirty-five patients (20 male, 15 female) were hospitalized during the initial 3 month period of functioning of the ICU. Colonization of the lower respiratory tract by Gram-negative bacteria was found in 29 of 35 patients (83 %) during the first 6–20 days (median 13 days) following admission to the ICU (13 patients with *Acinetobacter baumannii*, ten with *Pseudomonas aeruginosa*, three with *Enterobacter aerogenes*, two with *Klebsiella pneumoniae* and one with *Stenotrophomonas maltophilia*). Six of 29 patients (21 %) colonized with Gram-negative bacteria had blaVIM-2-positive *P. aeruginosa* isolates; one of these patients developed clinical infection due to this micro-organism. Previous use of carbapenems (*P*<0.01) or other β-lactams (*P*<0.03), as well as a stay in the ICU of >20 days (*P*<0.001), were associated with colonization with blaVIM-2-producing *P. aeruginosa*. In conclusion, colonization by Gram-negative bacteria of the respiratory tract by MBL-positive *P. aeruginosa* was common (83 %). Use of β-lactams, including carbapenems, was associated with subsequent colonization of the respiratory tract by MBL-positive *P. aeruginosa*.

**INTRODUCTION**

Colonization of the skin and mucosal surfaces of the human body by various micro-organisms is a natural process commencing at birth. The micro-organisms that colonize the epithelial and mucosal cells comprise the normal flora, which is made up of resident and transient flora (Bonten & Weinstein, 1996; Itokazu et al., 1996; Jarvis, 1996). The resident flora is complex and consists of a plethora of organisms, with a predominance of bacteria and fungi. The exact micro-organisms vary from patient to patient, but remain more or less constant in the undisturbed, non-manipulated host.

The transient microbial flora refers to the species of micro-organisms that colonize various parts of the human body for short periods of time. Colonization with transient flora usually lasts from a few hours up to several weeks. These micro-organisms originate from the environment and remain in balance with those of the resident flora for some time. The transient flora may lead to infection, especially if there are changes in the resident flora. The natural balance of micro-organisms on the human skin and mucosal surfaces is disturbed when a patient is admitted to hospital, especially in the environment of the intensive care unit (ICU). Patients are colonized by hospital bacteria, which may lead to serious infection (Flaherty & Weinstein, 1996; Jarvis, 1996; Itokazu et al., 1996; Bonten & Weinstein, 1996). The resident flora is complex and consists of a plethora of organisms, with a predominance of bacteria and fungi. The exact micro-organisms vary from patient to patient, but

**Abbreviations:** CLSI, Clinical and Laboratory Standards Institute; ICU, intensive care unit; MBL, metallo-β-lactamase; SAPS, simplified acute physiology score.
production of acquired metallo-
Gram-negative bacteria has been attributed partly to the
during the last decade, carbapenem resistance among
bacteria in the ICU environment is considered to be among
2001). Several factors have been associated with the
development of colonization of the mucosal surfaces of
patients at an early stage of their ICU stay; the presence of
bacteria in the ICU environment is considered to be among
them.

During the last decade, carbapenem resistance among
Gram-negative bacteria has been attributed partly to the
production of acquired metallo-β-lactamases (MBLs),
among which the most prevalent are IMP- and VIM-type
enzymes, whilst SPM, GIM and SIM types have been scarcely
reported from some regions. All of these enzymes except for
SPM are encoded on class 1 integrons (some IMP genes are
also encoded on class 3 integrons). The blaVIM gene was first
detected in 1996 in Portugal and then in Italy in 1997, whilst
the first report was in 1999 (Lauretto et al., 1999). A variant
gene (blaVIM-2) with 90% nucleotide identity with blaVIM-1
was detected some months later in France (Poirel et al.,
2000). Several variants of the blaVIM genes have since been
detected. For example, blaVIM-3 has two nucleotide
differences with blaVIM-2, whilst blaVIM-4 has one nucleotide
difference with VIM-1, resulting in an amino acid change of
serine to arginine at position 175 (Andrade et al., 2003;
Pournaras et al., 2002, 2003; Walsh et al., 2005; Yan et al.,
2001). To date, more than 12 VIM variants have been
identified, ranging in identity from 77 to 99%. These consist
of three separate groups, the VIM-1 group, the VIM-2 group
and VIM-7 (consisting of one member); VIM-7 is the most
divergent, with many substitutions of amino acids. Acquired
MBLs readily hydrolyse most β-lactams, including carba-
penems, and have been detected mostly among carbape-
nem-resistant nosocomial Pseudomonas aeruginosa
strains. In our region, several studies have shown a wide
dissemination of VIM-type MBLs among carbapenem-
resistant Gram-negative bacteria, whilst other MBLs have
not been detected (Pournaras et al., 2002, 2003; Walsh et al.,
2005).

We had the chance to work in a hospital (Sismanoglio
General Hospital, Athens, Greece) when a new ICU
was being established. Thus, we aimed to study the colonization
of the respiratory tract in patients admitted to this newly
established ICU during its first period of function, focusing
on Gram-negative bacteria, to evaluate how quickly
colonization occurred and by which bacteria, as well to
evaluate the frequency and predictors of colonization with
MBL-producing Gram-negative bacteria.

METHODOLOGY

Patient population. We studied all patients who were admitted to
a newly established ICU of a tertiary care hospital over the first
3 month period of its operation. Patients who were colonized
with VIM-2-producing P. aeruginosa had not been previously admitted to
a facility that harboured VIM-2-producing isolates. The ICU had
eight patient beds; four were in isolated rooms (with one patient
bed per room). Demographic and clinical data, including informa-
tion regarding the new simplified acute physiology score (SAPS II)
of the studied patients, were collected on their admission to the ICU
(Le Gall et al., 1993). The study protocol was reviewed in detail
regarding ethical issues and the expected scientific value of the
research findings, and was subsequently approved by the staff com-
mittee of the Department of Critical Care overseeing clinical studies.

Definitions of colonization and infection. Diagnosis of pneu-
monia required at least one of the following: new or progressive and
persistent infiltrate, consolidation, cavitation or pleural effusion,
fever >38°C with no other recognized cause or abnormal white
blood cell count (<4000 white blood cells mm−3) or
leukocytosis (≥12 000 white blood cells mm−3); and at least two of
the following: new onset of purulent sputum or change in character
of sputum, increased respiratory secretions or increased suctioning
requirements, new onset or worsening of cough or dyspnoea or
infiltrate, consolidation, cavitation or pleural effusion, reduced
micro-organisms from the tracheobronchial
exchange. Isolation of micro-organisms from the tracheobronchial
tree without the fulfilment of any of the combinations of the above
criteria was attributed to colonization of the respiratory tract.

Clinical specimen cultures. Tracheobronchial aspirate specimen
cultures were obtained every day for the first 3 days following
admission and subsequently every third day. An oral consent for
taking the above-mentioned specimens was given by the patients or
their relatives (when patients were unable to communicate) to one of
the authors of the study (M.H.).

Microbiological studies. Identification of the micro-organisms to
the species level was performed with the PASCO automated system
(Difco Laboratories) according to the manufacturer’s instructions,
and by testing for various properties using key tests prepared in
house. We focused only on Gram-negative bacteria, based on the
design of our study. The Gram-negative isolate with the highest
number of c.f.u. ml−1 of the tested specimen was considered to be
the main Gram-negative colonizing micro-organism and was exam-
ined further with antimicrobial-susceptibility testing. The MICs of
anti-pseudomonal drugs (cefazidime, piperacillin/tazobactam),
except carapenems, were determined with the PASCO microdilu-
tion system applying the criteria suggested by the Clinical and
Laboratory Standards Institute (CLSI). No in vitro susceptibility test-
ing of the studied isolates to ertapenem or other carbapenems under
development was performed. Susceptibility to imipenem and mer-
openem was determined by agar disc diffusion in accordance with
the CLSI recommendations. Isolates that were found to be resistant
to carapenems by this technique were further tested using Mueller–
Hinton agar containing serial twofold dilutions of antibiotic and a
final inoculum of 105 c.f.u. per spot to determine the MICs of imi-
penem, meropenem and other anti-pseudomonal drugs, based on a
CLSI-recommended agar dilution method.

Molecular assays. A blaVIM-specific product (587 bp) was ampli-
fied by PCR from crude DNA extracts, obtained after heating bacterial
suspensions in a 100°C heat block for 15 min, using published primers and conditions (Itokazu et al., 1996), whilst detection of
blaVIM alleles was performed with two different pairs of consensus primer sequences that amplify internal fragments of 261 and 510 bp,
respectively (Poirel et al., 2000; Tsakris et al., 2000). PCR was also
performed on the blaVIM-positive isolates using specific oligonucleo-
tide primers designed to amplify the entire sequences of the
blaVIM-1 (920 bp product) and blaVIM-2 (865 bp product) genes (Senda et al.,
1996). The amplicons were purified using a Qiagen II gel extraction kit
(Qiagen) and used as templates for sequencing of both strands using an ABI Prism 377 DNA sequencer (Perkin-Elmer). In PCR
assays, P. aeruginosa 174 strain (Mavroidi et al., 2000) and P. aerugi-
nosa 101/1477 strain (kindly provided by Dr N. Woodford,
Antibiotic Monitoring and Reference Laboratory, Central Public Health Laboratory, London, UK) were used as positive controls for detecting bla\textsubscript{VIM} and bla\textsubscript{IMP} respectively. As an internal control, primers for the cephalosporinase gene (de Champs et al., 2002), which is constantly present in Pseudomonas, were used.

**Statistical analysis.** Statistic analyses were performed using SPSS software version 11 (SPSS). Discrete data were expressed as percentages and compared using a $\chi^2$ test. A comparison of the distribution of quantitative variables was performed with the $t$ test and the Wilcoxon rank sum test (for normally and normally distributed variables, respectively). $P$ values were based on two-tailed test results.

**RESULTS**

Thirty-five patients (20 male, 15 female) were admitted to the ICU during the first 3 months of its operation. The demographic and clinical characteristics of the patients are shown in Table 1. Thirty-three of the 35 studied patients were intubated and mechanically ventilated for at least a part of their ICU stay. None of the newly admitted patients had positive cultures on admission to the ICU. All patients had a urinary catheter.

Bacteria were grown from 120 cultures of 420 specimens of tracheobronchial aspirates. In four of these 120 positive cultures on admission to the ICU. All patients had a tracheostomy, based on the criteria described above.

Forty-three imipenem-resistant Gram-negative bacteria were recovered from the 35 studied patients. Thirty-three of these bacteria were P. aeruginosa, five were K. pneumoniae and five were A. baumannii. The majority of the patients (60%) who were colonized with P. aeruginosa harboured a strain resistant to carbapenems, aminoglycosides, monobactams, quinolones, ureidopenicillins and anti-pseudomonal cephalosporins (the strains were only sensitive to colistin).

A bla\textsubscript{VIM} gene was detected in nine out of 33 (27%) imipenem-resistant P. aeruginosa isolates. All of these were isolated from six patients. Only one of the patients harbouring a bla\textsubscript{VIM} gene developed clinical infection. When specific primers for the whole bla\textsubscript{VIM-1} and bla\textsubscript{VIM-2} genes were used, only the latter gene was detected in all bla\textsubscript{VIM}-positive isolates. The results of sequencing showed that the 865 bp bla\textsubscript{VIM} products were identical in all cases to the bla\textsubscript{VIM-2} sequence available in the database (Gen Bank accession no. AF191564) (Walsh et al., 2005). No bla\textsubscript{IMP} gene was detected in any of the imipenem-resistant P. aeruginosa isolates. Testing for MBL genes showed negative results in all imipenem-resistant isolates of K. pneumoniae and A. baumannii.

In Table 2, we have presented the distribution of various possible risk factors in patients with or without colonization with bla\textsubscript{VIM-2}-carrying Gram-negative bacteria. Previous use of carbapenems ($P=0.01$) or other $\beta$-lactams (ceftazidime or piperacillin/tazobactam) ($P=0.03$) as well a stay in the ICU of $>20$ days ($P<0.001$) were associated with colonization with a bla\textsubscript{VIM-2}-positive bacterium. Mortality was not different between patients in the two groups (2/6, 33.3% versus 13/23, 56.5% $P=0.39$).

**DISCUSSION**

Colonization of the respiratory tract of patients in this newly established ICU was common (83%). Although our study lacked a control group of patients receiving care in a previously established ICU, comparison of our data with data from published historical controls suggests that there is no significant difference regarding colonization of the respiratory tract of patients who receive care in a newly established compared with a previously existing ICU (Jarvis et al., 1991). However, a point may be made about the relatively late occurrence of colonization of the respiratory tract of our patients (median 13 days) compared with the
Table 2. Predictors of colonization with VIM-producing Gram-negative bacteria

Results are given as number of patients (%), unless indicated otherwise.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>VIM+ (n=6)</th>
<th>VIM− (n=23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean ± SD)</td>
<td>49.5 ± 4.1</td>
<td>52.1 ± 3.5</td>
<td>0.73</td>
</tr>
<tr>
<td>No. of males</td>
<td>4 (67 %)</td>
<td>20 (87 %)</td>
<td>0.27</td>
</tr>
<tr>
<td>Morbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory dysfunction</td>
<td>3 (50 %)</td>
<td>15 (65 %)</td>
<td>0.65</td>
</tr>
<tr>
<td>Abdominal disorders</td>
<td>2 (33 %)</td>
<td>3 (13 %)</td>
<td>0.27</td>
</tr>
<tr>
<td>Stroke</td>
<td>1 (17 %)</td>
<td>4 (17 %)</td>
<td>0.99</td>
</tr>
<tr>
<td>Haematological disorders</td>
<td>0 (0 %)</td>
<td>1 (4 %)</td>
<td>0.99</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>1 (17 %)</td>
<td>2 (8 %)</td>
<td>0.52</td>
</tr>
<tr>
<td>SAPS II on admission to ICU (mean ± SD)</td>
<td>40 ± 3.5</td>
<td>39 ± 2.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous carbapenem administration</td>
<td>5 (83 %)</td>
<td>5 (22 %)</td>
<td>0.01</td>
</tr>
<tr>
<td>Previous β-lactam administration</td>
<td>6 (100 %)</td>
<td>11 (48 %)</td>
<td>0.03</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>5 (83 %)</td>
<td>9 (39 %)</td>
<td>0.08</td>
</tr>
<tr>
<td>Carbapenem/aminoglycosides</td>
<td>5 (83 %)</td>
<td>11 (48 %)</td>
<td>0.18</td>
</tr>
<tr>
<td>Low albumin (&lt; 3 g l−1)</td>
<td>4 (67 %)</td>
<td>12 (52 %)</td>
<td>0.66</td>
</tr>
<tr>
<td>Gastrostomy</td>
<td>2 (33 %)</td>
<td>12 (52 %)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hospitalization &lt; 20 days</td>
<td>0 (0 %)</td>
<td>23 (100 %)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

observations of other investigators who studied patients in previously existing ICUs (median 6 days) (Kerver et al., 1988). It should be noted that other factors, such as patient load and duration of the ICU stay, as well as infection control practices, may influence these observations.

Bonten et al. (1994) demonstrated that colonization originates from exogenous sources, and not from the gastrointestinal tract as is often assumed. Trautmann et al. (2001) examined water faucets as a source of P. aeruginosa infection in a surgical ICU. All water outlets harboured distinct genotypes of P. aeruginosa over prolonged time periods in that study; of interest, over a period of 7 months, five out of 17 patients (29 %) were infected with P. aeruginosa genotypes that were also detectable in tap water. Johanson et al. (1972) reported that 22 % of patients admitted to an ICU had their respiratory tract colonized with Gram-negative organisms over the first 24 h. Kerver et al. (1988) showed that 40−60 % of mechanically ventilated patients were colonized, mainly in the lower respiratory tract, by ICU-acquired organisms after day 5 following ICU admission, with the percentage rising to 100 % after day 10.

Colonization with potentially pathogenic Gram-negative organisms from external sources occurs rapidly after hospital admission. The mechanism involved may be related to a loss of Gram-positive organisms attributable to the widespread use of antibiotics, as well as to hydrolysis of fibronectin, a common feature in severely ill patients. Loss of fibronectin facilitates the exposure of surface receptors on epithelial cells, thus allowing Gram-negative organisms to bind (Woods et al., 1981). However, there are other major contributing factors, such as impaired natural host-defence mechanisms as a result of underlying disease, and medical and surgical interventions.

MBLs are emerging worldwide as determinants of acquired antimicrobial resistance among nosocomial strains of Gram-negative bacteria. Our results suggest that β-lactams other than carbapenems may also select for MBL-producing strains in a single patient. It should also be noted that all imipenem-resistant Gram-negative strains were isolated from patients with at least 20 days of hospitalization in the ICU, indicating that a long stay in the ICU is associated with factors that lead to the acquisition of blαVIM-positive bacteria. It is interesting that, in our study, colonization with MBL-producing Gram-negative bacteria did not seem to be associated with unfavourable patient outcome, although it should be emphasized that we studied a relatively small number of patients.

Our study is not without limitations. First, we did not directly compare the frequency and characteristics of colonization of patients in our newly established ICU with patients in another, previously existing ICU. In addition, our study focused only on Gram-negative bacteria, without examining colonization by Gram-positive bacteria and fungi. Also, we did not perform molecular clonality studies of the isolates and thus we cannot discern whether there was an outbreak situation during our study period.

In conclusion, despite the above limitations, our study provides information regarding colonization of the respiratory tract of patients in a newly established ICU by Gram-negative bacteria. In addition, our findings suggest that there is an association between exposure to carbapenems and other β-lactams, as well as a prolonged stay in the ICU,
and the development of colonization by VIM-producing *P. aeruginosa*.

**ACKNOWLEDGEMENTS**

M.H. and S.L. designed the study and collected the data. N.J.L., M.K. and A.T. performed the microbiological studies. M.H. and M.E.F. did the statistical analysis and wrote the first draft of the manuscript. All authors made revisions of the manuscript and approved its final version.

**REFERENCES**


