Emergence of multidrug-resistant Gram-negative bacteria during selective decontamination of the digestive tract on an intensive care unit

Nashwan Al Naiemi¹,², Edou R. Heddema¹, Aldert Bart¹, Evert de Jonge³, Christina M. Vandenbroucke-Grauls¹,², Paul H. M. Savelkoul² and Birgitta Duim¹*

¹Academic Medical Center, Department of Medical Microbiology, Amsterdam, The Netherlands; ²VU University Medical Center, Medical Microbiology and Infection Control, Amsterdam, The Netherlands; ³Academic Medical Center, Department of Intensive Care, Amsterdam, The Netherlands

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Objectives: During treatment with selective decontamination of the digestive tract (SDD), four multidrug-resistant (MDR) strains, three different Escherichia coli and one Klebsiella pneumoniae, were isolated from four patients not known as carriers of such MDR strains before their admission to the intensive care unit (ICU) in the Academic Medical Center (AMC) in Amsterdam. These isolates were extended-spectrum β-lactamase (ESBL)-positive. We investigated whether this was due to interspecies transfer of resistance genes.

Methods: The MDR strains were typed by amplified fragment length polymorphism (AFLP) analysis. The plasmids from these strains were characterized by restriction fragment length polymorphism and the resistance genes were characterized by PCR and sequence analysis.

Results: The strains were genetically unrelated and contained identical plasmids with ESBL genes.

Conclusions: We identified an outbreak of plasmid-mediated ESBL genes during SDD treatment in the ICU. The use of third-generation cephalosporins in SDD is associated with the emergence of ESBLs. We conclude that identification of emerging MDR Gram-negative bacteria and recognition of resistance plasmid transfer during SDD treatment are crucial for optimal application of this regimen in ICUs.

Keywords: ESBLs, SDD, intensive care, resistance

Introduction

Antimicrobial resistance is a complex and dynamic problem, which leads to excess morbidity, mortality and costs in many clinical settings. A strong association between the use of antibiotics and the emergence of antibiotic resistance has been demonstrated.¹ The prevalence of resistance is highest where antibiotic use is high, especially in intensive care units (ICUs).² Therefore, infection control measures to limit the emergence of antibiotic resistance are important issues in intensive care medicine. Selective decontamination of the digestive tract (SDD) is a prophylactic regimen that was introduced in intensive care medicine in 1984.³ The purpose of SDD is to eliminate potentially pathogenic aerobic microorganisms from the digestive tract without harming the anaerobic flora.⁴ SDD classically consists of oropharyngeal administration of non-absorbable antimicrobial agents that are active against most Gram-negative bacteria and fungi and decontamination of the rest of the gastrointestinal tract by local administration of the same antibiotics, combined during the first 3–4 days with a parenteral antibiotic to prevent early infections.⁴

We describe the emergence of four different strains of multidrug-resistant (MDR) Gram-negative bacteria (three Escherichia coli and one Klebsiella pneumoniae) at our ICU. These strains were isolated from patients not carrying these MDR bacteria before their treatment with SDD antimicrobials at the ICU. We investigated whether the isolates carried different resistance genes, indicating independent acquisition, or identical ESBL genes, which may result from horizontal transfer. We provide evidence that these strains harboured the same resistance...
plasmid, which carried identical extended-spectrum \( \beta \)-lactamase (ESBL) genes.

**Methods**

**Bacterial strains and SDD**

Four MDR Gram-negative bacterial strains (three *E. coli* and one *K. pneumoniae*) were isolated from four patients between August and December 2004, treated from the first day of admission until their discharge with SDD in the ICU (Table 1). These MDR Gram-negatives were isolated within a period of 5–8 days of treatment with SDD agents; there was no other antimicrobial therapy during the period of isolation.

The SDD regimen in our ICU consisted of application four times daily of approximately 0.5 g of an oral paste, containing 2% polymyxin E, 2% tobramycin and 2% amphotericin B, to the buccal cavity. Patients also received 100 mg of polymyxin E, 80 mg of tobramycin and 500 mg of amphotericin B through the gastric tube.

Cefotaxime 1000 mg four times daily was given intravenously throughout the first 4 days. For surveillance purposes, cultures from rectal swabs, throat swabs and sputum were taken at admission and twice weekly during the stay on the ICU. These were cultured on Columbia agar with tobramycin 4 mg/L for detection of tobramycin-resistant Gram-negative bacteria. All ICU patients with an expected duration of artificial ventilation of >48 h or an anticipated length of stay on the ICU of >72 h were treated with SDD from the first day of admission until discharge from the ICU.

The antimicrobial susceptibility tests in this study were done according to the NCCLS guidelines by the disc diffusion method. The Gram-negative bacteria were screened for ESBL production by the disc diffusion method on Mueller-Hinton agar plates with cefotaxime- and ceftazidime-containing discs (Rosco, Taastrup, Denmark). The double disc synergy test, with discs containing amoxicillin + clavulanate, cefotaxime, ceftazidime and cefepime, was used for the confirmation of ESBL production.

**Molecular typing**

The three MDR *E. coli* isolates were typed by amplified fragment length polymorphism (AFLP) of genomic DNA. Plasmids were isolated with the QIAgen Plasmid Midi Kit (QIAGEN, Westburg B.V., The Netherlands). Identity of the plasmids was investigated by restriction fragment length polymorphism (RFLP) patterns obtained by digestion with *Eco*RI and 1.0% agarose Tris-borate-EDTA gel electrophoresis. The plasmids were used for PCR and sequence analysis of \( \beta \)-lactamase genes as described previously.

**Results**

The four strains were ESBL-positive, intermediately susceptible to polymyxin E and resistant to the following antibiotics: tobramycin, gentamicin and ciprofloxacin (Table 1). AFLP analysis confirmed that the three MDR *E. coli* isolates represented three different strains (Figure 1a). Plasmids from all four strains had identical *Eco*RI RFLP patterns (Figure 1b). PCR and sequence analysis showed that all four strains harboured identical combinations of ESBL genes: CTX-M-15 and SHV-5.

**Discussion**

According to our information, this is the first description of infection by different bacterial strains carrying identical plasmids and ESBL genes during treatment with SDD antimicrobials wherein cefotaxime is included.

Besides the considerable inactivation of polymyxin E by faeces, the intermediate susceptibility to polymyxin E, the tobramycin resistance and the production of ESBLs explain the survival of these strains during the SDD regimen used in our ICU. Our results show that these four different MDR strains that were isolated from four different patients, who were treated with SDD antimicrobials for >4 days, contained the same plasmid with an identical ESBL gene (Figure 1 and Table 1).

In the routine surveillance cultures, obtained from these patients before SDD, the MDR Gram-negatives were not detected. This leaves three possibilities: (i) these strains were present below the detection level, and subsequently increased in numbers, above the detection level, during SDD therapy; (ii) the patients acquired the strains carrying the resistance plasmid; or (iii) the strains, already present in the patients, acquired the resistance plasmid. The identity of the resistance plasmids may suggest one of the latter two scenarios, although there are no data available on the presence of this resistance plasmid in the community. Any of the possibilities suggest a correlation between the treatment with the SDD antimicrobials and the emergence of these ESBL-producing Gram-negative bacterial strains.

The patients, from whom these MDR strains were isolated, were placed in isolation till their discharge from the ICU. During

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**Table 1. Characteristics of isolated strains**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Species</th>
<th>SDD-ICU(^a)</th>
<th>Isolation date</th>
<th>Antibiotic susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ECO</td>
<td>10/8–16/8</td>
<td>14/8</td>
<td>R R R R R R I S CTX-M-15/SHV-5</td>
</tr>
<tr>
<td>II</td>
<td>ECO</td>
<td>15/8–22/8</td>
<td>20/8</td>
<td>R R R R R R R I S CTX-M-15/SHV-5</td>
</tr>
<tr>
<td>III</td>
<td>KPN</td>
<td>27/9–2/10</td>
<td>1/10</td>
<td>R R R R R R R I S CTX-M-15/SHV-5</td>
</tr>
<tr>
<td>IV</td>
<td>ECO</td>
<td>15/10–6/12</td>
<td>22/10</td>
<td>R R R R R R R I S CTX-M-15/SHV-5</td>
</tr>
</tbody>
</table>

ECO, *E. coli*; KPN, *K. pneumoniae*; CAZ, ceftazidime; CTX, cefotaxime; CPD, cefpodoxime; FEP, cefepime; TOB, tobramycin; GEN, gentamicin; CIP, ciprofloxacin; POL, polymyxin E; FOX, cefoxitin; R, resistant; I, intermediate; S, susceptible. 

\(^a\)Period(s) of admission to ICU and use of SDD.
Emergence of MDR Gram-negatives under SDD

<table>
<thead>
<tr>
<th>% genetic homology</th>
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<tbody>
<tr>
<td>E. coli, C1</td>
</tr>
<tr>
<td>E. coli, I</td>
</tr>
<tr>
<td>E. coli, II</td>
</tr>
<tr>
<td>E. coli, C2</td>
</tr>
<tr>
<td>E. coli, IV</td>
</tr>
<tr>
<td>E. coli, C3</td>
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</table>

Figure 1. Different strains harbour identical plasmids. (a) AFLP patterns with fragments from 60 to 500 bp of the MDR Gram-negative bacterial strains E. coli I, II and IV. E. coli strains C1 and C2 are control strains related to the same period of the MDR Gram-negatives. E. coli strain C3 is reference strain ATCC 35218. (b) 1% agarose gel showing the EcoRI digest of plasmid DNA isolated from the MDR Gram-negative bacterial strains. E. coli strains I, II and IV and K. pneumoniae strain III; X, molecular weight marker X (Roche, 12.2 kb–0.1 kb).

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Transparency declarations

None to declare.

References


