Emergence of linezolid-resistant coagulase-negative Staphylococcus in a cancer centre linked to increased linezolid utilization

Victor E. Mulanovich 1*, Michael D. Huband 2, Sandra P. McCurdy 2, M. Megan Lemmon 2, MaryKay Lescoe 2, Ying Jiang 1, Kenneth V. I. Rolston 1 and P. Rocco LaSala 3

1 Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 1460, Houston, TX 77030, USA; 2 Pfizer Global R&D, Eastern Point Road, Groton, CT 06340, USA; 3 Department of Pathology, West Virginia University, Morgantown, WV 26505, USA

* Corresponding author. Tel: +1-713-794-4774; Fax: +1-713-794-4351; E-mail: vmulanov@mdanderson.org

Received 8 March 2010; returned 18 April 2010; revised 24 May 2010; accepted 3 June 2010

Objectives: The prevalence of linezolid-resistant coagulase-negative Staphylococcus (CoNS) in the MD Anderson Cancer Center rose from 0.6% in 2007 to 5.5% in 2009. The aim of our study was to analyse the relationship between linezolid use and an outbreak of linezolid-resistant CoNS.

Patients and methods: We retrospectively identified 27 infection or colonization events. Eleven isolates were available for supplemental investigation; species identification, clonal relatedness and linezolid resistance mutation analysis. The medical records of the affected patients were reviewed and linezolid utilization data were obtained from the pharmacy.

Results: Available isolates were confirmed as clonally related Staphylococcus epidermidis. Partial 23S rRNA gene sequencing found a G2576T mutation in all of the isolates tested. All patients received linezolid within 3 months prior to an event. Patients without a prior hospitalization had a longer time from admission to event; 29 versus 3.5 days ($P = 0.002$). The outbreak was preceded by a 51% increase in inpatient linezolid utilization and 64% of affected patients belonged to the leukaemia service, which had a utilization rate 3.1 times that of the other services (95% confidence interval: 2.96–3.23).

Conclusions: Increased linezolid utilization preceded the appearance of a linezolid-resistant CoNS clone. Patients probably acquired the clonal strain nosocomially, given the longer time from admission to event among patients with no previous admission to the MD Anderson Cancer Center. Linezolid administration then selected this strain, since all patients received linezolid prior to an event. A linezolid utilization rate of $\geq 13$ defined daily doses/100 patient-days was similar to that reported in two other outbreaks and may be the threshold required to generate an outbreak.

Keywords: coagulase-negative staphylococci, linezolid resistance, antibiotic usage

Introduction

Coagulase-negative Staphylococcus (CoNS) is the most frequent cause of nosocomial bacteraemia and catheter-related bloodstream infections (CRBSIs). 1 Patients with cancer are at particular risk of CoNS bacteraemia because of the extensive use of central venous catheters and prolonged neutropenia. 2

Linezolid is an oxazolidinone antimicrobial approved by the FDA in April 2000. The LEADER surveillance programme monitors linezolid resistance in US hospitals and reported an increase in linezolid-resistant CoNS isolates from 0.2% in 2004 to 1.63% in 2008. 3 The prevalence of linezolid-resistant CoNS in the MD Anderson Cancer Center increased from 0.6% in 2007 to 5.5% in the first quarter of 2009. The purpose of this study was to analyse the relationship between linezolid use and an outbreak of linezolid-resistant CoNS.

Patients and methods

The laboratory information system provided us with the total number of CoNS isolates between January 2006 and March 2009, as well as their individual linezolid MIC. Isolates for which the MIC was $\geq 8$ mg/L were considered resistant. Antimicrobial susceptibility testing was performed using Vitek Legacy (GPS-110; bioMérieux, Marcy l’Etoile, France) and/or Etest (AB Biodisk, Solna, Sweden). Pfizer Global Research and Development (R&D) performed the following tests: species identification by biochemical phenotyping [API Staph (bioMérieux), Phoenix NID Panel (Becton Dickinson)]; MIC determination by linezolid broth microdilution

© The Author 2010. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
and Etest (bioMérieux, AB Biodisk); standard PFGE; and PCR amplification of the cfr gene. Additional testing at the MD Anderson Cancer Center included: species identification by partial 16S rRNA gene sequencing using universal primers; and linezolid resistance mutation analysis by partial domain V 23S rRNA sequencing.

Approval was obtained from the Institutional Review Board at the MD Anderson Cancer Center (protocol DR09-0125). The medical records of patients identified as sources of the linezolid-resistant CoNS isolates were reviewed. We used routine quantitative blood cultures on all patient samples. BSI was diagnosed when clinical signs and symptoms of infection accompanied at least two sets of positive blood cultures, and CRBSI when the colony count from a quantitative blood culture drawn from the catheter lumen was at least 3-fold greater than the colony count from a blood culture obtained from a peripheral vein. Blood isolates not meeting these criteria were considered contaminants. We divided other episodes into colonizing events and probable infections, the latter requiring clinical signs and symptoms, isolation from a sterile body fluid or tissue culture and an absolute neutrophil count of <500 cells/μL. The defined daily dose (DDD) of linezolid was 1200 mg. The Department of Pharmacy Informatics provided us with the quarterly number of linezolid DDDs.

Student’s t-test or Wilcoxon rank sum test was used for comparing continuous variables, as appropriate. The incidence rates of linezolid utilization were calculated and compared by a normal-theory test using normal approximation to the binomial distribution. The Poisson method was used to construct the confidence interval (CI) for the incidence rate ratio. All tests were two-sided at a significance level of 0.05. The statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Microbiological data
We identified 35 individual positive cultures corresponding to 27 different events. Eleven isolates recovered from six patients were available for various supplemental investigations. Nine underwent biochemical phenotyping and were identified as Staphylococcus epidermidis. A single strain from each of four patients also underwent partial 16S rRNA gene sequencing, which confirmed the species identification. Among the nine strains analysed by PFGE, all were confirmed to be clonally related. Partial 23S rRNA gene sequencing performed in 10 strains from six patients confirmed the presence of a G2576T mutation in all. Other previously described 23S gene mutations near the 2576 position and associated with linezolid resistance were not observed. Nine isolates tested negative for the presence of the cfr gene using gene-specific PCR.

Patients
Twenty-two patients had 27 events during 25 hospital admissions. Two events occurred in 2006 in one patient and one event in July 2007. The outbreak started in July 2008. Bacteremia was the most common event (63%). Seven of 17 bacteremias (41%) were classified as BSI (5) or CRBSI (2). Six other events were classified as colonization and four as probable infections (Table 1).

Fourteen patients (64%) had leukaemia and three (14%) had lymphoma. The remaining five had solid tumours and were admitted under five different services. Only four events occurred in the intensive care unit (ICU). All patients received at least one course of linezolid during the 3 months prior to an event for a median duration of 16 days (range 8–37 days). Twenty-two of 27 events occurred in patients with at least one previous admission to the MD Anderson Cancer Center and five in patients with no previous admission. The median time from admission to positive culture was 3.5 days (range 0–39 days) for the 22 events having prior admission to the MD Anderson Cancer Center compared with 29 days (range 24–77 days) for 5 events without a prior admission (P=0.002).

Table 1. Classification of events in patients with linezolid-resistant CoNS

<table>
<thead>
<tr>
<th>Event</th>
<th>Number</th>
<th>Neutropenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>CRBSI</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Blood culture contaminant</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Probable cellulitis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Probable lung infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Polymicrobial empyema</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Colonizer</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>16</td>
</tr>
</tbody>
</table>

Discussion

Institutional linezolid use
The number of linezolid DDDs/100 patient-days was 4 in 2006 and 3.7 in 2007 and increased by 51%, to 5.6, in 2008. The increase in usage started in the second quarter of 2008, prior to the outbreak, and peaked at 7.0 DDDs/100 patient-days in the fourth quarter (Figure 1). The leukaemia service had the highest linezolid use, with 34% of linezolid DDDs and 13.3 DDDs per 100 patient-days in 2008, followed by the lymphoma service with 9% of DDDs and 5.8 DDDs per 100 patient-days. The linezolid utilization rate for the leukaemia inpatient service was significantly higher than for the rest of inpatients (13.3 versus 4.3 DDDs/100 patient-days, P<0.0001). The utilization rate ratio was 3.1 (95% CI: 2.96–3.23).

We identified the G2576T mutation in all 10 CoNS isolates related to the outbreak and the leukaemia service, which used three times more linezolid than the other services, accounted for
two-thirds of patients. The linezolid utilization rate in leukaemia (13 DDDs/100 patient-days) was similar to that reported in the ICU in two previous outbreaks (14 and 15 DDDs/100 patient-days),\(^5,6\) suggesting that a threshold in selection pressure is necessary for the occurrence of an outbreak. Patients probably acquired the clonal strain by nosocomial transmission from other patients, staff or the environment,\(^10\) given the significantly longer time from admission to event among patients with no previous exposure to the hospital environment. Linezolid administration then selected this strain, as evidenced by the fact that all patients received linezolid prior to an event.

We were unable to determine which units the affected patients were admitted to, and thus could not identify potential sources of the outbreak. The cancer centre has 550 beds, and given the number of different services represented by affected patients, it is unlikely that all or most patients were on the same ward. Empirical use of linezolid has increased in the MD Anderson Cancer Center because of the progressive rise in vancomycin MIC for methicillin-resistant \textit{S. aureus} and concerns about vancomycin-resistant enterococcal infections in colonized febrile neutropenic patients (V. E. Mulanovich, unpublished observation). An antibiotic stewardship programme that monitors antibiotic utilization and provides alternative recommendations may help prevent such outbreaks, especially if utilization thresholds are identified.

Our study had several limitations. This was a retrospective, single-centre investigation that included only a small cohort of patients. We were able to recover only 11 of 27 linezolid-resistant CoNS isolates identified during the study period for supplemental testing. Lastly, epidemiological information on affected patients was not available.

In summary, the establishment of a clonal strain of linezolid-resistant CoNS in the MD Anderson Cancer Center was preceded by a 51% increase in the inpatient linezolid utilization rate and 64% of affected patients belonged to the service having the highest linezolid utilization rate. Taken together our observations suggest that increased linezolid use created sufficient selective pressure to favour the appearance and persistence of a resistant clone. The fact that all patients received linezolid prior to an event further supports this conclusion. Inpatient person-to-person transmission probably contributed to the outbreak since patients without prior exposure required a longer time in the hospital to experience an event. A linezolid utilization rate of \(\geq 13\) DDDs/100 patient-days may be the threshold required to generate an outbreak.

**Funding**

This work was supported by the DNA Analysis Core Facility at the MD Anderson Cancer Center (Facility funded by NCI Grant CA-16672[DAF]) and Department of Pathology and Laboratory Medicine internal departmental funding (P. R. L.) at the MD Anderson Cancer Center.

Pfizer Global R&D provided PFGE, phenotypic testing and confirmatory susceptibility testing.

**Transparency declarations**

V. E. M. has received research support from Pfizer. K. V. I. R. has received support for grants/research support from Cubist, Astellas, Merck and JMI Laboratories. M. D. H., S. P. M., M. M. L. and M. L. are employed by Pfizer Global R&D and also hold stock in this company. P. R. L. and Y. J. have nothing to declare.

**References**


