Journal of Antimicrobial Chemotherapy (2008) **61**, 901–907
doi:10.1093/jac/dkn043
Advance Access publication 13 February 2008

JAC

An outbreak of colonization with linezolid-resistant *Staphylococcus epidermidis* in an intensive therapy unit

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Received 7 November 2007; returned 4 December 2007; revised 16 January 2008; accepted 16 January 2008

***Objectives*: To report an outbreak of colonization with linezolid-resistant *Staphylococcus epidermidis* in an intensive therapy unit (ITU).**

***Methods*: An outbreak of colonization with linezolid-resistant *S. epidermidis* affecting 16 patients in an ITU was investigated using PFGE. Environmental and staff screening was carried out as part of the investigation. Usage of linezolid in the hospital and in the ITU was reviewed. Resistant strains were screened for the presence of the G2576T mutation using PCR-RFLP genotyping. The interventions made to control the outbreak were restriction of linezolid prescription and specific infection control measures, including isolation of colonized patients and increased environmental cleaning.**

***Results*: Linezolid-resistant *S. epidermidis* strains from the 16 colonized patients were genetically related. The same strain was also cultured from environmental samples in the ITU. An increase in linezolid usage in the hospital and in the ITU occurred in the 6 months prior to the emergence of the resistant strain. Infection control measures and restriction of linezolid prescription controlled the outbreak. All resistant isolates contained the G2576T mutation.**

***Conclusions*: An outbreak of colonization with linezolid-resistant *S. epidermidis* occurred in the ITU in our institution. The resistant strain colonized the environment and probably spread from patient to patient. The outbreak was associated with an increase in the linezolid usage in the ITU and in the institution as a whole. Restriction of linezolid usage and infection control measures were introduced to control the outbreak. The emergence of linezolid resistance in *S. epidermidis* has implications for the use of linezolid as a therapeutic agent.**

Keywords: oxazolidinones, antibiotic usage, Gram-positive bacteria

Introduction

Linezolid, the first approved oxazolidinone antibiotic, is a useful therapeutic option in the management of infections caused by multidrug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and vancomycin-resistant enterococci.[1] Linezolid inhibits bacterial ribosomal protein synthesis.[2,3] The drug binds to rRNA, specifically to domain V of the 23S rRNA of the 50S ribosomal subunit. Mutations in the central loop of this domain confer resistance to linezolid.[4,5] However, nearly all bacteria possess multiple copies of the gene encoding 23S rRNA, and strains of *S. aureus* have five or six copies, which may explain why resistance is rare in clinical isolates of staphylococci.[6]

Linezolid resistance in coagulase-negative staphylococci (CoNS) is extremely rare. A recent report assessing isolates from 16 nations reported minimal resistance among a broad range of Gram-positive pathogens (4098 Gram-positive isolates) and no

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linezolid resistance among CoNS.[7] The previous SENTRY antimicrobial surveillance programme reported a single resistant isolate of *S. epidermidis* from the USA out of a total of 9833 Gram-positive isolates.[3] The LEADER 2004 surveillance programme also reported a single resistant isolate of *S. epidermidis* out of a total of 496 CoNS.[8] Fraimow *et al.*[9] reported 5 isolates of linezolid-resistant CoNS, all associated with the 23S rRNA G2576T mutation. A recently published study by Potoski *et al.*[10] identified a number of linezolid-resistant *S. epidermidis* isolates in patients in a hospital in the USA.

Although *S. epidermidis* is a constituent of normal skin flora, it is also a common cause of infection in hospitalized patients. It is a frequent pathogen in catheter-related bloodstream infections and is also associated with infective endocarditis, infections of prosthetic joints and osteomyelitis. Increasing antibiotic resistance in *S. epidermidis* has led to a decreasing range of antibiotic treatment options. In recent years, linezolid has been a useful option for antibiotic treatment of these infections and, in particular, for treatment of bone and joint infections. We have previously reported the emergence of linezolid-resistant *S. epidermidis* in our institution.[11] In this paper, we report an outbreak of colonization with linezolid-resistant *S. epidermidis* in an intensive therapy unit (ITU). The time course of the outbreak was from September 2005 to February 2006. To the best of our knowledge, this is the first such outbreak reported in Europe. We describe the identification of this outbreak and the control measures which were implemented. Our objectives in reporting this outbreak were to report the emergence of a new epidemic strain, to identify the likely causes of the outbreak, and to describe the interventions used to control it.

Methods

This is a report of an outbreak in an ITU. We describe the setting and time course of the outbreak and the clinical profile of the affected patients. Methods used to identify and investigate the outbreak are described. The interventions we made to control the outbreak are also outlined.

Setting

The Adelaide and Meath Hospital Incorporating the National Children's Hospital (AMNCH), Tallaght, Ireland, is a 600 bed university teaching hospital. The ITU is a 9 bed open medical-surgical unit with two isolation rooms. Patients are admitted from within the hospital and from the community or from other hospitals through the accident and emergency department. The ITU has a bed occupancy of 120%. The hospital infection control team comprises two consultant microbiologists, an infection control officer and three infection control nurses.

Time course of the outbreak

Between September and December 2005, we identified three separate patient blood culture isolates of *S. epidermidis* that were resistant to linezolid. From January to February 2006, we identified a further 10 patients in the ITU who were colonized with linezolid-resistant *S. epidermidis*. The last patient colonized with linezolid-resistant *S. epidermidis* during the outbreak in the ITU was identified on 8 February 2006. During the outbreak period, two cases were identified on other wards in the hospital. Both patients had been admitted to the ITU during the outbreak period. A further patient with nasal colonization was identified on a hospital ward on 16 February 2006. This patient had been an inpatient in the ITU in January 2006. In total, 16 patients were diagnosed with colonization with linezolid-resistant *S. epidermidis*.

Case definitions

Colonization was defined as isolation of linezolid-resistant *S. epidermidis* from either screening cultures or culture of potentially infected sites. Linezolid resistance was defined as MIC >4 mg/L. Infections were defined according to standard criteria.[12] No cases of infection requiring specific antimicrobial therapy were identified.

Patients

During this outbreak, 16 patients were identified as colonized with linezolid-resistant *S. epidermidis* (Table 1). Of these patients, all but one patient (Patient 1) had been admitted to the ITU. However, this patient had been a patient on ward A at the same time as Patient 3. Twelve patients (75%) were males and 4 (25%) were females (Table 1). The resistant strain was isolated from a variety of culture sites, but the commonest sites were blood cultures (five patients) and central venous catheter tips (five patients) (Table 1).

Microbiological detection of linezolid-resistant S. epidermidis

Isolates of CoNS from patient cultures (blood, central venous catheter tips etc.) were identified as *S. epidermidis* by the VITEK 2 Advanced Expert System (bioMérieux), which was also used to confirm linezolid resistance (MIC $>$ 4 mg/L). Linezolid Etest strips (AB Biodisk, Solna, Sweden) were used to determine the MICs. The first three resistant isolates that were obtained from blood cultures were referred to the UK Reference Laboratory (Centre for Infections, Health Protection Agency, UK), who confirmed MICs >4 mg/L using an agar dilution method.[13]

Outbreak investigation

Following identification of the outbreak, we carried out an investigation of the outbreak which included: molecular analysis of the linezolid-resistant *S. epidermidis*; bacteriological surveillance of other patients in the ITU, patients from other wards, staff in the ITU and the environment to determine the extent of colonization with the resistant strain and to elucidate possible modes of transmission; examination of the linezolid treatment profile of colonized patients and of linezolid usage data in the ITU and in the hospital as a whole.

Molecular analysis of linezolid-resistant S. epidermidis

PFGE was used for molecular typing of linezolid-resistant *S. epidermidis* isolates, as described previously.[14,15] The PFGE types were defined on the basis of the DNA banding patterns in accordance with the criteria of Tenover *et al.*[16]

Detection of 23S rRNA mutation

Linezolid-resistant *S. epidermidis* isolates from all the affected patients were screened for the G2576T mutation, which leads to a G2576U change in the 23S rRNA. PCR-RFLP genotyping was

Table 1. Profile of 16 patients colonized with linezolid-resistant *S. epidermidis* including number of doses of linezolid received and linezolid MIC for resistant *S. epidermidis*

Patient	Sex	Age (years)	Ward	Culture site	Date of culture (dd/mm/yyyy)	Linezolid doses	Linezolid MIC (mg/L)
Patient 1	F	63	ward A	blood	04/09/2005	0	16
Patient 2	M	53	ITU	blood (two samples)	05/11/2005	166	>256
Patient 3	M	42	ITU (ward A)	blood	08/11/2005	9	>32
Patient 4	M	67	ITU	urine	09/01/2006	53	>256
Patient 5	F	45	ITU	CVC tip and blood	16/01/2006	10	96
Patient 6	F	63	ITU	CVC tip	19/01/2006	12	>256
Patient 7	M	64	ITU	CVC tip	19/01/2006	0	8
Patient 8	M	76	ITU	CVC tip	20/01/2006	32	12
Patient 9	F	57	ITU	blood, CVC tip	20/01/2006	9	12
Patient 10	M	75	ITU	nose	27/01/2006	0	16
Patient 11	M	57	ITU	nose	27/02/2006	0	>256
Patient 12	M	75	ward A (ITU)	wound	21/01/2006	9	32
Patient 13	M	82	ward B (ITU)	blood	02/02/2006	66	8
Patient 14	M	55	ITU	arterial line tip	08/02/2006	0	8
Patient 15	M	79	ITU	vascath tip	08/02/2006	0	24
Patient 16	M	45	ward C (ITU)	nose	16/02/2006	84	32

CVC, central venous catheter; vascath, vascular catheter.

carried out for the detection of the G2576T mutation, as described previously.[17]

Bacteriological surveillance

Patients. When the outbreak was identified in the ITU, screening samples were obtained from all patients in the ITU who had not already had cultures positive for linezolid-resistant *S. epidermidis*. A total of five patients were screened in the ITU. Surveillance was also carried out in a number of other wards at the same time in order to ascertain the incidence of colonization with the resistant strain throughout the hospital. Nasal swabs were obtained from patients in five wards, which included two medical wards, two surgical wards and one long-stay ward. Patients were chosen at random with an equal number of male and female patients screened. A total of 62 patients outside the ITU were screened. To obtain screening samples, a nasal swab moistened in saline was used. The swab was plated onto a selective medium. The selective medium was composed of blood agar supplemented with linezolid at a concentration of 2 mg/L.

Healthcare staff. Healthcare staff working in the ITU during the outbreak period were also screened for nasal carriage of linezolid-resistant *S. epidermidis* using the same screening method used for screening patients. Fifty-eight staff members across all disciplines working in the ITU were screened.

Environment. Environmental sampling was carried out in the ITU during the outbreak period. Samples were taken from the beds and equipment of all patients in the ITU. Samples were obtained from the bed rails and wheels, monitor touch keys, heparin syringe drivers, thermometers, mattresses, blood pressure cuffs and ventilators. Environmental sampling was extended to include the nurses' station and sinks in the ITU. Air sampling using the SAS Super 100 air sampler was carried out in the patient bed areas and at the nurses' station. Settle plates were also used in the patient bed areas. For environmental air sampling, both the selective medium and plain blood agar were used. Repeat environmental screening of the same sites including air sampling and settle plates was carried out 3 months after the outbreak.

Linezolid treatment profile

We collected data on the linezolid treatment profile of patients colonized with linezolid-resistant *S. epidermidis*. The number of doses of linezolid received by each patient was documented. We also examined the relationship between the number of doses of linezolid received by the colonized patients and the linezolid MIC of the colonizing strain.

Linezolid usage data

We examined the pattern of linezolid usage in the hospital as a whole and in the ITU from 2001 when linezolid was introduced to the hospital in order to determine whether there was a trend in the usage and whether this might be related to the emergence of linezolid resistance in *S. epidermidis* in our institution. The number of 600 mg doses of linezolid prescribed from 1 July 2001 to 31 December 2005 was documented in 6 monthly periods.

Interventions

The interventions that we made to control the outbreak included both infection control measures and changes in antibiotic prescribing practice. Patients who were colonized with linezolid-resistant *S. epidermidis* were nursed in isolation where possible. Contact precautions including the use of aprons and gloves as recommended for the management of patients colonized with MRSA were used.[18] Hand washing procedures were reinforced. Following discharge of colonized patients, cleaning and disinfection of the patient area was carried out. Hypochlorite (1 in 1000) was used for disinfection of the patient area. During the peak of the outbreak in February 2006, a single cleaning and disinfection of the entire ITU was also carried out. When the outbreak in the ITU was identified in January 2006, the use of linezolid was restricted to prescription by the consultant

microbiologist only. During the months of February and March 2006, no linezolid was prescribed in the ITU.

Results

Results of outbreak investigation

Molecular analysis. Using standard criteria for PFGE comparison,[16] linezolid-resistant *S. epidermidis* isolates from 16 colonized patients were identical (Figure 1). However, some patients had more than one strain (Patients 2, 5 and 9). A number of different but closely related pulsotypes were identified (Table 2). Most of the pulsotypes differed by no more than two bands, indicating a close genetic relationship. One pulsotype (AA) from Patient 5 showed significant band differences[5,6] from the predominant pulsotype (Table 2 and Figure 2). This may indicate an unrelated strain. However, band similarities indicate that it may well be related but is significantly divergent from the parental strain. PFGE of environmental strains of linezolid-resistant *S. epidermidis* demonstrated that the strains cultured from the environment in the ITU were identical to the predominant pulsotype of the patient isolates (Figure 2 and Table 2). Linezolid-susceptible control strains showed clear differentiation from the resistant strains (Figure 2). All the linezolid-resistant *S. epidermidis* isolates from the colonized patients had the G2576T mutation detected by PCR-RFLP genotyping.

Results of surveillance cultures

Patients. Patients in the ITU at the time of the outbreak were screened for nasal carriage of the resistant strain. Of the nine

Table 2. Pulsotypes of linezolid-resistant *S. epidermidis* strains from patients and ITU environmental screening samples

Source	Pulsotype
Patient 1	A
Patient 2	A, A3
Patient 3	A
Patient 4	A
Patient 5	A1, AA
Patient 6	A
Patient 7	A1
Patient 8	A
Patient 9	A1
Patient 10	A1
Patient 11	A1
Patient 12	A1
Patient 13	A
Patient 14	A
Patient 15	A1
Patient 16	A2
Environmental (six sites)	A

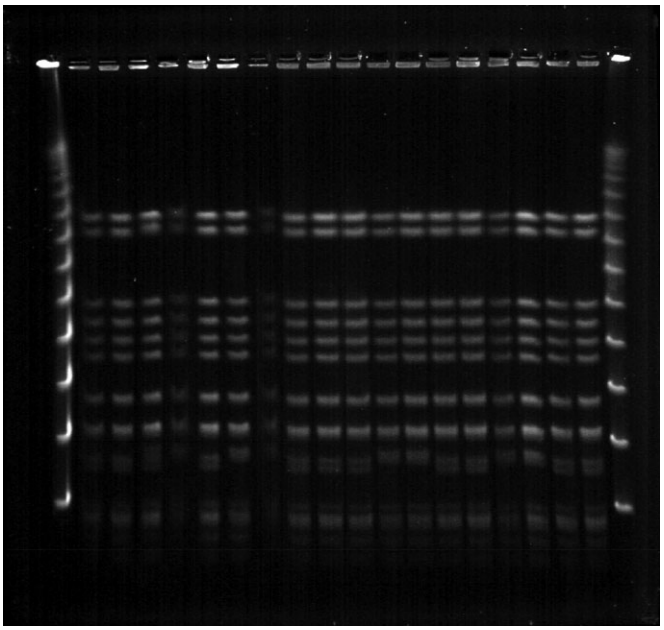

Figure 1. PFGE of linezolid-resistant *S. epidermidis* from 16 colonized patients (includes duplicate strains from two patients). Lane 1, molecular weight marker; lanes 2–19, linezolid-resistant *S. epidermidis* from Patients 1–16 [includes duplicate strains from two patients (Patients 2 and 9), where resistant strain was isolated from two samples]; lane 20, molecular weight marker.

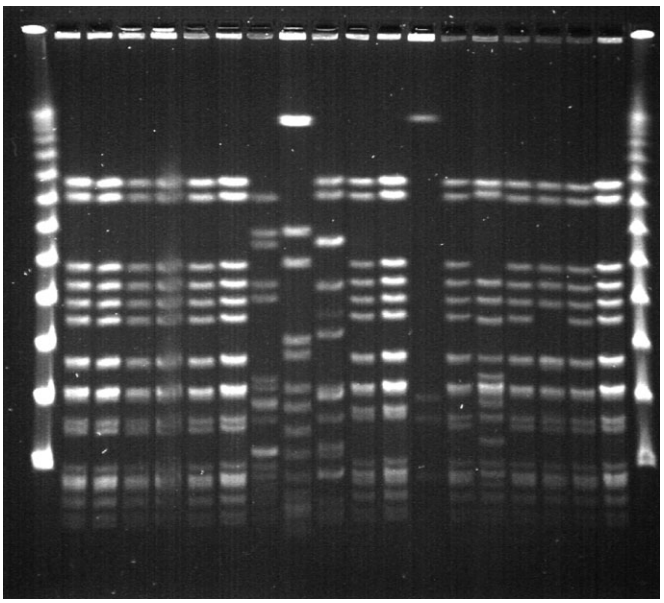

Figure 2. PFGE of environmental isolates and patient isolates of linezolid-resistant *S. epidermidis* including linezolid-susceptible control isolates. Lane 1, molecular weight marker; lane 2, environmental screen (bed); lanes 3–5, environmental screen (bed); lanes 6 and 7, environmental screen (nurses' station and computer keyboard); lane 8, linezolid-susceptible control strain (ITU patient); lane 9, linezolid-susceptible control strain; lane 10, linezolid-susceptible control strain (ITU patient); lane 11, Patient 12; lane 12, Patient 11; lane 13, linezolid-susceptible control strain; lane 14, Patient 16; lane 15, Patient 5 (different pulsotype from predominant pulsotype); lane 16, Patient 6; lane 17, Patient 2; lane 18, Patient 6; lane 19, environmental screen (bed); lane 20, molecular weight marker.

patients in the ITU, four had been identified as part of the outbreak. The other five patients were screened for nasal carriage, and two of these patients had the resistant strain cultured from nasal swabs. These two patients were included in the outbreak numbers (Patients 10 and 11). Neither of these patients had received linezolid, so it was concluded that these patients had probably acquired the resistant strain by cross-infection. Screening of patients in five other wards (medical, surgical and long-stay wards) for nasal carriage was also carried out and included a total of 62 patients. Only one of these patients was positive for nasal carriage of the resistant strain (Patient 16). This patient had spent time in the ITU, where he had shared accommodation with other colonized patients and he had also received linezolid. All 61 other patients screened in these wards were negative for nasal carriage of the resistant strain.

Healthcare staff. None of the 58 ITU staff screened for nasal carriage of linezolid-resistant *S. epidermidis* was colonized with the resistant strain.

Environment. Linezolid-resistant *S. epidermidis* was cultured from the environment in the ITU. Air samples taken in the vicinity of one bed occupied by a colonized patient grew the resistant strain. Settle plates that were placed in the vicinity of four beds, which were occupied by colonized patients, cultured the resistant strain. The resistant strain was also cultured from a computer keyboard at the nurses' station in the ITU. Linezolid-resistant *S. epidermidis* was not grown from any of the other environmental swabs. Repeat environmental screening of the same sites 3 months later did not yield any linezolid-resistant *S. epidermidis*.

Results of examination of linezolid treatment profile and linezolid usage data

Linezolid treatment profile of colonized patients. The majority of colonized patients (62.5%) had received courses of linezolid prior to colonization with the resistant isolate. However, 6 (37.5%) of the 16 patients had not received linezolid, suggesting possible cross-infection (Table 1). Some patients who received higher numbers of doses of linezolid were colonized with strains that had higher linezolid MICs (Table 1). The patient who had received the highest number of doses (166 doses), Patient 2, was colonized with a strain that had an MIC of >256 mg/L. However, this correlation did not occur in all cases. Of note, some patients who had received a relatively lower number of doses were colonized with more resistant strains (Patient 6) and one patient who had not received any linezolid (Patient 11) was colonized with *S. epidermidis* with a linezolid MIC of >256 mg/L (Table 1).

Hospital and ITU linezolid usage. We reviewed the hospital-wide usage of linezolid in our hospital since the drug was first introduced in 2001. In the 6 months prior to the emergence of linezolid-resistant *S. epidermidis* (1 January 2005 to 30 June 2005), we noted a significant increase in the total usage of linezolid throughout the hospital relative to the previous 6 month period (63% increase) (Figure 3). Since the introduction of linezolid in our hospital in 2001, the usage of linezolid had been steadily increasing in the ITU. In the 18 months prior to emergence of the linezolid-resistant *S. epidermidis* (1 January 2004 to 30 June 2005), the linezolid usage had not increased (Figure 3). However, during the 6 month period in which linezolid-resistant *S. epidermidis* emerged in the ITU (1 July 2005 to 31 December 2005), there was a marked increase in the usage of linezolid in the ITU (66% increase) (Figure 3).

Results of interventions

Following our interventions to control the outbreak, which included both infection control measures and restriction of prescription of linezolid, no further cases were identified in our ITU while the restriction of linezolid prescription was in place, i.e. for a 5 month period from February to July 2006. Following re-introduction of linezolid in the unit in July 2006, the resistant *S. epidermidis* strain re-emerged and we were obliged to restrict linezolid use again and this restriction has remained in place up to the present time.

Discussion

Resistance to linezolid in *S. epidermidis* has rarely been reported worldwide.[7,8,10] To the best of our knowledge, this is the first outbreak of linezolid-resistant *S. epidermidis* reported in Europe. In our hospital, the emergence of this strain was associated with a hospital-wide increase in linezolid usage in the 18 months prior to emergence of the resistant strain and with a very significant increase (66%) in linezolid usage in our ITU in the 6 month period during which the resistant strain emerged. Restriction of linezolid usage was associated with disappearance of the resistant strain from the ITU. However, not all patients who were colonized with the resistant strain had received linezolid and it is likely that these patients acquired the resistant *S. epidermidis* due to cross-infection. PFGE showed that the linezolid-resistant strains isolated from all 16 colonized patients were genetically related (Figure 1).

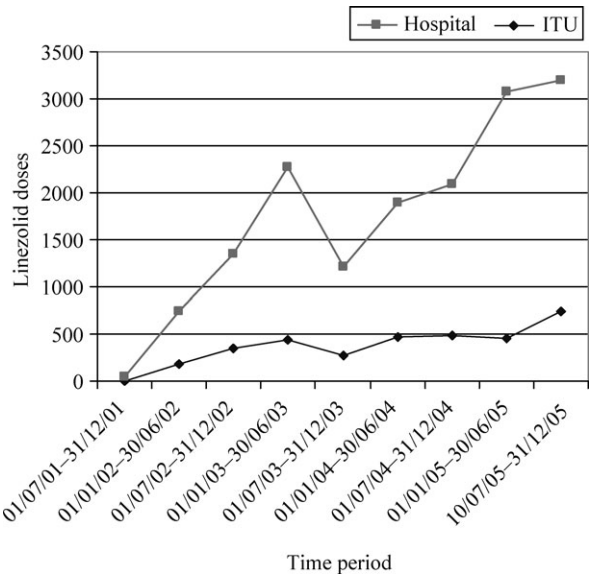

Figure 3. Linezolid usage data in AMNCH 2001–05: total hospital usage and usage in ITU (linezolid doses: number of 600 mg linezolid dosage units).

A number of mutations in the domain V region of the 23SrRNA gene that are associated with oxazolidinone resistance have been described, including G2447U in *S. aureus*,[5] G2505A in *Enterococcus faecium*,[19] G2576T, C2512U, G2513U and C2610G in *Enterococcus faecalis*,[19] and G2576T and C2534U in *S. epidermidis*.[3] However, in the clinical setting, only the G2576T mutation has been described in *E. faecium*,[20,21] *S. aureus*[17] and *S. epidermidis*.[9] This is the mutation found in the linezolid-resistant *S. epidermidis* described in this paper also which confirms previous observations that, although other mutations may occur on *in vitro* exposure to linezolid, the emergence of G2576T seems to be favoured in the clinical setting.[22]

Transmission of *S. epidermidis* from patient to patient on the hands of healthcare personnel during outbreaks has been reported previously.[23–25] We also cultured the resistant isolate from the environment in the vicinity of colonized patients and on a computer keyboard used by staff in the ITU. PFGE demonstrated that the linezolid-resistant *S. epidermidis* cultured from the environment was indistinguishable from the strain that had been cultured from the colonized patients. *S. epidermidis* may be shed from the skin into the environment on skin squamae, and it is possible that contaminated environmental surfaces could serve as a potential reservoir for these microorganisms, but the potential role of the inanimate environment as a source of nosocomial CoNS has received little attention.[26] Increased environmental cleaning was one of the measures implemented in our ITU to prevent further spread of the resistant strain. Repeat screening of the ITU environment 3 months after the outbreak did not yield any linezolid-resistant *S. epidermidis*. Although we did not culture the resistant strain from any of the ITU staff screened, we only screened for nasal carriage and it is likely that transient carriage of the resistant strain on the hands of healthcare personnel could have occurred in the early stages of the outbreak before implementation of control measures. The ITU has a high bed occupancy rate and this is associated with an increased likelihood of cross-infection.

The emergence of linezolid resistance in *S. epidermidis* in our institution was associated with increased usage of linezolid, specifically in our ITU, which may have exerted a selective pressure. Cross-infection was probably related to environmental contamination with the resistant strain and the ease with which *S. epidermidis* can be transmitted on the hands of healthcare workers. Infection control measures and restriction of linezolid usage were introduced to control the outbreak. It is possible that the absence of the selective pressure caused by intense usage of linezolid led to the replacement of the resistant strain by more susceptible strains.

Staphylococci have multiple copies of the gene that encodes domain V of the 23S rRNA, the location of the target for linezolid. A gene dosage effect has been described, whereby linezolid MICs increase with the number of gene copies that have mutations.[19,27–29] Although the linezolid-resistant *S. epidermidis* isolates described in this outbreak belonged to a single strain, isolates from different patients had different MICs. The differences in the linezolid MICs are most likely due to different numbers of gene copies possessing the mutation in the domain V gene in the resistant isolates from different patients.

The emergence of linezolid resistance in *S. epidermidis* has important implications for the use of linezolid as a therapeutic agent for the treatment of infections due to *S. epidermidis*, which is a frequent cause of bloodstream infections associated with central venous catheters and a range of other infections including endocarditis and infections of implanted devices. Linezolid resistance has also been reported to occur quite frequently in vancomycin-resistant *E. faecium*.[7,28,30–32] Reports of linezolid resistance in MRSA to date have been limited to case reports.[17,33,34] The possibility that a linezolid-resistant strain of MRSA could emerge and cause an outbreak in a manner similar to the outbreak described in this paper is a matter of concern. In order to preserve the usefulness of linezolid as a therapeutic agent, judicious use of this antibiotic and careful stewardship of its use within individual institutions and units are important as our experience has demonstrated. Surveillance for the emergence of resistant strains is necessary to identify their emergence at an early stage so that appropriate measures can be taken to prevent their spread.

Transparency declarations

J. C. received a grant from the Cystic Fibrosis Association of Ireland and P. G. M. received a grant in 2003 from Pharmacia for a study proposal of linezolid use in cystic fibrosis. P. G. M. also received a grant from Pfizer in 2007. All other authors have none to declare.

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