Factors Influencing Linezolid-Nonsusceptible Coagulase-Negative Staphylococci Dissemination Among Patients in the Intensive Care Unit: A Retrospective Cohort Study

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Key Words

Coagulase-negative staphylococci · Linezolid · Resistance · Clones · Intensive care unit · Risk factors

Abstract

Background: The aim of the present study was to identify risk factors for linezolid-nonsusceptible coagulase-negative staphylococci (CNS) dissemination in the intensive care unit.

Methods: Among the 246 patients included, 33 revealed a linezolid-nonsusceptible CNS-positive culture specimen, 68 were positive for linezolid-susceptible CNS and 145 served as controls. Isolates were characterized by phenotypic and genotypic methods to species level, susceptibility to anti-staphylococcal agents and clones. **Results:** Among the 33 linezolid-nonsusceptible CNS patients, 29 revealed *Staphylococcus epidermidis* and 4 *Staphylococcus capitis*. All *S. epidermidis* strains belonged to the ST22 clone (by multilocus sequence typing), 26 carried both C2534T and T2504A and 3 strains were C2543T mutations. *S. capitis* strains were stratified as a common pulsed-field gel electrophoresis type and carried the G2576T mutation. Risk factors for linezolid-nonsusceptible CNS isolation were linezolid administration and

mean number of linezolid-nonsusceptible CNS-positive patients in nearby beds per day. **Conclusions:** These results reinforce the aspect of rational antibiotic usage, but also highlight the need for strict infection control measures to prevent the dissemination of linezolid-nonsusceptible CNS.

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Introduction

Linezolid, a synthetic agent belonging to the oxazolidinone family, was introduced in the clinical practice in 2000 because of its specific activity against multiresistant Gram-positive cocci, especially glycopeptide-resistant enterococci and methicillin-resistant staphylococci [1]. It is a protein synthesis inhibitor binding to the 50S ribosomal subunit, specifically to the peptidyl transferase center of bacteria (domain V), thus blocking the precise po-

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sitioning of t-RNA to the A-site [2]. The development of resistance to linezolid is associated with the presence of mutations in 23S rRNA nucleotides, of which the most frequently reported are G2061, C2452, A2503, U2504, G2505, A2062, G2447, A2453, C2499, U2500 and G2576U [2]. Other mechanisms of linezolid resistance include mutations in genes encoding L3 and L4 ribosomal proteins and the presence of the transferable *cfr* gene which encodes an rRNA methyltransferase [2, 3]. Most reports underline the role of linezolid usage in hospitals for the emergence of resistance among Gram-positive bacteria [1–4].

Coagulase-negative staphylococci (CNS) are part of the normal skin flora. However, they cause severe infections in hospitalized patients (catheter-related bloodstream infections, endocarditis, prosthetic joint infections, etc.). Often, they are multidrug resistant, leaving as the only therapeutic options newer antimicrobials such as linezolid [1, 4, 5]. Reports from all over the world have identified low numbers of linezolid-resistant CNS and their presence is usually associated with hospitalized patients in intensive care units (ICU) [1, 4–7]. Previous Greek studies have reported mutations conferring resistance to linezolid among *Staphylococcus epidermidis* from ICU wards of different hospitals. More specifically, the mutations T2504A and C2534T are closely associated with linezolid exposure and circulation of ST2 and ST22 clones (sequence types) [4, 8].

In the present study, the risk factors for developing infection or colonization with linezolid-nonsusceptible CNS during hospitalization in the adult medical/surgical ICU of a tertiary care university hospital were analyzed. The aim was to identify the resistance mechanisms of such strains and their genetic relationship in relation to antibiotic usage and other factors contributing to linezolid-nonsusceptible CNS spread in our setting.

Methods

Patients

This single-center retrospective cohort study was conducted in the adult ICU of the University Hospital of Patras, Greece, a 700-bed teaching hospital, from March 2010 to February 2012. The ethics committee of the University Hospital of Patras approved the study and waived the need for informed consent (approval No. 571).

Patients with an ICU stay of at least 6 days were classified into three groups. Group 1 (case patients) comprised 33 patients with positive CNS clinical specimens (intravenous catheter tips and/or blood) that exhibited reduced susceptibility to linezolid (minimum inhibitory concentration, MIC ≥ 2 mg/l), group 2 consisted

of 68 ICU patients with intravenous catheter tip or blood cultures positive for linezolid-susceptible CNS (MIC ≤ 1 mg/l) and patients with no CNS isolation served as group 3 (controls). Duplicate isolates of the same patient were excluded from further analysis. Bloodstream infection, catheter-related or not, was associated with systemic symptoms or signs (fever, tachycardia, tachypnea, hypotension, leukocytosis) with at least two positive blood cultures on separate occasions or a positive blood culture along with a positive culture by the same organism from an infected catheter [9]. Positive blood or catheter tip cultures not meeting the above criteria were considered as contaminants.

Phenotypic Identification and Antibiotic Susceptibility Testing

Of the 33 case patients, 15 had a linezolid-susceptible CNS isolate prior to final linezolid-nonsusceptible CNS recovery. All isolates (48) were identified to species level by the Vitek 2 Advanced Expert System (bioMérieux SA, Marcy l'Étoile, France), while antibiotic susceptibility testing was performed by the disk diffusion method against the following antistaphylococcal agents: cefoxitin, tetracycline, rifampicin, gentamicin, kanamycin, erythromycin, clindamycin, fusidic acid, ciprofloxacin and sulfamethoxazole-trimethoprim [10]. MICs of oxacillin, vancomycin, linezolid and daptomycin were determined by Etest (bioMérieux) [10].

Molecular Analysis

All linezolid-nonsusceptible CNS were identified to species level by sequence analysis of the *tuf* gene [11], whereas mutations in region V of 23S rDNA were investigated by PCR and sequence analysis [8]. Sequence data were analyzed using Chromas (www.technelysium.com.au/chromas.html). The presence of the *cfr* gene was tested by PCR [3]. The possible presence of mutations in ribosomal protein L4 was investigated by PCR followed by sequence analysis [12].

Clonal Identification

Clones were identified by means of *Sma*I DNA macrorestriction patterns and pulsed-field gel electrophoresis (PFGE) [13]. Linezolid-nonsusceptible and representative linezolid-susceptible *S. epidermidis* from different pulsotypes and phenotypes were further typed by multilocus sequence typing (<http://www.mlst.net>).

Epidemiological ICU Data

Epidemiological data were collected from the ICU computerized database (Criticus™, University of Patras, Greece) and patients' chart reviews. Parameters assessed included demographic characteristics (age, sex), severity scores of illness on admission, e.g. APACHE II (Acute Physiology and Chronic Health Evaluation II) score, SAPS II (Simplified Acute Physiology Score II) score, chronic illness prior to admission (e.g. diabetes mellitus, chronic obstructive pulmonary disease, chronic heart failure, chronic renal failure, malignancy), cortisone use, obesity, surgery, length of hospitalization, presence of invasive catheters, endotracheal intubation, tracheotomy, type of antibiotic administration and enteral or parenteral nutrition. Enteric colonization by vancomycin-resistant enterococci and nasal carriage of methicillin-resistant *Staphylococcus aureus* were also assessed by obtaining rectal and nasal samples from each patient upon admission and weekly afterwards, according to the surveillance program in the ICUs. The role of colonized or infected patients in nearby beds in dissemination of linezolid-nonsusceptible CNS strains was also investigated. Days

Table 1. Phenotypic and genotypic characteristics of CNS isolated from the 33 study cases

Species	Mutation	MIC, mg/l	PFGE	ST	Resistance pattern (disk diffusion method)	Clinical samples	Colonizing/ infecting, n
<i>S. epidermidis</i>	C2534T, T2504A	256 (26)	A (26)	22	FOX, GM, KAN, CC, FA, CIP, SXT	blood (19) catheter tip (7)	15/4 6/1
	C2534T	2 (1), 4 (1)	C (2)	22	FOX, GM, KAN, CC, CIP, SXT	blood (2)	2/0
	C2534T	3 (1)	E (1)	22	FOX, KAN, CC, E	blood (1)	1/0
	–	<1 (15)	B (11)	45	FOX, GM, KAN, CC, FA, CIP, SXT, RA, E	blood (9) catheter tip (2)	9/0 2/0
	–		D (4)	45	FOX, GM, KAN, CC, FA, CIP, SXT, E	blood (4)	4/0
<i>S. capitis</i>	G2576T	6 (2), 32 (2)	k (4)	n.a.	FOX, GM, KAN, CC, FA, CIP, SXT, RA	blood (4)	4/0

Values in parentheses indicate number of isolates. MIC: linezolid MIC identified by Etest. ST = Sequence types, identified by multi-locus sequence typing; n.a. = not applicable; FOX = ceftiofur; RA = rifampicin; GM = gentamicin; KAN = kanamycin; E = erythromycin; CC = clindamycin; FA = fusidic acid; CIP = ciprofloxacin; SXT = sulfamethoxazole-trimethoprim.

at risk were defined as days in the ICU until linezolid-nonsusceptible CNS or linezolid-susceptible CNS isolation for the resistant and the susceptible study cases, respectively, and total ICU stay for the control group.

Statistical Analysis

SPSS version 19.0 (SPSS, Chicago, Ill., USA) software was used for data analyses. Categorical variables were analyzed by using the Fisher exact test or the χ^2 test and continuous variables with the Mann-Whitney U test, as appropriate. Three different univariate analyses were performed: the first comprised linezolid-nonsusceptible study cases versus controls, the second nonsusceptible study cases versus susceptible ones and, finally, susceptible study cases versus controls. Factors contributing to multicollinearity were excluded from the multivariate analysis. Backward stepwise multiple logistic regression analysis used all variables with a $p < 0.1$ from the univariate analysis. Odds ratios and 95% confidence intervals were calculated to evaluate the strength of any association. All statistical tests were 2-tailed and $p < 0.05$ was considered statistically significant.

Results

Susceptibility Testing and Molecular Analysis

Among the 246 studied patients, 33 (13.4%) exhibited at least 1 positive linezolid-nonsusceptible CNS culture (linezolid MIC ≥ 2 mg/l). From this group, 29 *S. epidermidis* and 4 *S. capitis* strains were isolated; 5 patients had bloodstream catheter-related infection (identified by clinical signs and positive blood and intravenous catheter tip culture with the same strain), while the remaining 28 cases were judged to be colonized (table 1) [9].

Overall, 26 *S. epidermidis* strains showing linezolid MIC >256 mg/l carried both C2534T and T2504A mutations (*Escherichia coli* 23S rRNA gene numbering; table 1), while 3 carried only the C2534T mutation in the 23S rDNA genes exhibiting linezolid MICs 2–4 mg/l. Linezolid-resistant *S. capitis* strains (MICs: 6 and 32 mg/l) carried the G2576T mutation. No strain carried the *cfr* gene or any mutation in the L4 ribosomal protein gene. All linezolid-nonsusceptible *S. epidermidis* strains belonged to the ST22 clone and to 3 PFGE types (A, C and E). Antibiotic resistance patterns of isolates were related to their PFGE types (table 1). Linezolid-resistant *S. capitis* strains belonged to a common PFGE type, 'k'. From the group of 33 case patients, 15 had a linezolid-susceptible *S. epidermidis* that belonged to 2 (unrelated to A, C and E) PFGE types (B and D) and were classified as ST45 clone (table 1). All CNS were methicillin resistant. Mean MIC values among linezolid-nonsusceptible CNS were 1.7 mg/l for vancomycin (range 0.75–3 mg/l) and 0.4 mg/l for daptomycin (range 0.064–1 mg/l). The respective values for linezolid-susceptible CNS were 1.6 mg/l (range 1.25–2 mg/l) and 0.3 mg/l (range 0.19–1 mg/l).

Risk Factors for Colonization or Infection by Linezolid-Nonsusceptible CNS

Risk factors associated with linezolid-nonsusceptible CNS colonization or infection from the univariate analyses are shown in table 2, whereas results from multivariate

Table 2. Univariate analysis for risk factors for linezolid-nonsusceptible CNS isolation during ICU hospitalization

Characteristics	Linezolid-nonsusceptible CNS (n = 33)	Linezolid-susceptible CNS (n = 68)	Controls (n = 145)	p ^a	p ^b	p ^c
Days at risk	23.8±13.7	11.1±5.2	12.8±6.9	<0.001	<0.01	<0.05
Demographics						
Age, years	47.5±18.0	54.1±18.6	56.8±19.9	<0.05	n.s.	n.s.
Male gender	22 (66.7)	53 (77.9)	88 (60.7)	n.s.	n.s.	n.s.
Comorbidities, number	1.0±1.0	0.9±1.1	0.8±1.1	n.s.	n.s.	n.s.
Diabetes mellitus	5 (15.2)	8 (11.8)	17 (11.7)	n.s.	n.s.	n.s.
Chronic obstructive pulmonary disease	3 (9.1)	12 (17.6)	21 (14.5)	n.s.	n.s.	n.s.
Chronic heart failure	2 (6.1)	6 (8.8)	12 (8.3)	n.s.	n.s.	n.s.
Chronic renal failure	2 (6.1)	2 (2.9)	4 (2.8)	n.s.	n.s.	n.s.
Malignancy	3 (9.1)	5 (7.4)	14 (9.7)	n.s.	n.s.	n.s.
Obesity	12 (36.4)	21 (30.9)	27 (18.6)	<0.05	n.s.	n.s.
Cortisone use	4 (12.1)	4 (12.1)	12 (8.3)	n.s.	n.s.	n.s.
Admission data						
APACHE II score upon admission	17.9±9.7	15.9±6.5	15.6±6.6	n.s.	n.s.	n.s.
SAPS II score upon admission	39.0±15.6	37.1±10.3	37.3±12.7	n.s.	n.s.	n.s.
Previous hospitalization	15 (45.5)	36 (52.9)	67 (46.2)	n.s.	n.s.	n.s.
Prior emergency surgery	15 (45.5)	27 (39.7)	51 (35.2)	n.s.	n.s.	n.s.
Prior abdominal surgery	12 (36.4)	17 (25.0)	32 (22.1)	n.s.	n.s.	n.s.
Number of antibiotics administered in ICU	5.4±1.8	3.3±1.1	3.2±1.4	<0.001	n.s.	n.s.
Carbapenems	32 (97.0)	62 (91.2)	123 (84.8)	0.082	n.s.	n.s.
Quinolones	7 (21.2)	10 (14.7)	22 (15.2)	n.s.	n.s.	n.s.
3rd- or 4th-generation cephalosporins	3 (9.1)	4 (5.9)	19 (13.1)	n.s.	n.s.	n.s.
β-Lactam/lactamase inhibitors	10 (30.3)	24 (35.3)	52 (35.9)	n.s.	n.s.	n.s.
Colistin	27 (81.8)	21 (30.9)	34 (23.4)	<0.001	<0.001	n.s.
Aminoglycosides	23 (69.7)	22 (32.4)	32 (22.1)	<0.001	0.001	n.s.
Glycopeptides	24 (72.7)	64 (94.1)	117 (80.7)	n.s.	<0.01	<0.05
Metronidazole	9 (27.3)	7 (10.3)	20 (13.8)	0.070	<0.05	n.s.
Tigecycline	7 (21.2)	2 (2.9)	7 (4.8)	<0.01	<0.01	n.s.
Linezolid	25 (75.8)	5 (7.4)	30 (20.7)	<0.001	<0.001	<0.05
ICU procedures						
Tracheotomy	29 (87.9)	41 (60.3)	87 (60.4)	<0.01	<0.01	n.s.
Presence of catheters	26 (78.8)	38 (55.9)	77 (53.1)	0.010	<0.05	n.s.
Hemodialysis during ICU stay	11 (33.3)	7 (10.3)	16 (11.1)	<0.01	<0.05	n.s.
Cortisone administration	25 (75.8)	40 (58.8)	82 (56.6)	<0.05	n.s.	n.s.
Parenteral nutrition	23 (69.7)	32 (47.1)	59 (40.7)	<0.01	<0.05	n.s.
Enteral nutrition	27 (81.8)	53 (77.9)	109 (75.2)	n.s.	n.s.	n.s.
Colonization/infection data						
VRE rectal colonization	9 (27.3)	20 (29.4)	31 (21.4)	n.s.	n.s.	n.s.
VRE infection	1 (3)	2 (2.9)	1 (0.7)	n.s.	n.s.	n.s.
MRSA nasal colonization	0 (0.0)	6 (8.8)	8 (5.5)	n.s.	n.s.	n.s.
MRSA infection	0 (0.0)	0 (0.0)	1 (0.7)	n.s.	n.s.	n.s.
Mean number of linezolid-nonsusceptible CNS-positive patients in nearby beds per day	0.4±0.3	0.1±0.2	0.1±0.2	<0.001	<0.05	n.s.

Data are presented as number of patients (with percentages in parentheses) or means ± SD. Colistin: only IV administration of colistin is included. Presence of catheters: all patients after ICU admission were intubated, mechanically ventilated and continuously monitored with a central venous catheter, an arterial catheter and a urinary catheter. Presence of catheters does not include the aforementioned catheters. VRE = Vancomycin-resistant *enterococcus*; MRSA = methicillin-resistant *S. aureus*; n.s. = nonsignificant.

^a Comparison between linezolid-nonsusceptible CNS patients vs. controls. ^b Comparison between linezolid-nonsusceptible CNS patients vs. linezolid-susceptible CNS patients. ^c Comparison between linezolid-susceptible CNS patients vs. controls.

Table 3. Multivariate analysis of risk factors for linezolid-nonsusceptible CNS isolation during ICU stay

Characteristics	p	OR (95% CI)
Linezolid-nonsusceptible CNS vs. controls		
Hemodialysis during ICU stay	0.048	3.7 (1.0–13.3)
Aminoglycoside administration	0.006	4.9 (1.6–15.3)
Linezolid administration	0.001	6.8 (2.2–21.5)
Mean number of linezolid-nonsusceptible CNS-positive patients in nearby beds per day	<0.001	23.4 (4.1–134.8)
Linezolid-nonsusceptible CNS vs. linezolid-susceptible CNS		
Days at risk	0.003	1.2 (1.1–1.3)
Linezolid administration	<0.001	27.2 (5.3–138.8)
Mean number of linezolid-nonsusceptible CNS-positive patients in nearby beds per day	0.011	32.6 (2.3–472.5)
Linezolid-susceptible CNS vs. controls		
Obesity	0.012	2.6 (1.2–5.3)
Male gender	0.003	2.9 (1.4–5.9)
Glycopeptide administration	0.003	5.7 (1.8–18.2)

OR = Odds Ratio; CI = confidence interval.

Fig. 1. Isolation of linezolid-nonsusceptible CNS per month.

analyses appear in table 3. Compared to controls, hemodialysis during ICU stay, aminoglycoside and/or linezolid administration and number of linezolid-nonsusceptible CNS-positive patients in nearby beds per day were significantly associated with linezolid-nonsusceptible CNS isolation. Multivariate analysis of linezolid-nonsusceptible CNS-positive and linezolid-susceptible CNS-positive patients revealed that days at risk, linezolid administration and number of linezolid-nonsusceptible CNS-posi-

tive patients in nearby beds per day were significantly higher in the first group. Obesity, male gender and glycopeptide administration were independently associated with isolation of linezolid-susceptible CNS compared to controls. No statistically significant differences were detected in the univariate analysis between infected and colonized patients by linezolid-nonsusceptible CNS.

Figure 1 shows the number of new linezolid-nonsusceptible CNS cases per month. During the last 12 months

of the study (March 2011 to February 2012), 21 out of 112 patients (18.8%) were linezolid-nonsusceptible CNS-positive compared to 12 out of 124 (9.7%) during the first 12 months ($p = 0.038$).

Discussion

The widespread use of linezolid due to the reduced activity of glycopeptides against Gram-positive cocci has led to the emergence and dissemination of linezolid resistance. Recently, Gu et al. [1] reported a total of 351 linezolid-resistant CNS cases from Europe, North America, South America and Asia (53.6, 42.5, 2.8 and 1.1%, respectively). All isolates were resistant to methicillin, as well as to a wide range of antistaphylococcal agents, underlying the need for accurate detection of resistance to linezolid and judicious use of the drug, since prolonged treatment induces resistance [1]. The isolates of the present study were also methicillin resistant, with antibiotic resistance patterns correlating to their PFGE types. Previous studies in Greece, including strains from our institution, identified linezolid-resistant CNS carrying C2534T, G2576T and T2504A mutations, the latter of which is associated with high levels of linezolid MIC [4, 8]. This fact is also verified in the present study, where 26 cases of *S. epidermidis* expressing high levels of linezolid MIC carried both C2534T and T2504A mutations. Furthermore, the *S. epidermidis* ST22 clone seems to circulate steadily in our setting [4]. Although 3 *S. epidermidis* strains were linezolid susceptible (MIC: 2–4 mg/l), they were positive for the presence of the C2534T mutation. In a study by Ferreira et al. [14] among 101 staphylococci phenotypically tested, the range of linezolid MIC was 0.5–4 mg/l, but no molecular analysis was performed. In another study, linezolid nonsusceptibility among staphylococci was associated with the G2576T mutation in 23S rDNA, whereas 5 isolates carried the mobile *cfr* element [15]. Additionally, the presence of the G2576T mutation in 23S rDNA (mutations in L3 or L4 genes) resulted in reduced susceptibility to linezolid among enterococci [16, 17].

Importantly, we report for the first time that linezolid-resistant *S. capitis* strains bear the G2576T mutation and belong to a common PFGE type. Resistance to linezolid among *S. capitis* was also identified in China due to the presence of a novel C2104T mutation [18].

Linezolid administration is reported to be one of the most important risk factors for linezolid-resistant Gram-positive cocci isolation in hospital outbreaks [4–7, 19].

However, it should be noted that there are not enough data regarding the risk factors contributing to the emergence of linezolid-resistant CNS [4–7].

In our studied group, 8 out of 33 patients did not receive linezolid for at least 6 months prior to admission and during their ICU stay, suggesting that linezolid administration, although important, is not by itself the only factor contributing to the spread of linezolid-resistant pathogens. Additionally, ICUs remain the source of silent dissemination; therefore, the determination of risk factors involved in potential linezolid-resistant CNS outbreaks in such settings provides essential information for understanding the emergence and evolution of future outbreaks [4–6].

Furthermore, even though it has been previously suggested [4, 6, 19], this is the first study proving, with multivariate analysis, that the presence of linezolid-nonsusceptible CNS-positive patients in nearby beds plays an important role in the dissemination of these pathogens.

Increased length of stay was also found to be an important risk factor for the evolution and isolation of linezolid-nonsusceptible CNS. As mentioned in other studies regarding multidrug-resistant pathogens, being hospitalized near an already colonized patient usually increases the chances of acquiring the microorganism. Prolonged ICU stay predisposes to infection, especially when patients receive linezolid as an anti-Gram-positive coverage [20, 21].

An interesting finding was that colistin and aminoglycosides administration was identified by univariate analysis as a risk factor for the isolation of linezolid-nonsusceptible CNS compared to patients with linezolid-susceptible isolates and controls (table 2). This result can be explained by the fact that colistin and aminoglycosides are administered in combination with linezolid to patients not responding to carbapenems and glycopeptides. Therefore, no correlation exists between colistin or aminoglycoside administration and the spread of CNS resistant to linezolid.

It is noteworthy that all linezolid-nonsusceptible CNS belonged to common clones (ST22 for *S. epidermidis* and pulsotype k for *S. capitis*). Also, susceptible strains from the same patients belonged to different PFGE and sequence types (ST45), suggesting a clonal-related dissemination of linezolid-resistant strains [4, 6]. However, since different mutations were simultaneously detected among these strains, a continuous evolution of multidrug-resistant bacteria also occurs under the pressure of antibiotic usage among critically ill ICU patients. The number of

infected patients by linezolid-nonsusceptible CNS was low in the present study, as also reported by Potoski et al. [19].

Conclusions

Besides linezolid usage, patient-to-patient transmission of linezolid-nonsusceptible CNS occurs among ICU patients, suggesting the necessity for strict infection control measures such as adequate central venous catheter handling and hand hygiene of health care workers.

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Disclosure Statement

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