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In vitro susceptibility of Gram-positive pathogens to linezolid and teicoplanin and effect on outcome in critically ill patients

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***Objectives*: To determine the prevalence of teicoplanin and linezolid resistance amongst Gram-positive pathogens isolated in the intensive care unit (ICU) and the impact of any resistance on clinical outcome.**

***Methods*: Gram-positive isolates were collected from two critical care units over 1 year. All patients were screened weekly for methicillin-resistant *Staphylococcus aureus* (MRSA). Susceptibility to teicoplanin and linezolid was tested by Etest. The length of hospital and critical care unit stay and the use of antibiotics in each patient were recorded.**

***Results*: Reduced susceptibility to teicoplanin (MIC ≥ 16 mg/L) was found in 21 [3.3% (95% CI 2.0–5.0%) 6 patients] of 643 strains of MRSA versus none of 374 methicillin-susceptible *S. aureus* (MSSA) [<0.3% (95% CI 0–0.9%)]. Of 49 enterococci 3 were teicoplanin-resistant. All Gram-positive isolates were susceptible to linezolid. The length of treatment with teicoplanin and outcome of patients infected with these strains were similar to that of susceptible strains. MRSA was a more common cause of infection than MSSA but a less frequent colonizer.**

***Conclusions*: Resistance to teicoplanin remains at a comparatively low level and there was no clear relationship between susceptibility and outcome in this critically ill population. There was no resistance in Gram-positives to linezolid but this should be kept as a reserve antibiotic to maintain its activity.**

Keywords: critical care, MRSA, glycopeptides

Introduction

Heterogeneous resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) to teicoplanin is increasingly common, particularly in critical care, and isolates appear susceptible on disc testing.[1] Etest susceptibility testing is the most reliable method of detecting these organisms but is expensive. Linezolid has good activity against glycopeptide-resistant strains including staphylococci, enterococci and streptococci. As oxazolidinones are chemically unrelated to any other available antibiotic, cross-resistance is not expected. The observed spontaneous mutation rate of staphylococci after exposure to linezolid at twice the MIC has been reported to be as low as $<1 \times 10^{-9}$.[2] Nevertheless, the first cases of linezolid-resistant MRSA have been reported.[2,3]

The clinical importance of intermediate resistance to teicoplanin is unclear and the use of linezolid has been limited by adverse effects and cost. A randomized controlled trial of treatment of serious Gram-positive infection was undertaken comparing teicoplanin and linezolid over 1 year at University College London Hospitals.[4] During the study all Gram-positive isolates from microbiological specimens or screening swabs were collected to determine the prevalence of resistance to each antibiotic and any relationship to outcome.

Materials and methods

From June 2000 to June 2001 Gram-positive organisms isolated from all patients admitted for more than 48 h to the general intensive care units (ICUs) at University College Hospital NHS Trusts were analysed. The study was in support of a randomized double-blind trial comparing linezolid and teicoplanin in the treatment of Gram-positive infections.[4] Ethics approval, including for consent

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arrangements, was obtained from the UCLH Committee. All antibiotic treatment, duration and doses were recorded. The incidence of staphylococcal colonization and infection were determined from (i) screening samples (nose and groin swabs) taken on admission, weekly thereafter and at discharge and (ii) samples taken as clinically indicated, e.g. sputum, wound and blood cultures. Colonization with MRSA was defined by the presence of MRSA in nose, groin, sputum, wound or other sites that did not require treatment with an appropriate antibiotic. Infection was described as the presence of the pathogen in any clinical isolate coinciding (within 5 days) with treatment with an appropriate antibiotic (e.g. for MRSA, glycopeptide, linezolid or rifampicin/trimethoprim). *S. aureus* was detected using nutrient broth with salt (2.5% with aztreonam 75 mg/L) incubated at 37°C overnight. After overnight incubation at 37°C, the salt broth was subcultured onto mannitol salt agar (without oxacillin). Suspect colonies on the original plate at 24 and 48 h were identified and subcultured on a blood agar plate with oxacillin disc and incubated at 30°C overnight. The salt broth subculture was incubated for a further 24 h and re-examined on day 4. Patients staying more than 48 h in the ICU had swabs taken on discharge from ICU to identify MRSA acquisition in the ICU.

All Gram-positive isolates from clinical samples and screens were tested for teicoplanin and linezolid susceptibility. Initially susceptibility was determined on all isolates by the standard disc test (linezolid disc 10 μg, teicoplanin 30 μg) and then Etest (AB Biodisk, Solna, Sweden). In accordance with the manufacturer's instructions, a 0.5 McFarland inoculum was used on Mueller–Hinton agar for linezolid, reading to the hazy zone edge at 80% inhibition at 16–18 h. For teicoplanin, a 2.0 McFarland inoculum was used on brain–heart infusion agar, reading to the point of complete inhibition at 48 h. The breakpoints for linezolid and teicoplanin were 4 mg/L[5] and 8 mg/L, respectively. The Antibiotic Resistance Monitoring and Reference Laboratory confirmed teicoplanin resistance in MRSA isolates by agar dilution (Dr D. Livermore, Health Protection Agency, Centre for Infections, Colindale, UK).

Coagulase-negative staphylococci or enterococci were judged clinically significant by standard criteria, i.e. patient requiring antibiotic treatment.

Results

Antibiotic susceptibility

A total of 2569 specimens (including 179 blood, 110 sputum/tracheal aspirate and 117 wound specimens) were tested from 917 patients. *S. aureus* was isolated from 1017 specimens. Of 2023 nose/groin screening swabs, 689 (34.0%) produced a growth of *S. aureus*. The MICs of linezolid were consistently below the breakpoint for all isolates collected [MRSA n = 643, MIC_{90} 2.0 mg/L, range 0.4–4.0; methicillin-susceptible *S. aureus* (MSSA) n = 374, MIC_{90} 1.5 mg/L, range 0.8–3.0]. No resistance of *S. aureus* to teicoplanin was detected on disc testing. However, 21 [3.3%, (95%CI 2.0–5.0%) 6 patients] of the 643 strains of MRSA were found to have an MIC of teicoplanin of ≥16 mg/L by Etest versus none of 374 MSSA [0% (95%CI 0–1.0%)] (MRSA MIC_{90} 6.0 mg/L, range 0.09–32; MSSA MIC_{90} 4.0 mg/L, range 0.4–8.0) (Table 1). Of the 177 isolates (75 patients) of clinically significant coagulase-negative staphylococci, reduced susceptibility to teicoplanin (MIC ≥ 16 mg/L) was found in 72 (40.7%) strains (30 patients) by Etest (MIC_{90} 24 mg/L, range 0.8–128), compared with only 12 isolates shown to be resistant on disc testing. Linezolid was active against all strains (MIC_{90} 1.0 mg/L, range 0.2–3.0). Three (6%) of 49 isolates (2 patients) of *Enterococcus* spp. were resistant to teicoplanin on both disc testing and Etest (MIC 96–256 mg/L) but were susceptible to linezolid (MIC 0.8–2.0 mg/L).

Treatment and outcome

There were 1601 daily defined doses (DDD) of antibiotics with Gram-positive activity (median course 4 days, 0–16 days), including 147 courses of teicoplanin (median 7 days, 0–24 days), 63 courses of linezolid (median 8 days, 1–24 days) and 29 courses of vancomycin (median 5 days, 0–30 days). Staphylococci (all coagulase negative) resistant to teicoplanin (MIC ≥ 32 mg/L by Etest) were nevertheless treated with teicoplanin in three cases. One of these patients failed treatment, one improved and one was cured (as defined in an earlier paper).[4] Of a total of 27 patients infected with staphylococci with intermediate susceptibility to teicoplanin (MIC 16 mg/L, 23 coagulase-negative staphylococci, 4 MRSA), 14 were treated with teicoplanin alone for a median of 8 days (108 DDD). There was no significant difference in the length of hospital or ICU stay ($P > 0.1$, Mann–Whitney test) or mortality ($P > 0.2$, χ^2 test) for patients infected by coagulase-negative staphylococci or MRSA with reduced susceptibility to teicoplanin compared with those infected by fully susceptible strains (Table 1).

Table 1. Clinical outcome of patients according to susceptibility of the infecting pathogen to teicoplanin

Isolate:	Coagulase-negative staphylococci 177 isolates (75 patients)			MRSA 643 isolates (143 patients)		
Etest MIC:	Susceptible ≤8 mg/L	Intermediate 16 mg/L	Resistant ≥32 mg/L	Susceptible ≤8 mg/L	Intermediate 16 mg/L	Resistant ≥32 mg/L
Total isolates	100	61	11	622	19	2
Total patients	35	23	7	137	4	2
Median length of ICU stay, days (quartiles)	14 (9, 24)	22 (12, 33)	29 (23, 33)	7 (3, 19)	10 (6,20)	(10, 66)
Length of hospital stay, days (quartiles)	40 (28, 74)	40 (20, 71)	36 (34, 51)	28 (16, 47)	59 (12, 106)	(17, 84)
Patients died[a]	12	11	3	44	2	2

[a]Where more than one isolate per patient, the highest MIC was used.

Table 2. Susceptibility of *S. aureus* to linezolid and teicoplanin by site of isolation

Site	Patients (*n*, %) *n* = 917	Pathogen	Teicoplanin MIC (mg/L)			Linezolid MIC (mg/L)		
			MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}	range
Blood	22 (2.4%)	MRSA	3	4	1.5–16	1.5	2	0.75–2
	9 (1.0%)	MSSA	3	6	2–6	2	2	0.75–3
Catheter tip	20 (2.2%)	MRSA	3	8	1.5–24	1.5	2	0.38–2
	2 (0.2%)	MSSA			3–6			1
Nose/perineum	166 (18.1%)	MRSA	3	8	1.5–32	1.5	2	0.38–3
	246 (26.8%)	MSSA	3	4	0.38–8	1	1.5	0.8–3
Sputum/tracheal aspirate	48 (5.2%)	MRSA	3	8	1.5–32	1.5	2	0.75–3
	9 (1.0%)	MSSA	1.5	6	2–6	2	3	1–3
Wound	35 (3.8%)	MRSA	4	8	0.25–24	1.5	2	0.75–4
	3 (0.3%)	MSSA	3	3	2–3	1.5	2	1–2

In the ICU population, MSSA was more common in screening specimens than MRSA (Table 2). However, MRSA was significantly more frequent than MSSA in blood, catheter tip, respiratory and wound infections. The susceptibility of *S. aureus* strains to linezolid or teicoplanin was not significantly affected by the site of isolation. However the range of susceptibilities of MRSA to teicoplanin was greater than that of MSSA.

Discussion

The present study demonstrates 3–6% resistance to teicoplanin in Gram-positive isolates in the ICU. Teicoplanin-intermediate susceptible *S. aureus* has been reported from this unit.[1] No resistance to linezolid was found. However, the teicoplanin resistance did not translate into any clear clinical consequences either in the present study or in the accompanying double-blind prospective trial that showed teicoplanin to have similar efficacy to linezolid.[4] The level of resistance to teicoplanin is modest given that it has been the main antibiotic used for treatment of Gram-positive infection in these ICUs since 1990.

As expected, there was a wider range of susceptibility to teicoplanin among staphylococci than to linezolid. Increasing resistance of coagulase-negative staphylococci to teicoplanin has been observed in a longitudinal study comparing isolates over 10 years, with a fifth of isolates becoming resistant.[6] Using Etest, strains heterogeneously resistant to glycopeptides have been found in 7.6% of 250 MRSA isolates in the Netherlands, the MIC_{90} of teicoplanin being 8 mg/L with a maximum of 96 mg/L compared with 4 and 16 mg/L, respectively, of vancomycin.[7] The poor correlation between teicoplanin resistance demonstrated by disc testing and Etest is well recognized.[6] Given the potential risk of inadequate treatment of bacteraemia due to MRSA with intermediate resistance, Etest has been recommended in preference to disc testing.

Comparing fully susceptible strains of staphylococci with those with reduced susceptibility to teicoplanin, there was no significant difference in the length of hospital stay or mortality. Other manoeuvres such as intravenous line removal would contribute to outcome. Numbers are small and no allowance has been made for potential confounders for clinical outcomes. In a study of 535 patients with bacteraemia caused by coagulase-negative staphylococci, the mortality rate in the 20 patients with resistant strains was not significantly different from controls (25% versus 18%).[8] Resistance was associated with previous use of glycopeptides but not the amount of use of glycopeptides on the ward.

The predominance of MRSA in blood isolates, but not on the skin, suggests that there are differences in the invasiveness of MRSA and MSSA. A higher rate of bacteraemia in carriers of MRSA (38%) than in carriers of MSSA (9.5%) has been observed in an MRSA outbreak in 147 ICU patients.[9]

The emergence of glycopeptide intermediate-resistant and now fully resistant *S. aureus* demonstrates the importance of keeping an effective reserve antibiotic for the treatment of Gram-positive infection in the critically ill.[10] Some strains of staphylococci with reduced susceptibility to teicoplanin remain susceptible to vancomycin.[1] However, linezolid is currently active against almost all staphylococci, irrespective of their susceptibility to glycopeptides. Therefore it can be recommended in units where teicoplanin resistance is problematic. Despite 15 years of use, teicoplanin resistance in our ICUs remains uncommon and resistance appeared to have few clinical consequences. The use of linezolid therefore should be restricted to ensure that its activity is preserved.

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Transparency declarations

There were no conflicts of interest for any of the authors in the study.

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