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In vitro Gram-positive antimicrobial activity of evernimicin (SCH 27899), a novel oligosaccharide, compared with other antimicrobials: a multicentre international trial

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The antimicrobial activity of evernimicin (formerly SCH 27899), a novel oligosaccharide antimicrobial of the everninomicin class, was evaluated against four groups of Gram-positive pathogens: (i) *Streptococcus pneumoniae* (*n* = 1452); (ii) methicillin- or oxacillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (MR-CoNS; *n* = 1427); (iii) enterococci (*n* = 1517); and (iv) non-pneumococcal streptococci (*n* = 1388), using the Etest method at each study centre throughout Eastern and Western Europe, Scandinavia, South Africa, Turkey and North America. Comparative MICs were determined for a variety of reference compounds, including vancomycin, quinupristin/dalfopristin, chloramphenicol, penicillin, ampicillin, oxacillin, ceftriaxone and ciprofloxacin. Evernimicin was highly active against all strains tested, with MIC_{90} values ≤1.0 mg/L, ranging from 0.047 mg/L against *S. pneumoniae* to 1.0 mg/L against MRSA/MR-CoNS and enterococci. Compared with the reference agents, the MIC_{90} of evernimicin were lower against all species. Against MRSA and MR-CoNS the MIC_{90}s of evernimicin, quinupristin/dalfopristin and vancomycin (the three most active agents) were 1.0, 1.5 and 3.0 mg/L, respectively. Against all species tested, the relative activities and spectra of these agents were: evernimicin > vancomycin > quinupristin/dalfopristin. The Etest proved to be reliable and reproducible, despite occasional interpretive difficulties caused by observer inexperience. Quality control results were excellent among the 33 participant sites. The results of this *in vitro*, multicentre, multinational study demonstrate that evernimicin possesses high antimicrobial activity against Gram-positive organisms that compares favourably with established antibacterial treatments and newer agents such as quinupristin/dalfopristin. Further clinical investigations of everninomicin class compounds appear warranted.

Introduction

Despite many recent advances in antimicrobial therapy, resistance among Gram-positive pathogens continues to pose a serious and growing clinical problem.[1–6] Indeed, over the past 15 years, there has been a steady erosion in the activity of many antimicrobials against certain Gram-positive pathogens.[1,2] The clinical impact of this escalating resistance is underscored by the findings that *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), streptococci and enterococci are implicated as the aetiological organisms in ⩾52% of nosocomial bloodstream infections and account for nearly half of all nosocomial infections in the USA.[2,3,5,7] In addition, almost 50% of all infections in European intensive care units are attributed to staphylococci alone.[6] Resistance to antimicrobial agents among Gram-positive bacteria is increasing, particularly for many commonly prescribed antimicrobial agent classes, including the penicillinase-resistant penicillins (oxacillin and methicillin), other β-lactams, the fluoroquinolones

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(ciprofloxacin and ofloxacin) and macrolides (erythromycin, azithromycin and clarithromycin).[5] Alarmingly, resistance to antimicrobials of 'last resort' such as the glycopeptides (vancomycin and teicoplanin), prevalent among enterococci for some time, has now emerged in strains of methicillin-resistant *S. aureus* (MRSA) in Japan,[8] although similar strains among CoNS were reported over a decade earlier.[9] This serious therapeutic challenge of glycopeptide resistance in enterococci has already been accompanied by an increased infection-related morbidity and mortality, a prolongation in the duration of hospitalization and an escalation in healthcare costs.[10]

Clearly, new antimicrobials or modifications of existing compounds, are needed to treat the growing number of resistant Gram-positive pathogens effectively. The everninomicins are oligosaccharide antimicrobials, isolated from *Micromonospora carbonacea*.[11–13] The structure and stereochemistry of this class of antimicrobial have been studied extensively.[14–16] Evernimicin (SCH 27899), a novel oligosaccharide analogue of the everninomicin class, displays activity against a wide range of Gram-positive pathogens, including vancomycin-resistant enterococci (VRE), staphylococci and penicillin-resistant pneumococci.[17–21]

In this era of rapidly changing antimicrobial susceptibilities, it is important to build a baseline database of sufficient size and geographical scope, early in the development of a new antimicrobial class.[22] Future surveillance work can then, with confidence, be compared with early benchmark susceptibility test results. The purpose of the present study was to collect a large series of recent Gram-positive isolates and compare the antimicrobial activity of evernimicin against these isolates with those of several commonly used antimicrobial agents using the Etest (AB Biodisk, Solna, Sweden) method, a stable gradient MIC technology.[23]

Materials and methods

A total of 33 laboratories (11 in North America, 21 in Europe or Turkey, and one in South Africa) were recruited to provide data on the susceptibility to evernimicin and other selected antimicrobial agents. Each participant contributed results from 200 recently isolated Gram-positive clinical strains.

Organisms tested

Where possible, each participating laboratory tested 50 isolates in each of four groups of organisms: (i) methicillin- or oxacillin-resistant *S. aureus*/coagulase-negative staphylococci (MRSA/MR-CoNS); (ii) enterococci; (iii) *Streptococcus pneumoniae*; and (iv) various non-pneumococcal streptococci. The latter group included a variety of streptococci considered of clinical importance, including β-haemolytic streptococci, viridans group streptococci, *Streptococcus bovis* and *Streptococcus milleri*.

Organisms were identified to the species level by the routine methods available within each laboratory. Where species identification was not possible, the isolates were recorded as non-speciated isolates of the identified genus.

Generally, 50 recent clinical isolates in each group were collected and tested by each centre. In certain centres where collection of a particular group of organisms was problematic (as for MRSA in Scandinavia), participating laboratories were allowed to submit additional isolates from the three other groups of organisms.

Susceptibility testing methodology

The Etest allows a determination of MIC based on the inhibition created when a plastic carrier strip impregnated with a gradient antimicrobial concentration is placed on an agar plate inoculated with a particular organism and incubated overnight.[23] The MIC is defined as the point where the zone of inhibition intersects the numerical scale (15 $\log_2$ dilution steps) on the edge of the strip. For the study drugs whose MICs were to be determined, the Etest strip method was used. The 80% inhibition endpoint was used for evernimicin as recommended by the manufacturer. The following antimicrobial agents were tested using Etest strips: ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, quinupristin/dalfopristin, erythromycin, oxacillin, penicillin G, evernimicin and vancomycin. Additional diffusion discs (BD Microbiology Systems, Cockeysville, MD, USA) were placed on the 150 mm agar plates to aid in the characterization of some clinical isolates. The following antimicrobial agents were tested using conventional discs: ceftizoxime (30 μg), clindamycin (2 μg), gentamicin (120 μg) and streptomycin (300 μg). The presence of an oxacillin Etest strip on the staphylococcal plate, along with the ceftizoxime disc, permitted greater accuracy in the identification of MRSA and MR-CoNS in the absence of salt-containing agar.[24] A staphylococcal isolate was considered methicillin resistant if it was classified as 'intermediate' or 'resistant' by either method.

The use of high-concentration gentamicin and streptomycin discs on the enterococcus test plate allowed the identification of high-level aminoglycoside resistance (synergy testing) among the enterococci. National Committee for Clinical Laboratory Standards (NCCLS) guidelines[24,25] served as the method and interpretive criteria for each antimicrobial tested. The susceptibility criteria used for evernimicin were as follows: $\leqslant$4 mg/L, susceptible; 6 or 8 mg/L, intermediate; and $\geqslant$12 mg/L ($\geqslant$16 mg/L, rounded), resistant. These are proposed criteria and await regulatory approval.

Quality control

Each laboratory was provided with a set of study and manufacturer instructions for the Etest susceptibility method. To provide uniformity of testing, each laboratory was also

asked to provide three replicates of MIC data on supplied quality control organisms (*Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 29213, and *S. pneumoniae* ATCC 49619.[18,19,24] North American sites were additionally asked to provide data on seven challenge organisms (two staphylococci, three enterococci and two streptococci) chosen specifically to highlight potential susceptibility categorization problems. To pass the quality control phase of the study and proceed to the collection and testing of clinical isolates, each laboratory had to score ⩾90% for quantitative and/or susceptibility categorical accuracy.

For each laboratory, if the geometric mean MIC result for a particular group of organisms was four-fold, or more, greater or less than the geometric mean of the total study population, the entire group was excluded from the final analysis. Furthermore, laboratories were asked to send isolates with evernimicin MICs above previously specified levels (MRSA/MR-CoNS, 1 mg/L; enterococci and non-pneumococcal streptococci, 0.5 mg/L; and *S. pneumoniae*, 0.25 mg/L) to a central reference laboratory [University of Iowa College of Medicine (UICOM), Iowa City, IA, USA] for confirmation of susceptibility and further characterization. Reference laboratory values were substituted in the analysis of data where strains were repeated.

Results

A total of 6526 clinical isolates was collected and tested at 33 participating laboratories. The isolates included 1659 enterococci, 1685 MRSA/MR-CoNS, 1573 *S. pneumoniae* and 1609 non-pneumococcal strains of streptococci.

Quality control and validation of organisms

Data from three laboratories were eliminated from the survey because they failed to reach the 90% quantitative accuracy threshold[18,19,24] specified in the study protocol using ATCC control strains. Data from two laboratories (a single group of organisms each) were also excluded from the final analysis because the geometric mean MIC was either four times higher (MRSA/MR-CoNS) or four times lower (MRSA/MR-CoNS) than the all-sites geometric mean MIC for that group of organisms. Data from two other laboratories approached, but did not reach, these exclusion values. When compared with the geometric mean MIC for the group, 17 of the remaining 30 laboratories had higher geometric means and 13 had lower geometric mean results. A review of the non-pneumococcal streptococci results by the reference laboratory (UICOM) led to the elimination of 80 isolates, each of whose susceptibility pattern was not consistent with the strain as it was identified (each clearly an enterococcus).

Forty-four of 52 isolates (84.6%), originally determined to have MICs above the evernimicin MIC values specified previously, were forwarded for retesting of susceptibility by UICOM. All except one were found to be fully susceptible to evernimicin (MIC ⩽ 1 mg/L). The exception was an isolate of *S. bovis* against which the MIC of evernimicin was consistently 1.5 mg/L. Evernimicin MICs against two strains of MRSA, when retested, were 1 mg/L. In most cases, the discrepancy seems to have arisen as a result of difficulty in reading the 80% inhibition breakpoint for haemolytic isolates. All of the remaining isolates having values greater than specified MICs could not be confirmed and were eliminated from the analysis.

Of the 44 strains for which repeat MICs were performed, 25 were isolated in North America and 19 were from Europe and South Africa. These isolates consisted of 22 MRSA/MR-CoNS, 16 enterococci and six streptococci.

Susceptibility of MRSA and MR-CoNS to evernimicin

MIC_{50} and MIC_{90} values (concentrations inhibiting 50 and 90% of tested strains, respectively) for MRSA and MR-CoNS for all tested antimicrobial agents are displayed in Table I. Not all laboratories identified staphylococcal isolates to the species level. The MR-CoNS group included seven major species or groups: *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus warnerii*, *Staphylococcus* spp. and CoNS. Methicillin resistance was assessed by determining susceptibility to oxacillin and ceftizoxime and then analysing three subsets of isolates: (i) those susceptible to both oxacillin and ceftizoxime (dropped from the analysis); (ii) those resistant to both agents; and (iii) those intermediate or resistant to either cited β-lactam alone.

Evernimicin had the lowest MICs when tested against the isolates in both staphylococcal groups, with MIC_{90}s of 0.75 and 1.0 mg/L against MRSA and MR-CoNS, respectively. A significant proportion of all MRSA and MR-CoNS showed intermediate susceptibility (16.6 and 6.6%, respectively) or resistance (0.7 and 1.1%, respectively) to quinupristin/dalfopristin. Almost two-thirds of MRSA and MR-CoNS isolates (67.3%) were resistant to ciprofloxacin.

Examining the susceptibility of MRSA and MR-CoNS isolates according to the geographical distribution of study centres showed that resistance to quinupristin/dalfopristin in Scandinavia and North America was greater than that in western or eastern Europe (Table II). Resistance to erythromycin was widespread throughout the study centres, although noticeably less in Scandinavia. High susceptibility to evernimicin was maintained across all regions.

Enterococcal susceptibility to evernimicin

MIC_{50} and MIC_{90} values were ⩽1.0 mg/L and consistently lower for evernimicin than for all other tested agents against unspeciated enterococcus isolates, *Enterococcus*

Table I. Overall susceptibility of *S. aureus* isolates characterized as oxacillin- (methicillin-)resistant[a] tested against evernimicin and six comparators

	Coagulase-negative staphylococci					Oxacillin- (methicillin-)resistant				
Test method/ antimicrobial agent	no. tested	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	%R[b]	no. tested	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	%R[b]
Etest MICs	664					763				
evernimicin		0.38	1.0	100.0	0.0		0.25	0.75	100.0	0.0
quinupristin/dalfopristin		0.38	1.0	92.3	1.1		0.75	1.5	82.7	0.7
vancomycin		2.0	3.0	99.2	0.2		1.5	2.0	100.0	0.0
oxacillin[a]		256	>256	1.5	98.5		>256	>256	1.8	98.2
erythromycin		>256	>256	26.5	71.8		>256	>256	11.3	86.9
ciprofloxacin		4.0	>32	46.2	53.8		>32	>32	21.0	79.0
Antimicrobial disc	615					763				
ceftizoxime (30 μg)[a]		NA	NA	4.6	85.9		NA	NA	0.8	97.4

Abbreviations: S, susceptible; R, resistant; NA, not applicable.
[a] Strains were categorized as resistant if the oxacillin MIC was >2 mg/L or the ceftizoxime disc zone was <20 mm.[24]
[b] Susceptibility according to NCCLS criteria.[24] Proposed susceptibility criteria for evernimicin: ≤4 mg/L, susceptible; ≥16 mg/L, resistant.

faecium and *E. faecalis* (Table III). A susceptibility rate of 100% for evernimicin was recorded for all three groups. Whereas the *Enterococcus* spp. group and *E. faecalis* were both highly susceptible to vancomycin and ampicillin, susceptibility rates were markedly reduced for *E. faecium* (59.5 and 17.3%, respectively) indicating that this species has developed widespread resistance. Of the antimicrobials tested, the quinupristin/dalfopristin combination produced the lowest susceptibility rates for all three enterococcus groups tested, with *E. faecalis* being almost totally resistant to this newer agent.

Ampicillin-resistant, quinupristin/dalfopristin-susceptible enterococci (*E. faecium*) were analysed according to their vancomycin-susceptibility phenotype (data not shown). This purer population of highly probable *E. faecium* isolates[26] was fully susceptible to evernimicin, with MIC_{50} and MIC_{90} below 0.5 mg/L, regardless of glycopeptide susceptibility pattern.

Table II lists the worldwide patterns of resistance in isolates of *Enterococcus* spp. to the antimicrobials tested in this survey. There was uniform reporting of high resistance rates to quinupristin/dalfopristin throughout Europe and North America (10.3–14.4% susceptibility overall; all among *E. faecium* isolates). Significant variations in the pattern of susceptibility to chloramphenicol were noted for eastern European isolates, where only 42.5% of isolates were susceptible (57.3–65.4% for other regions).

There were very few reports of vancomycin-resistant enterococci (13 isolates) from centres in Europe (six nations) and South Africa. In contrast, each centre in the USA reported at least one VRE isolate (111 strains altogether). Of the North American VRE strains, 86.5% were *E. faecium* and the remaining strains were unspeciated. Only one vancomycin-resistant *E. faecalis* strain was documented from a medical centre in Russia. Evernimicin was highly active (MIC_{90} 0.75–1.0 mg/L) against enterococci throughout all geographical regions; no resistant strains were reported.

Susceptibility of S. pneumoniae to evernimicin

S. pneumoniae isolates were divided for analyses according to their susceptibility to penicillin (Table IV). In total, only 66.9% of *S. pneumoniae* isolates tested were susceptible to penicillin in this worldwide random sample. Patterns of resistance to penicillin did not affect the susceptibility of *S. pneumoniae* to evernimicin, quinupristin/dalfopristin or vancomycin. For evernimicin, the MIC_{50} and MIC_{90} were <0.1 mg/L in all groups. Penicillin-intermediate and -resistant isolates of *S. pneumoniae* also had marked cross-resistance to erythromycin, with only 54.9 and 35.0% of isolates, respectively, susceptible. Ceftriaxone retained some activity against penicillin-intermediate strains of *S. pneumoniae* (67.3% susceptible) but had virtually no activity against penicillin-resistant strains (4.3%).

Examination of the geographical distribution of *S. pneumoniae* resistance showed no evidence of isolates resistant to evernimicin in eastern or western Europe, Scandinavia or North America (Table II). For quinupristin/dalfopristin, highly resistant (MIC ≥ 4 mg/L) strains of *S. pneumoniae* were confined to eastern Europe, but strains in the intermediate (2 mg/L) category for quinupristin/dalfopristin were observed in all regions monitored (data not shown). Although resistance to erythromycin was apparent in both North America and Europe, more macrolide-susceptible strains were reported at centres in eastern Europe (89.1% susceptible). Erythromycin only showed 0.2—17.7% additional activity compared with penicillin against *S. pneumo-*

Table II. Worldwide susceptibility (% susceptible)[a] of *Streptococcus pneumoniae*, enterococcal and MRSA/MR-CoNS isolates to antimicrobial agents studied

	S. pneumoniae				Enterococci				MRSA/MR-CoNS			
Antimicrobial agent	central Europe (n = 366)	eastern Europe (n = 311)	Scandinavia (n = 247)	North America (n = 528)	central Europe (n = 408)	eastern Europe (n = 308)	Scandinavia (n = 243)	North America (n = 558)	central Europe (n = 355)	eastern Europe (n = 301)	Scandinavia (n = 231)	North America (n = 540)
Evernimicin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Quinupristin/dalfopristin	92.9	93.9	97.2	96.4	10.3	10.4	14.4	14.0	91.5	92.0	84.4	82.8
Vancomycin	100.0	99.4	100.0	99.2	98.5	98.4	96.5	78.7	100.0	99.0	99.1	100.0
Ampicillin	–[b]	–	–	–	90.4	78.9	83.5	74.8	–	–	–	–
Ciprofloxacin	–	–	–	–	–	–	–	–	14.9	48.5	55.4	25.9
Chloramphenicol	–	–	–	–	57.3	42.5	65.4	57.7	–	–	–	–
Erythromycin	73.2	86.5	78.5	68.8	–	–	–	–	15.5	15.6	42.4	11.5
Oxacillin	–	–	–	–	–	–	–	–	1.7	1.0	3.9	1.1
Ceftizoxime	–	–	–	–	–	–	–	–	0.3	2.4	4.5	3.0
Penicillin	73.0	71.4	66.0	60.4	–	–	–	–	–	–	–	–
Ceftriaxone	90.4	93.6	78.5	76.5	–	–	–	–	–	–	–	–
Gentamicin	–	–	–	–	79.9	58.1	88.0	73.8	–	–	–	–
Clindamycin	78.5	93.5	81.8	91.5	–	–	–	–	–	–	–	–
Streptomycin	–	–	–	–	62.3	60.1	68.7	59.7	–	–	–	–

[a] Susceptibility according to NCCLS criteria.[24] Proposed susceptibility breakpoints for evernimicin: ≤4 mg/L, susceptible: ≥16 mg/L, resistant.
[b] Not tested.

Table III. Overall susceptibility of enterococci to evernimicin and six comparators

Test method/ antimicrobial agent		*Enterococcus* spp.					*Enterococcus faecium*					*Enterococcus faecalis*				
		n	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[a]	%R[a]	*n*	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[a]	%R[a]	*n*	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[a]	%R[a]
Etest MICs																
evernimicin	Van-S	495	0.38	1.0	100.0	0.0	157	0.25	0.75	100.0	0.0	726	0.38	1.0	100.0	0.0
	Van-R	28	0.19	0.5	100.0	0.0	107	0.25	1.0	100.0	0.0	3	0.5	–[b]	100.0	0.0
quinupristin/dalfopristin	Van-S	496	16	>32	7.1	83.5	157	1.5	8.0	45.2	23.6	726	16	>32	1.7	92.6
	Van-R	28	1.	4	39.3	17.9	107	1.0	8.0	54.2	17.8	3	16	–	0.0	100.0
vancomycin	Van-S	484	2.0	3.0	100.0	0.0	157	1.0	2	100.0	0.0	721	2.0	3.0	100.0	0.0
	Van-R	28	>256	>256	0.0	100.0	107	>256	>256	0	100.0	3	6.0	—	0	100.0
ampicillin	Van-S	495	1.0	2	93.3	6.7	155	24	>256	25.9	74.1	721	1.0	1.5	97.2	2.8
	Van-R	27	64	>256	29.6	70.4	107	>256	>256	5.6	94.4	3	0.75	–	100.0	0.0
chloramphenicol	Van-S	496	8.0	64	51.8	19.0	155	8.0	24	60.6	8.4	725	8.0	128	54.8	22.1
	Van-R	28	4	12	85.7	3.6	107	6	24	64.5	4.7	3	4.0	–	100.0	0.0
Antimicrobial discs																
gentamicin	Van-S	445	–	–	80.0	16.0	157	–	–	66.9	32.5	726	–	–	77.1	22.3
	Van-R	28	–	–	46.4	50.0	107	–	–	49.5	50.5	3	–	–	100.0	0.0
streptomycin	Van-S	446	–	–	69.7	22.4	157	–	–	33.1	65.6	726	–	–	69.4	28.1
	Van-R	28	–	–	46.4	50.0	107	–	–	24.3	75.7	3	–	–	66.7	33.3

[a] Susceptibility according to NCCLS criteria.[24] Proposed susceptibility breakpoints for evernimicin: ≤4 mg/L, susceptible; ≥16 mg/L, resistant.
[b] Not applicable.

Table IV. Overall susceptibility of 'other clinically significant streptococci' and *Streptococcus pneumoniae*

	'Other clinically significant streptococci'									*S. pneumoniae*								
	penicillin-susceptible[a] (n = 1218)			penicillin-intermediate[a] (n = 137)			penicillin-resistant[a] (n = 29)			penicillin-susceptible[a] (n = 971)			penicillin-intermediate[a] (n = 364)			penicillin-resistant[a] (n = 117)		
Test method antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]
Etest MICs																		
evernimicin	0.047	0.125	100.0	0.094	0.38	100.0	0.125	0.5	100.0	0.023	0.047	100.0	0.032	0.094	100.0	0.032	0.064	100.0
quinupristin/dalfopristin	0.38	0.75	97.2	0.75	1.5	89.1	0.5	1.5	88.9	0.5	1.0	95.2	0.5	1.0	95.6	0.5	1.0	93.2
vancomycin	0.5	1.0	98.7	0.5	1.0	96.6	0.5	1.0	100.0	0.5	0.75	99.7	0.5	0.75	99.2	0.5	1.0	100.0
ceftriaxone	0.047	0.19	98.7	0.38	2.0	62.8	3.0	16	0.0	0.023	0.047	99.9	0.38	1.0	67.3	1.0	4.0	4.3
erythromycin	0.125	8.0	72.9	0.25	16	53.3	4.0	>256	17.2	0.125	0.38	88.7	0.25	>256	54.9	4.0	>256	35.0
Antimicrobial disc																		
clindamycin	–[c]	–	85.7	–	–	81.0	–	–	69.0	–	–	92.6	–	–	72.0	–	–	72.4

[a] Definitions of susceptible (≤0.064 mg/L), intermediate (≤0.094–1.0 mg/L) and resistant (≥1.5 mg/L) were taken from the NCCLS adapted to Etest.
[b] Susceptibility according to NCCLS criteria.[24] Proposed susceptibility breakpoints for evernimicin: ≤4 mg/L, susceptible; ≥16 mg/L, resistant.
[c] Not applicable.

niae, with the greatest advantage being in eastern Europe and the least advantage in central European sites.

Penicillin non-susceptible strains were more prevalent in North America (39.6%) and among the strains reported from one site in Scandinavia (34.0%). All other geographical areas had <30% penicillin-intermediate or -resistant isolates combined.

Evernimicin activity against non-pneumococcal streptococci of clinical significance

In total, 1394 strains of streptococci (other than *S. pneumoniae*) that were of clinical significance were isolated during this study (Table IV). The distribution of species was as follows: *Streptococcus agalactiae* (serogroup B; 379 strains); *Streptococcus pyogenes* (serogroup A; 455 strains); other β-haemolytic streptococci (serogroup C; 41 strains); serogroup F (nine strains); serogroup G (64 strains); other non-specified β-haemolytic streptococci (nine strains) and *S. bovis* (19 strains). Remaining streptococci were classified in the viridans group.

Bacterial isolates within this non-pneumococcal streptococci group were highly susceptible to evernimicin, regardless of their susceptibility to penicillin. The evernimicin MIC_{50} and MIC_{90} were $\leq$0.5 mg/L in all tabulated groups. All other antimicrobials showed lower *in vitro* potency against penicillin-intermediate or -resistant strains, with the quinupristin/dalfopristin results heavily and negatively influenced by the resistances among *S. bovis* strains.

Discussion

Gram-positive bacterial resistance continues to be an important clinical therapeutic problem. Increasing multidrug resistance in MRSA, MR-CoNS and enterococci and the increasing incidence of β-lactam and macrolide resistance in *S. pneumoniae* are at the forefront of current treatment concerns.[1,2,4,10] Glycopeptides such as vancomycin and teicoplanin are still considered the drugs of 'last resort' for serious infections caused by drug-resistant Gram-positive strains because of their excellent activity against these pathogens. Recently, the incidence of nosocomial VRE has been increasing, especially in some intensive care units, oncology and transplant wards.[2,3,5,10,26] Glycopeptides were used clinically for almost 30 years before the emergence of high-level resistance in *E. faecalis* and *E. faecium*.[3,5] A current concern is that high-level vancomycin resistance genes found in enterococci may be transferred to more virulent species, such as *S. aureus* or *S. pneumoniae*, which may also be resistant to other antimicrobial classes.[27]

This global study was designed to assess the *in vitro* antimicrobial activity of evernimicin against Gram-positive pathogens. The results were compared with those of other commonly used antimicrobial agents, including vancomycin, and a new streptogramin combination, quinupristin/dalfopristin.[10,26,28,29] The worldwide distribution of the survey centres in this surveillance programme also permitted analysis of global patterns of resistance to the studied compounds.

MRSA and MR-CoNS are an increasing therapeutic problem in both the USA[17,26] and Europe.[2,5–8,30] The rates of susceptibility to evernimicin among both MRSA and MR-CoNS were excellent during this study throughout both cited regions, with MIC_{90}s $\leq$ 1.0 mg/L for each species group. While the activity of quinupristin/dalfopristin against MRSA was also good, with results consistent with those published previously,[26] the frequency of strains with quinupristin/dalfopristin MICs > 1 mg/L (17.3%) appears to have increased, but only 0.7% of strains were resistant ($\geq$4 mg/L).

Although initially susceptible to the fluoroquinolones, many strains of staphylococci, particularly MRSA and MR-CoNS, now show high levels of resistance to these agents.[2,30,31] During this study, MRSA and MR-CoNS co-resistant to ciprofloxacin or erythromycin were common throughout Europe and North America. These results are in accordance with those of earlier studies with fluoroquinolones conducted in the USA and Europe.[5,26,30,32] In a CDC-based USA study, the reported resistance among MRSA to ciprofloxacin was as high as 80% (see Table I).[30]

The susceptibility results for *S. aureus*, analysed according to susceptibility to oxacillin and ceftizoxime, indicate that only the oxacillin- and ceftizoxime-susceptible population differed significantly (were more susceptible) in the rates of co-resistances. The recently modified oxacillin breakpoint criteria for CoNS[24] and the ceftizoxime susceptibility data were used to identify accurately *mecA* gene expression. The results indicate that ceftizoxime resistance may be an additional sensitive indicator of the *mecA* gene in these pathogens when compared with, or used with, oxacillin tests.

Enterococci colonize the bowel of >90% of healthy adults with *E. faecalis* accounting for >80% of enterococci in clinical infections.[3,5] The incidence of *E. faecalis* infections has also increased in recent years, probably because of widespread use of antimicrobial agents inactive against the enterococci.[2,3] Furthermore, enterococci, one of the leading causes of hospital-acquired infections, have become increasingly resistant to β-lactams primarily as a consequence of altered penicillin-binding proteins (PBPs). They are generally regarded as intrinsically resistant to cephalosporins and more recently have developed resistance to vancomycin. In contrast to the incidence of clinical enterococcal species, vancomycin resistance is more common in *E. faecium* (>50% of strains) than in *E. faecalis* (<5% of strains).[3,5]

During this study, evernimicin was highly effective *in vitro* against all *Enterococcus* spp. tested, regardless of their vancomycin resistance phenotype. In contrast, isolates of *E. faecalis* were almost completely resistant to

quinupristin/dalfopristin.[26] Activity against *E. faecium* was also lower for quinupristin/dalfopristin than for evernimicin. The activity and bactericidal action of quinupristin/dalfopristin can be greatly influenced by macrolide co-resistance in *E. faecium*,[28] and resistance has emerged on chemotherapy.[29] Examination of the susceptibility rates for *E. faecium* in relation to the quinupristin/dalfopristin and ampicillin-resistance phenotypes, revealed potential misidentification of this species in approximately 20% of cases (i.e. *E. faecalis* identified as *E. faecium*).[26] The vast majority of vancomycin resistance among enterococci, usually *E. faecium*, was reported from centres in North America.

Penicillin susceptibility or resistance had no effect on the potency of evernimicin, quinupristin/dalfopristin or vancomycin *in vitro* when tested against *S. pneumoniae*. The absence of co-resistance with these cited agents and penicillin confirms earlier reports.[26] In contrast, penicillin resistance was associated with varying degrees of co-resistance to 'third-generation' cephalosporins (ceftriaxone), erythromycin and clindamycin, which may be geographically specific. When a similar analysis was performed on the non-pneumococcal streptococci subset of strains, evernimicin also maintained its activity against those pathogens regardless of associated penicillin resistance.

Owing to their broad spectrum of activity against contemporary Gram-positive pathogen isolates, compounds like evernimicin may be a suitable alternative to glycopeptides. However, like vancomycin, evernimicin has limited activity against Gram-negative strains;[17–21] thus, when mixed bacterial infections are detected, combination therapy would be required. Nevertheless, these results are encouraging in that they suggest that agents of the everninomicin class may prove to be candidates for managing the growing number of resistant Gram-positive pathogens.

For all the species tested, the relative potencies or activities among the three most effective antimicrobials were consistent: evernimicin > quinupristin/dalfopristin > vancomycin, whereas the spectrum rank order was: evernimicin > vancomycin > quinupristin/dalfopristin. These results confirm previous findings which showed that evernimicin displayed potent activity against Gram-positive organisms,[17,20,21] exceeding the current activity documented for vancomycin. Evernimicin was clearly more active by weight than vancomycin against all tested species, including enterococci (MIC_{90} 1.0 and 4.0 mg/L for evernimicin and vancomycin, respectively) and *S. pneumoniae* (MIC_{90} 0.047 and 0.75 mg/L, respectively).

This study also confirmed previous findings by showing the Etest to be a reliable, reproducible and possibly the preferred method for assessing the evernimicin MIC.[3,19,23] Evernimicin MIC readings for staphylococci were occasionally more difficult to determine when the edges of the zones of inhibition were diffuse, but application of the 80% inhibition criteria recommended by AB Biodisk enhanced accuracy. Overall, this study confirms that the Etest was suitable for use in microbiological oligosaccharide activity surveillance programmes.[18,19] In addition to providing data on the geographical patterns of pathogen incidence, worldwide monitoring of bacterial resistance patterns (as reported in this paper) can provide early recognition of evernimicin resistance in specific bacteria,[32] which may reduce the inappropriate use of certain antibacterial agents and/or direct epidemiological interventions.[5,22]

In conclusion, the results of this *in vitro* surveillance study demonstrate that evernimicin exhibits consistent, complete antimicrobial activity against tested contemporary Gram-positive bacterial isolates. The *in vitro* efficacy of evernimicin compares favourably with that of alternative antibacterial treatments, including vancomycin and the streptogramin combination, quinupristin/dalfopristin. These results support the continued investigation of the clinical success and tolerability of compounds in the everninomicin class and provide a benchmark for future surveillance programmes of these important agents having potency against the troublesome Gram-positive cocci.

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