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Multicentre surveillance of antimicrobial resistance in enterococci and staphylococci from Colombian hospitals, 2001–2002

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Invasive isolates of staphylococci and enterococci were collected from 15 tertiary care centres in five Colombian cities from 2001 to 2002. A total of 597 isolates were available for analysis. Identification was confirmed by both automated methods and multiplex PCR assays in a central laboratory. *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) corresponded to 49.6% and 29.6% of isolates, respectively, and 20.8% were identified as enterococci. MICs of ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, linezolid, oxacillin, rifampicin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole (SXT) and vancomycin were determined using an agar dilution method as appropriate. Screening for vancomycin-resistant *S. aureus* was also carried out on brain–heart infusion agar plates supplemented with vancomycin. The presence of *mecA* and *van* genes was investigated in methicillin-resistant staphylococci and glycopeptide-resistant enterococci (GRE), respectively. All staphylococci were susceptible to vancomycin, teicoplanin and linezolid. No VISA isolates were found. In *S. aureus* and CoNS, the lowest rates of resistance were found for SXT (7.4%) and chloramphenicol (10.7%), respectively. Resistance to oxacillin in *S. aureus* and CoNS was 52% and 73%, respectively. The *mecA* gene was detected in 97.5% of methicillin-resistant *S. aureus* isolates. In enterococci, resistance to glycopeptides was 9.7%: *vanA* (58.3%) and *vanB* (41.7%) genes were found. Pulsed-field gel electrophoresis indicated that the GRE isolates were closely related. Rates of resistance to ampicillin, ciprofloxacin, chloramphenicol, rifampicin and high levels of gentamicin and streptomycin were 9.7%, 27.4%, 8.9%, 43%, 17% and 28.2%, respectively. All enterococci were susceptible to linezolid.

Keywords: staphylococci, enterococci, resistance, Colombia

Introduction

Antimicrobial resistance in staphylococci and enterococci is a growing problem worldwide with serious implications at the clinical level. The dramatic reduction of therapeutic options to treat patients infected with these microorganisms is of great concern. Specific problems in staphylococci include methicillin, glycopeptide and to a lesser extent linezolid resistance.[1,2] In enterococci, multi-resistance is now increasingly common and includes resistance to β-lactams, aminoglycosides (high level), glycopeptides and most disturbingly to oxazolidinones.[3,4] Quinupristin/dalfopristin, a streptogramin antibiotic, also has a spectrum of *in vitro* activity against clinically relevant Gram-positive organisms, including staphylococci, streptococci and vancomycin-resistant enterococci (excluding *Enterococcus faecalis*).[5] However, resistance to these groups of antibiotics has also been reported.[6,7] Resistance determinants are widely distributed in isolates of both

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enterococci and staphylococci. High-level resistance to methicillin requires the presence of the *mecA* gene, which can be transferred horizontally.[8,9] In enterococci, the *van* genes are usually associated with mobile elements, which can also disseminate successfully.[10]

Colombia has particular characteristics that directly influence the emergence and dissemination of antibiotic resistance, including: (i) availability of 'over the counter' compounds; (ii) a high referral rate between medical institutions, which is likely to favour the dissemination of resistant clones; and (iii) lack of multicentre surveillance data on antimicrobial resistance for several microorganisms, which prevents closer monitoring of the problem.

We carried out the first multicentre surveillance of antimicrobial resistance in *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS) and *Enterococcus* spp. in Colombian hospitals spanning a year (March 2001–March 2002). The study included 15 tertiary care hospitals in five major cities across the country.

Materials and methods

Study design

From March 2001 to March 2002, tertiary care hospitals in Bogotá (eight hospitals), Cali (three hospitals), Medellín (one hospital), Bucaramanga (two hospitals) and Cartagena (one hospital) were asked to collect up to 60 consecutive clinical isolates (defined as likely to be causing infection for which there was an intention to treat) of either *S. aureus*, CoNS or *Enterococcus* spp. from the following clinical samples: blood, surgical wound, urine, peritoneal fluid, abdominal abscess, joint aspirate, osteomyelitis aspirate, bronchoalveolar lavage, pleural fluid, pericardial effusion, cerebral abscess and cerebrospinal fluid. Isolates excluded from the study included duplicate organisms from the same patient and those coming from sputum, catheters or skin (unless originating in an infected surgical wound). Each hospital identified the microorganisms using either automated (Vitek or MicroScan) or manual methods. Once an isolate was included in the study, the corresponding hospital sent the isolate to the reference laboratory (located in Bogotá) via courier, using a transport medium (Amies, BBL, Franklin Lakes, NJ, USA). Upon arrival, the reference laboratory confirmed the purity of the isolate and confirmed identification by the Vitek Gram-positive identification card (bioMérieux, Marcy l'Étoile, France) and by molecular methods using multiplex PCR for enterococci and staphylococci (see below).

Susceptibility testing by the reference laboratory and screening for VISA isolates

Susceptibility tests for staphylococci and enterococci were carried out using an agar dilution method following the recommendations of the NCCLS with an inoculum of 10^4 cfu/spot.[11] For staphylococci, Mueller–Hinton agar (ICN Biomedicals, Inc., Aurora, OH, USA) was supplemented with 2% NaCl. For both staphylococci and enterococci, isolates were incubated in ambient air at 35°C and MIC results were read after 20 h incubation. For vancomycin, teicoplanin and oxacillin, results were read at 24 h. The following antimicrobial agents were tested against staphylococci: ciprofloxacin, chloramphenicol, erythromycin, gentamicin, linezolid, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole (SXT), oxacillin, rifampicin and vancomycin. Enterococcal isolates were tested against ampicillin, ciprofloxacin, chloramphenicol, linezolid, rifampicin, teicoplanin, tetracycline and vancomycin. High levels of resistance to streptomycin (2000 mg/L) and gentamicin (500 mg/L) were also investigated in all enterococci as described previously.[11,12] All MIC determinations were carried out with the inclusion of reference strains as controls. These included *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212.

All methicillin-resistant *S. aureus* (MRSA) isolates were screened for intermediate levels of resistance to vancomycin (VISA isolates) following the published recommendations of Tenover *et al.*[13] Briefly, 10 μL of a bacterial suspension at a turbidity equivalent to that of a 0.5 McFarland Standard was inoculated in plates of brain–heart infusion agar (BHI, ICN Biomedicals, Inc.) containing 2, 4 or 6 mg/L vancomycin. Further quality control was achieved by carrying out susceptibility testing of 10% of all isolates at the National Microbiology Reference Laboratory, Instituto Nacional de Salud, Bogotá, Colombia.

Molecular methods

Staphylococci. All isolates of *S. aureus* and CoNS were subjected to a multiplex PCR assay following the protocol of Martineau *et al.*,[14] which allows species-specific identification of *S. aureus* and *Staphylococcus epidermidis* and detection of the *mecA* gene. Oxacillin-resistant staphylococci in which *mecA* was not detected were subjected to a second multiplex PCR assay, which included primers for the *blaZ* gene instead of those for the *mecA* gene.[14] Methicillin-resistant *S. aureus* ATCC 43300 was used as a positive control for all experiments.

Enterococci. Identification of all isolates of enterococci was confirmed by PCR as described previously.[15,16] Vancomycin-resistant isolates (MIC ≥ 4 mg/L) were further characterized by PCR to detect specific genotypes.[15] *Enterococcus faecium* BM4147 (*vanA*), *E. faecalis* V583 (*vanB*) and *Enterococcus gallinarum* BM4174 (*vanC-1*) were used as control strains. Molecular typing of both glycopeptide-resistant *E. faecalis* and *E. faecium* was carried out by pulsed-field gel electrophoresis (PFGE) as described previously.[17] Restriction of DNA was carried out with *Sma*I. Fragments were separated

by agarose gel electrophoresis (CHEF DRII apparatus, Bio-Rad Laboratories, Richmond, CA, USA) at 6 V/cm with switch times ramped from 1 to 35 s over 23 h at 14°C. Following staining with ethidium bromide, the restricted DNA fragments were visualized under UV light and photographed. A previously characterized glycopeptide-resistant strain of *E. faecium* (known to be the first GRE isolated in the country) was included in the electrophoresis gel.[17] The interpretation of the band patterns was carried out according to the criteria of Tenover *et al*.[18]

Statistical analyses

Differences in resistance patterns between isolates from Bogotá and Cali were calculated using the χ^2 test (Epi info 6.04d, CDC, Atlanta, GA, USA) for each antimicrobial agent. A P value of <0.05 was considered statistically significant.

Results

Bacterial isolates

A total of 663 isolates were sent to the reference laboratory from the participating hospitals, of which 66 were discarded due to contamination or misidentification. Only isolates with agreement between microbiological and molecular identification methods were included. From the 597 that were available for evaluation and susceptibility tests, *S. aureus* comprised 296 (49.6%) of the isolates. Surgical wound infection, blood and joint aspirate were the most common sources, accounting for 36%, 30% and 8%, respectively. A total of 177 (29.6%) isolates were identified as CoNS, of which the majority (62%) were *S. epidermidis*. Other species of CoNS identified by the automated Vitek system included *Staphylococcus saprophyticus*, *Staphylococcus auricularis*, *Staphylococcus haemolyticus*, *Staphylococcus sciuri*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus simulans* and *Staphylococcus warneri*. Most CoNS were isolated from blood (60%), surgical wound infection (16%) and urine (6%).

Enterococci comprised 20.8% (124) of isolates. The majority (82%) were *E. faecalis* isolated mostly from urine (33%) and surgical wounds (27%). *E. faecium* comprised 14% (18) of enterococcal isolates. Most common sources of *E. faecium* included surgical wound infection (33%), urine (22%) and blood (17%), and were sent mainly from hospitals in Bogotá (17 isolates). *Enterococcus avium* (also identified by PCR), *Enterococcus hirae* and *Enterococcus durans* accounted for the remaining isolates.

Susceptibilities, resistance genes and genotyping

S. aureus. Table 1 shows the MIC distributions and resistance rates for *S. aureus*. The overall prevalence of MRSA amongst consecutive isolates of *S. aureus* was 52%. In contrast to methicillin-sensitive *S. aureus* (MSSA), MRSA isolates exhibited higher rates of resistance to most antibiotics (Table 1). The highest rates of resistance were found with erythromycin (89%), gentamicin (86%) and ciprofloxacin (83%). Resistance rates for SXT and rifampicin were 8% and 17%, respectively (Table 1). As expected, MRSA isolates were all susceptible to glycopeptides and linezolid. No VISA isolates were found. MICs of linezolid were between 0.25 and 4 mg/L. No specific difference was found between MSSA and MRSA, although one MSSA isolate exhibited an MIC of 0.12 mg/L (tested three times). Linezolid MIC_{90}s for MRSA and MSSA were 2 and 4 mg/L, respectively. Among MRSA, 97.5% (151 isolates) carried the *mecA* gene. The remaining isolates (four) were positive for the *blaZ* gene.[19] Oxacillin MICs for these four isolates were ≥64 mg/L. The *mecA* gene was not detected in any of the MSSA isolates.

CoNS. MIC distributions and resistance rates for CoNS are shown in Table 2. A high proportion of isolates were methicillin resistant (73%). High resistance rates were also found to erythromycin, SXT and gentamicin. CoNS were less resistant to ciprofloxacin than MRSA (29% versus 83%, respectively) (Table 2). Apart from glycopeptides and linezolid, rifampicin and chloramphenicol exhibited the lowest rates of resistance (Table 2). No teicoplanin or vancomycin resistance was observed. The majority of isolates exhibited linezolid MICs between 0.12 and 2 mg/L. Only one isolate had an MIC of 4 mg/L (re-tested and confirmed).

PCR for the *mecA* gene in CoNS indicated that 87.7% (114 isolates) of methicillin-resistant isolates carried the *mecA* gene. The *blaZ* gene was detected in nine methicillin-resistant CoNS that did not yield positive results for the *mecA* gene. We were unable to detect either *blaZ* or *mecA* in seven methicillin-resistant CoNS. Oxacillin MICs for these isolates ranged from 0.5 to 1 mg/L and were non-*epidermidis* species (three *S. hominis*, one *S. haemolyticus*, one *S. sciuri* and two not identified at the species level).

Enterococci. Resistance rates and MIC distributions for the enterococci are shown in Table 3. All *E. faecalis* were susceptible to ampicillin. Resistance to ciprofloxacin was 25%. In contrast, for *E. faecium*, resistance rates for the same antibiotics were 67% and 55%, respectively. From 18 *E. faecium* isolates collected during the year, nine (50%) exhibited high-level resistance to streptomycin (>2000 mg/L), three (17%) to gentamicin (>500 mg/L) and one to both antibiotics. The phenotypic and genotypic characteristics of the GRE are shown in Table 4. Five vancomycin-resistant *E. faecalis* isolates were received from hospitals in Bogotá. Two were isolated from surgical wound infections. Urine, peritoneal fluid and blood were the sources of the other three isolates. Multiplex PCR for the *van* genes revealed the presence of the *vanB* gene in all five isolates that correlated with the pheno-

Table 1. MIC distribution for *S. aureus*

Organism (no. of isolates)	Antimicrobial agent	No. of isolates with indicated MIC (mg/L)																	
		0.0075	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	≥1024	
			0.015/0.3	0.03/0.6	0.06/1.2	0.12/2.4	0.25/4.8	0.5/9.5	1/19	2/38	4/76	8/152	16/304	32/608	64/1216[a]					%R
S. aureus	oxacillin	–	–	–	2	28	56	48	6	1	4	2	5	5	**139**	–	–	–	–	52
(296)	erythromycin	–	–	2	2	8	53	58	15	6	2	5	–	8	**137**	–	–	–	–	51
	tetracycline	–	–	–	–	5	46	110	33	4	12	8	13	31	**34**	–	–	–	–	26
	chloramphenicol	–	–	–	–	1	–	1	2	14	87	108	14	14	**51**	4	–	–	–	23
	gentamicin	–	–	–	–	15	56	61	4	4	4	1	8	4	4	24	44	**50**	17	51
	vancomycin	–	–	–	–	–	5	48	202	**31**	10	–	–	0	–	–	–	–	–	0
	teicoplanin	–	–	–	1	6	23	84	**166**	11	5	–	–	0	–	–	–	–	–	0
	linezolid	–	–	–	–	1	11	18	91	142	**33**	–	–	0	–	–	–	–	–	0
	ciprofloxacin	–	–	3	1	11	55	67	14	12	2	9	83	**39**	–	–	–	–	–	45
	rifampicin	10	39	72	62	57	6	4	4	11	11	5	2	2	1	7	3	–	–	10
	SXT	–	1	2	11	43	84	99	**33**	1	4	2	1	3	12	–	–	–	–	7
MRSA	oxacillin	–	–	–	–	–	–	–	–	–	4	2	5	5	**139**					100
(155)	erythromycin	–	–	1	–	–	2	6	3	4	1	3	–	6	**129**	–	–	–	–	89
	tetracycline	–	–	–	–	4	19	62	13	2	11	6	9	**13**	16	–	–	–	–	24
	chloramphenicol	–	–	–	–	–	–	–	–	5	33	40	9	14	**50**	4	–	–	–	44
	gentamicin	–	–	–	–	3	7	8	1	–	2	–	4	3	–	22	41	**48**	16	86
	vancomycin	–	–	–	–	–	2	24	102	**21**	6	–	–	0	–	–	–	–	–	0
	teicoplanin	–	–	–	1	6	12	49	**76**	8	3	–	–	0	–	–	–	–	–	0
	linezolid	–	–	–	–	–	4	8	58	**74**	11	–	–	0	–	–	–	–	–	0
	ciprofloxacin	–	–	–	–	1	6	9	3	7	1	9	83	**36**	–	–	–	–	–	83
	rifampicin	8	25	33	24	21	3	3	3	9	10	4	1	1	1	6	3	–	–	17
	SXT	–	–	1	1	7	36	75	**23**	–	2	1	–	3	6	–	–	–	–	8
MSSA	oxacillin	–	–	–	2	28	56	**48**	6	1	0	–	–	–	–	–	–	–	–	0
(141)	erythromycin	–	–	1	2	8	51	52	12	**2**	1	2	–	2	8	–	–	–	–	8
	tetracycline	–	–	–	–	1	27	48	20	2	1	2	4	18	**18**	–	–	–	–	28
	chloramphenicol	–	–	–	–	1	–	1	2	9	54	**68**	5	0	1	–	–	–	–	1
	gentamicin	–	–	1	–	12	48	53	3	4	2	1	4	1	4	2	3	2	1	12
	vancomycin	–	–	–	–	–	3	24	**100**	10	4	–	–	0	–	–	–	–	–	0
	teicoplanin	–	–	–	–	–	11	35	**90**	3	2	–	–	0	–	–	–	–	–	0
	linezolid	–	–	–	–	1	7	10	33	68	**22**	–	–	0	–	–	–	–	–	0
	ciprofloxacin	–	–	3	1	10	49	58	**11**	5	1	–	–	3	–	–	–	–	–	3
	rifampicin	2	14	39	38	**36**	3	1	1	2	1	1	1	1	–	1	–	–	–	4
	SXT	–	–	2	10	36	48	24	**10**	1	2	1	1	–	6	–	–	–	–	7

[a]Dilution ranges of SXT (trimethoprim/sulfamethoxazole).
Breakpoint (mg/L) underlined; MIC_{90} values in bold.

Table 2. MIC distribution for CoNS

Organism (no. of isolates)	Antimicrobial agent	No. of isolates with indicated MIC (mg/L)																	%R
		0.0075	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	≥1024	
		0.015/0.3		0.03/0.6	0.06/1.2	0.12/2.4	0.25/4.8	0.5/9.5	1/19	2/38	4/76	8/152	16/304	32/608	64/1216[a]					
CoNS (177)	oxacillin	–	–	–	1	25	21	11	25	20	10	7	4	8	**45**	–	–	–	–	73
	erythromycin	–	–	1	2	9	34	18	4	2	3	3	5	8	**88**	–	–	–	–	59
	tetracycline	–	–	–	1	1	19	34	18	28	17	4	7	19	**29**	–	–	–	–	31
	chloramphenicol	–	–	–	–	5	–	2	18	51	48	27	7	5	11	3	–	–	–	11
	gentamicin	–	–	–	–	46	16	3	1	5	5	6	23	23	21	**11**	10	6	1	54
	vancomycin	–	–	–	–	4	3	16	69	**78**	7	–	–	–	–	–	–	–	–	0
	teicoplanin	–	–	–	–	7	9	13	53	54	**41**	–	–	–	–	–	–	–	–	0
	linezolid	–	–	–	–	7	6	37	85	**41**	1	–	–	–	–	–	–	–	–	0
	ciprofloxacin	–	4	5	7	26	53	21	4	5	6	11	9	**26**	–	–	–	–	–	29
	rifampicin	–	–	–	–	118	–	4	11	18	4	2	–	**2**	2	9	7	–	–	15
	SXT	–	2	3	8	8	14	23	20	4	18	14	15	20	**28**	–	–	–	–	54

[a]Dilution ranges of SXT (trimethoprim/sulfamethoxazole).
Breakpoint (mg/L) underlined; MIC_{90} values in bold.

type: high-level resistance to vancomycin (≥256 mg/L) and susceptibility to teicoplanin (1 mg/L). Genotyping by PFGE yielded a similar DNA banding pattern in all five vancomycin-resistant *E. faecalis* isolates (designated pattern A, Table 4). One band difference was noted in two isolates (pattern A1, Table 4), and one isolate exhibited a difference in two DNA fragments (pattern A2). The results indicated that all isolates were closely related.[18]

Resistance to glycopeptides in *E. faecium* was 39% (seven isolates). Four were isolated from surgical wounds, two from blood and one from pleural fluid. High-level resistance to both vancomycin (≥512 mg/L) and teicoplanin (≥32 mg/L) and the *vanA* gene were found in all of them. All seven isolates were resistant to ampicillin, streptomycin (high levels), ciprofloxacin and rifampicin. Only one isolate was resistant to high levels of gentamicin. All glycopeptide-resistant *E. faecium* were susceptible to chloramphenicol and linezolid (MICs ranged from 0.5 to 4 mg/L). Genotyping by PFGE also showed that the isolates were closely related. Five isolates had an identical banding pattern on PFGE (designated pattern F, Table 4). The remaining two isolates exhibited two and three band differences (patterns F1 and F2, respectively) (Table 4). Interestingly, the isolate with restriction pattern F2 could be distinguished phenotypically from the others for its resistance to high levels of gentamicin (Table 4, isolate 404). All glycopeptide-resistant *E. faecium* were isolates from hospitals in Bogotá and were genotypically different (more than four DNA fragment differences on PFGE) from the first Colombian GRE, which emerged in the city of Medellín in 1998.[17]

Discussion

The impact of antimicrobial resistance in a particular region ranges from failure in an individual patient to respond to therapy, to serious implications for prescribing, to hospital costs and the choice of optimal empirical therapy.[20,21] The design of surveillance studies should be goal-orientated.[21] Colombia is a country where antibiotic prescription policies are very relaxed ('over the counter' antibiotics are widely available and self-medication is an accepted practice in the population) and information on antimicrobial resistance rates at a national level is lacking for many microorganisms (national surveillance data on antimicrobial resistance is only available for pneumococci). The aims of this study were: (i) to determine the frequency of isolation of invasive isolates of staphylococci and enterococci; (ii) to characterize resistance patterns to antibiotics; and (iii) to investigate genetic determinants of resistance to β-lactams and glycopeptides (in staphylococci and enterococci, respectively), which are currently prevalent among hospital isolates. All isolates were studied and characterized in a central (reference) laboratory with a very strict methodology for identification to avoid bias and inter-laboratory variations. Since there is significant

Table 3. MIC distributions for *E. faecalis* and *E. faecium*

		No. of isolates with indicated MIC (mg/L)																	
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024		
Organism (no. of isolates)	Antimicrobial agent															>500[a]		>2000[a]	%R
E. faecalis (101)	ampicillin	–	–	–	–	29	45	4	**19**	4	0	–	–	–	–	–	–		0
	chloramphenicol	–	–	–	–	–	2	7	29	36	16	7	4	–	–	–	–		11
	streptomycin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26	26
	gentamicin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	18	–		18
	vancomycin	–	–	–	–	10	32	42	**12**	–	–	0	–	–	4	1	–		5
	teicoplanin	1	–	–	32	40	**24**	3	1	–	–	0	–	–	–	–	–		0
	linezolid	–	–	–	5	1	16	68	**11**	–	–	0	–	–	–	–	–		0
	ciprofloxacin	–	–	2	–	15	34	25	2	–	2	**19**	2	–	–	–	–		25
	rifampicin	1	–	–	–	4	20	34	16	**21**	2	1	–	1	–	–	1		42
E. faecium (18)	ampicillin	–	–	–	–	1	1	1	3	–	1	–	1	**9**	1	–	–		67
	chloramphenicol	–	–	–	–	–	1	7	3	**5**	2	0	–	–	–	–	–		0
	streptomycin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	50
	gentamicin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–		17
	vancomycin	–	–	–	–	4	4	3	–	–	–	0	–	–	–	**7**	–		39
	teicoplanin	–	–	–	1	4	5	1	–	–	–	7	–	–	–	–			39
	linezolid	–	–	–	–	1	3	11	**3**	–	–	0	–	–	–	–	–		0
	ciprofloxacin	1	–	–	1	1	2	3	1	–	–	**9**	–	–	–	–	–		55
	rifampicin	2	–	–	1	2	–	1	2	**8**	1	–	–	1	–	–	–		67

[a]High-level resistance to gentamicin (>500 mg/L) and streptomycin (>2000 mg/L).
Breakpoint (mg/L) underlined; MIC_{90} values in bold.

Table 4. Phenotypic and genotypic characteristics of GRE from Bogotá isolated from March 2001 to March 2002

Species	Isolate code	Hospital	Antimicrobial susceptibility[a]		*Sma*I pattern
			R	S	
E. faecium	272	INC	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F
	273	INC	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F
	351	INC	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F
	352	INC	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F
	382	HSI	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F
	477	HSI	AMP, STR, VAN, TEI, CIP, RIF	GEN, CHL, LNZ	F1
	404	HMC	AMP, STR, GEN, VAN, TEI, CIP, RIF	CHL, LNZ	F2
E. faecalis	380	FSFB	VAN, STR, GEN, CIP	AMP, CHL, LNZ, TEI, RIF	A
	381	FSFB	VAN, STR, GEN, CIP	AMP, CHL, LNZ, TEI, RIF	A2
	383	HMC	VAN, STR, GEN, CIP	AMP, CHL, LNZ, TEI, RIF	A
	384	HMC	VAN, STR, GEN, CIP	AMP, CHL, LNZ, TEI, RIF	A1
	403	HMC	VAN, STR, GEN, CIP	AMP, CHL, LNZ, TEI, RIF	A1

INC, Instituto Nacional de Cancerología; HSI, Hospital San Ignacio; FSFB, Fundación Santa Fé de Bogotá; HMC, Hospital Militar Central. AMP, ampicillin; STR, streptomycin; VAN, vancomycin; TEI, teicoplanin; CIP, ciprofloxacin; RIF, rifampicin; GEN, gentamicin; CHL, chloramphenicol; LNZ, linezolid; R, resistant; S, susceptible.
[a]High-level resistance to gentamicin and streptomycin: >500 and >2000 mg/L, respectively.

variation in policies across Colombian cities regarding antibiotic usage by hospitals and physicians, hospitals from different regions were included in this surveillance. It is clear that regional differences are important: for example, when rates of oxacillin resistance in *S. aureus* isolates from Cali (population ~2 million) and Bogotá (~8 million) were compared, MRSA were significantly more frequent in Cali than in Bogotá (78% versus 43%, respectively) ($P < 0.05$).

The overall rate of methicillin resistance among *S. aureus* in our study was 52%, which is significantly higher than in other parts of the world.[22] As found by others, higher resistance rates to other antibiotics were seen for MRSA than for MSSA.[23,24] Resistance to ciprofloxacin, erythromycin and gentamicin was found in >83% of the MRSA isolates. Nonetheless, rates of resistance to SXT and rifampicin were relatively low in Colombian MRSA. Hence, as in other parts of the world, these antibiotics might be used as alternatives for the treatment of MRSA in selected cases.[25] None of the *S. aureus* isolates was resistant to teicoplanin or vancomycin. Screening for VISA isolates was negative, indicating that reduced susceptibility to glycopeptides has not yet emerged in this country. In South America, VISA isolates have already been reported in Brazil.[26] Linezolid also exhibited good activity against all *S. aureus* isolates with MICs ≤ 4 mg/L.

Antibiotic resistance in MRSA is directly related to the successful dissemination of specific clones. In Colombia, the first study on the molecular characterization of MRSA isolates (recovered in Bogotá between 1996 and 1998) showed the prevalence of a single multiresistant clonal type (designated II::NH::D).[27] This clone was previously described among paediatric (almost exclusively) MRSA isolates recovered in the early 1990s in European, New York and South American hospitals. The MRSA isolates in this study, however, differed phenotypically from the clone II::NH::D. Resistance to rifampicin, SXT and tetracycline was lower compared with other antibiotics (17%, 8% and 24%, respectively). The same study also noted that a new clonal type emerged in 1998 in a single isolate: the organism showed no similarity to any of the major international clones identified previously, and exhibited resistance to oxacillin, ciprofloxacin and erythromycin but not to rifampicin, SXT or tetracycline: a pattern that resembles the antibiotic profile of the isolates of the current study.[27] Molecular epidemiology analysis of the isolates is currently under way to determine whether a clonal shift in Colombian MRSA has occurred.

As expected, the vast majority of MRSA in the current study carried the *mecA* gene. We were unable to detect the *mecA* gene in four MRSA isolates. The presence of the *blaZ* gene in these organisms indicates that they are likely to produce β-lactamase. These four isolates were also resistant to erythromycin and tetracycline but susceptible to the other antibiotics.

The resistance rate to oxacillin among CoNS was 73%, using the NCCLS breakpoint (≥0.5 mg/L). Among the nine isolates that were negative for the *mecA* gene and positive for the *blaZ* gene, oxacillin MICs were ≥64 mg/L. Seven isolates (all non-*epidermidis*) were negative for both *mecA* and *blaZ*, with MICs ranging between 0.5 and 1 mg/L; these isolates would have been reported susceptible if a breakpoint of ≤2 mg/L (e.g. British Society for Antimicrobial Chemotherapy recommendations) had been used. All isolates were susceptible to the glycopeptides (teicoplanin and vancomycin)

and linezolid. Chloramphenicol and rifampicin exhibited low levels of resistance against CoNS (11% and 15%, respectively). Rifampicin has been widely used in combination for the treatment of CoNS infections.[28–30] The clinical value of chloramphenicol in this setting is unclear.

The first cluster of glycopeptide-resistant *E. faecium* in Colombia was identified in the city of Medellín in a single hospital in 1998.[17] These isolates, which belonged to a unique clonal type, harboured the *vanA* gene cluster and were resistant to all antibiotics except chloramphenicol, linezolid and nitrofurantoin.[17] This hospital did not participate in the current surveillance study; all the GRE reported here were identified at hospitals in Bogotá (Table 4). Amongst the *vanA*-carrying *E. faecium*, all were resistant to ampicillin (MIC > 128 mg/L) and high levels of streptomycin but only one was resistant to high levels of gentamicin. PFGE analysis indicated that all isolates were closely related, suggesting that clonal dissemination of GRE has already occurred amongst hospitals in Bogotá. The genotyping results also indicate that a different clonal type of GRE from that of Medellín is present in Bogotá. These data suggest that the prevalence of GRE in Colombia is likely to increase as specific clones disseminate to other hospitals as well.

Apart from linezolid, chloramphenicol was active against all isolates of *E. faecium* (including glycopeptide resistant). Several reports have documented the efficacy of chloramphenicol for the treatment of VRE infections, including endocarditis and infections in immunocompromised patients.[31–33] Although large clinical trials are not available to recommend chloramphenicol as first line therapy for VRE in Colombia, it emerges as an interesting alternative.

Unlike *E. faecium*, the majority of *E. faecalis* isolates were susceptible to all antibiotics tested. Resistance to vancomycin was present in five isolates. All of them exhibited the VanB phenotype, harboured the *vanB* gene and were resistant to ciprofloxacin and high levels of gentamicin and streptomycin. Ampicillin, teicoplanin, linezolid and rifampicin were active against all VanB *E. faecalis*. As occurred with *E. faecium*, PFGE analysis indicated that all *E. faecalis* isolates were closely related, suggesting that a single clonal type was present in the two hospitals where the organisms were isolated.

Linezolid, a member of the oxazolidinone group, was recently launched in Colombia for the treatment of Gram-positive infections. The antibiotic showed activity against all isolates in this study, which indicates that it is a promising therapeutic alternative in particular clinical settings. It is important to note, however, that resistance to linezolid has already been reported in both enterococci and staphylococci clinical isolates and was associated with prolonged courses.[1,4,34] Attention to proper dosing, specific indications and monitoring of susceptibility is recommended for all patients chosen to be treated with linezolid.

Quinupristin/dalfopristin consists of a 30:70 ratio of two different streptogramin antibiotics that bind to separate sites on the bacterial ribosome and are active against a broad variety of multidrug-resistant Gram-positive organisms.[6] Quinupristin/dalfopristin has been available in other parts of the world for the treatment of infections caused by vancomycin-resistant *E. faecium*. However, this antibiotic has not been released in Colombia and it is not available in this country.

In summary, *S. aureus* was the most prevalent Gram-positive invasive organism in Colombian hospitals during this surveillance. High rates of resistance to methicillin were observed. GRE are emerging pathogens in Colombian hospitals, although most isolates remained susceptible to other antibiotics. Linezolid was the only compound with activity against all staphylococci and enterococci.

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