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Prospective Study Using Standardized Methodology for Antimicrobial Susceptibility of Gram-positive Cocci Isolated from The Puerto Rico Medical Center

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The Gram-positive cocci (GPC), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*, have become important causes of community and nosocomial-acquired infections. The prevalence of multiple resistant isolates to standard antimicrobial drugs has significantly increased over the past decades. Few prospective studies have been performed in Puerto Rico (PR) concerning the GPC antimicrobial susceptibilities pattern. The purpose of this study was to evaluate the *in vitro* susceptibility of GPC clinical isolates from PR to selected standard antibiotics and to the new antimicrobial agents, linezolid (LZ), quinupristin/dalfopristin (Q/D) and gemifloxacin (GM). The *in vitro* susceptibility utilizing disk diffusion and Etest methods to selected antibiotics was determined for a total of 429 isolates obtained during a period of 5 months from the Puerto Rico Medical Center Bacteriology Laboratory. The distribution of GPC collected was as follows: 213 *S. aureus* isolates, 162 *E. faecalis*, 16 *E. faecium* and 38 *S. pneumoniae*. The results of the susceptibility test demonstrated: 1) that in *S. aureus*, 100% of the isolates were susceptible to vancomycin (VAN), LZ and Q/D; 93% to GM; and 61% to methicillin/oxacillin; 2) in *S. pneumo*niae, 100% were susceptible to LN and GM; 87% to Q/D; and 53% to penicillin; 3) in *E. faecalis*, 99% were susceptible to ampicillin; 93% to LZ; 79% to GM; 78.6% to VAN; and 0% to Q/D. Sixty eight and 66% of the *E. faecalis* isolates were susceptible to gentamicin and streptomycin respectively; and 4) in *E. faecium*, 100% were susceptible to LZ; 94% to Q/D; 69% to GM; 37.5% to VAN and 20% to ampicillin. In *E. faecium* isolates, 50% and 31% were susceptible to gentamicin and streptomycin, respectively. Of the vancomycin resistant enterococci, 88.9% and 21% of *E. faecium* and *faecalis* showed VanA phenotypic resistance, respectively. These results show that there is a significant degree of antimicrobial resistance in GPC, including 38% methicillin resistance in *S. aureus*, a near 50% penicillin resistant *S. pneumoniae*, and a significant resistance of enterococcal species to VAN. The new agents, LZ, Q/D and GM, proved to be effective against both, *S. aureus* and *S. pneumoniae*. For *E. faecium*, both, LZ and Q/D were active, while for *E. faecalis*, only LZ showed consistent activity.

Key words: Gram-positive cocci, Antibiotic resistance, Antimicrobial agents, Antimicrobial resistant phenotype, Antimicrobial surveillance

The Gram-positive cocci, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*, are important causes of morbidity and mortality in community as well as in nosocomial-acquired infections (1-3). The prevalence of strains with multiple resistances to standard antimicrobial drugs has significantly increased over the past several years. In some institution, over 40% of the *S. aureus* isolates have shown resistance to methicillin-oxacillin (MRSA) (1,4). Enterococcal isolates resistant to penicillins, aminoglycosides (high-level aminoglycoside resistance, HLAR) and to vancomycin (VRE) has been responsible for serious infections (5-8). A worldwide significant increase in penicillin resistant strains of *S. pneumoniae* (PRP) has been reported during the last two decades (1-3,9).

Two new drugs have recently been approved for the treatment of serious infections caused by VRE and/or MRSA: quinupristin/dalfopristin (Synercid, Rhône-Poulenc Rorer Pharmaceuticals, Inc.) a streptogramin

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(10,11,15) antibiotic and the oxazolidinone, linezolid (Zyvox, Pharmacia Corporation) (12-15).

Third generation fluoroquinolones have demonstrated consistent activity against PRP and other gram-positive and gram-negative bacteria. Gemifloxacin (Glaxo-SmithKline Pharmaceuticals), a new fluoroquinolone not yet approved by the FDA, has excellent *in vitro* and *in vivo* activity against PRP (16-18).

Few prospective studies have been performed in Puerto Rico concerning the antimicrobial resistance pattern of these Gram-positive cocci. The objectives of this study were to evaluate the *in vitro* susceptibility of clinical isolates of *Staphylococcus aureus*, *Enterococcus faecalis, Enterococcus faecium* and *Streptococcus pneumoniae* to selected standard antibiotics and to the new antimicrobial agents quinupristin/dalfopristin, linezolid, and gemifloxacin, and to identify the proportion of MRSA, VRE, HLAR and PRP in our studied population.

Methods

Gram-positive bacterial isolates and their corresponding susceptibility reports were obtained from The Puerto Rico Medical Center Bacteriology Laboratory for a period of 5 months (June to October, 2001). The following bacteria were included in the study: *Staphylococcus aureus*, *Enterococcus faecalis, Enterococcus faecium*, and *Streptococcus pneumoniae*. The *in vitro* antimicrobial susceptibility for quinupristin/dalfopristin and oxacillin was performed using the disk diffusion method following NCCLS (National Committee for Clinical Laboratory Standards) approved procedures. The Elipsometer test (Etest, AB Biodisk, Solna, Sweden) MIC susceptibility method was used according to the manufacturer's instructions for linezolid, gemifloxacin, benzylpenicillin, vancomycin and teicoplanin antimicrobial testing.

For the detection of penicillin resistant pneumococci (PRP), a screening test was performed utilizing the 1-µg oxacillin disk on Mueller Hinton agar supplemented with 5% sheep blood. To discriminate between intermediate- and high-level resistant isolates, the benzylpenicillin Etest was performed in Mueller Hinton agar supplemented with 5% sheep blood. The MIC interpretation for PRP using benzylpenicillin Etest strip was as follows: sensitive MIC £ of 0.06, intermediate MIC of 0.1 to 1 µg/ml and resistant MIC ³ 2 µg/ml.

For the enterococcal isolates, the susceptibilities to vancomycin, gentamicin and streptomycin were obtained from the PR Medical Center Bacteriology Laboratory. This information was evaluated and tabulated to determine the presence of VRE and high-level aminoglycoside resistance (HLAR).

To determine the vancomycin resistance phenotypes the Etest method was used according to the manufacturer's instructions. The Etest strips for vancomycin and for teicoplanin were placed over the agar surface and incubated at 35°C for 24 hours to detect VanA phenotype and at 48h to detect VanB. The MIC interpretation was as follows:

Resistance phenotype	Vancomycin (mg/ml)	Teicoplanin (mg/ml)
VanA	>256	16-256
VanB	8-256	0.125-8

High-level aminoglycoside resistance to enterococci was determined utilizing the antimicrobial susceptibility pattern reported by the Puerto Rico Medical Center Bacteriology Laboratory. An MIC to gentamicin > 500 µg/ml or > 2000 µg/ml to streptomycin is by definition considered high-level resistance to these antibiotics (HLAR).

The gemifloxacin Etest MIC interpretation was as follows: MIC £ 0.25 µg/ml for susceptible isolates, and MIC > 0.25 µg/ml for non-susceptible bacteria.

The linezolid Etest MIC interpretation was as follows: MIC £ 2 µg/ml for susceptible isolates of enterococci and pneumococci, and MIC £ 4 µg/ml for susceptible staphylococcal isolates.

The quinupristin/dalfopristin susceptibility interpretation utilizing the disk diffusion method was as follows: ³ 19 mm for susceptible, 16-18 mm for intermediate and £ 15 mm for resistant isolates.

The following American Type and Culture Collection (ATCC) strains were used for quality control: *S. aureus* ATCC 29213 and ATCC 43300, *S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and ATCC 51299.

Results

A total of 429 Gram-positive clinical isolates were obtained from The Puerto Rico Medical Center Bacteriology Laboratory in a period of 5 months. The distribution of the bacterial isolates was as follows: *Staphylococcus aureus* 213 (49.7%), *Enterococcus faecalis* 162 (37.8%), *Enterococcus faecium* 16 (3.7%) and *Streptococcus pneumoniae* 38(8.9%). The antimicrobial susceptibility results of the studied bacteria was as follows:

Staphylococcus aureus: Table 1 summarizes the antimicrobial pattern of *S. aureus* against selected antimicrobial agents. As shown in this table, 80 (39%) were MRSA, of which 56 (70%) were obtained from hospitalized patient and 24 (30%) from ambulatory subjects. Resistance to cefazolin and erythromycin was observed in 39% and 45% of all *S. aureus*, respectively.

Table 1. *Staphylococcus aureus* antimicrobial susceptibility

Antimicrobial agents	Total number and (%)		
	Susceptible	Intermediate	Resistant
Oxacillin	127(61)	0	80 (39)
Cefazolin	122 (61)	0	79 (39)
Vancomycin	203 (100)	0	0
Erythromycin	98 (47)	17 (8)	93 (45)
Linezolid	213 (100)	0	0
Quinupristin/Dalfopristin	212 (99.5)	1 (0.5)	0
Gemifloxacin	191 (93)	0	14 (7)

All isolates were susceptible to vancomycin, quinupristin/dalfopristin and linezolid. Ninety three percent of the analyzed *S. aureus* isolates (191), were sensitive to gemifloxacin. All methicillin susceptible *S. aureus* (MSSA) were also susceptible to gemifloxacin.

Enterococcal species: The antimicrobial susceptibility of the enterococcal isolates is shown in Table 2. For *Enterococcus faecalis,* ampicillin and linezolid showed the highest antimicrobial activity with over 90% of the isolates being susceptible to them. Vancomycin resistance was detected in 19 (12%) of these isolates. Of the 19 vancomycin resistant isolates, 18 (94.7%) were susceptible to ampicillin. Seventy nine percent were susceptible to gemifloxacin. Quinupristin/dalfopristin showed no adequate antimicrobial activity against *E. faecalis* (0%). High-level aminoglycoside resistance (HLAR) to gentamicin and/or streptomycin was identified in 32% and 34%, respectively, of the *E. faecalis* isolates.

Enterococcus faecium represented only 16 (9%) of the identified enterococcal species. Fifty six percent showed resistance to vancomycin (VRE), and 80% to ampicillin. Linezolid and quinupristin/dalfopristin showed the highest antimicrobial activity with over 90% of the isolates being susceptible to them. Sixty nine percent of the isolates were susceptible to gemifloxacin. High-level aminoglycoside resistance to gentamicin and/or streptomycin was identified in 50% and 69%, respectively, of the *E. faecium* isolates.

The VRE phenotypes are shown in Table 3. VanA phenotype was observed in 21% and 88.9% of *E. faecalis* and *E. faecium*, respectively, while VanB was detected in 79% and 11.1%.

Table 3. Vancomycin resistance phenotype among enterococcal isolates

Enterococcus spp.	VanA	VanB
E. faecalis	4 (21)	15 (79)
E. faecium	8 (88.9)	1 (11.1)

Streptococcus pneumoniae: The *S. pneumoniae* antimicrobial susceptibility is shown in Table 4. A total of 38 *S. pneumoniae* isolates were collected, 16 from blood cultures and 22 from the respiratory tract. Sixteen (42%) were highly resistant, 2 (5%) were intermediate and 20 (53%) were susceptible to penicillin. Activity of the third generation cephalosporins ceftriaxone and cefotaxime remained high, with 12.5% and 6% of resistance respectively. One hundred percent of the isolates were susceptible to gemifloxacin, gatifloxacin, vancomycin, levofloxacin, linezolid and 87% were susceptible to quinupristin/dalfopristin.

Table 2. *Enterococcus spp.* antimicrobial susceptibility

Antimicrobial agents	*E. faecalis*			*E. faecium*		
	Total number and (%)			Total number and (%)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin	158 (99.4)	0	1 (0.6)	3 (20)	0	12 (80)
Vancomycin	125 (78.6)	15 (9.4)	19 (12)	6 (37.5)	1 (6.25)	9 (56.3)
Strepto-2000	101 (66)	0	52 (34)	4 (31)	0	9 (69)
Genta-500	108 (68)	0	50 (32)	6 (50)	0	6 (50)
Linezolid	150 (92.9)	11 (6.8)	1 (0.6)	16 (100)	0	0
Quinupristin/Dalfopristin	0	0	162 (100)	15 (94)	0	1 (6)
Gemifloxacin	72 (79)	0	19 (21)	11 (69)	0	5 (31)

Table 4. *Streptococcus pneumoniae* antimicrobial susceptibility

Antimicrobial agents	Total number and (%)		
	Susceptible	Intermediate	Resistant
Benzylpenicillin	20 (53)	2 (5)	16 (42)
Oxacillin	19 (50)	0	19 (50)
Ceftriaxone	7 (87.5)	0	1 (12.5)
Cefotaxime	16 (94)	0	1 (6)
Vancomycin	32 (100)	0	0
Levofloxacin	19 (100)	0	0
Gatifloxacin	6 (100)	0	0
Linezolid	38 (100)	0	0
Quinupristin/dalfopristin	33 (87)	0	5 (13)
Gemifloxacin	38 (100)	0	0

Discussion

Bacteria resistant to multiple antibiotics have become a major health issue. Infections with these organisms are associated with increase in hospitalization costs, length of the hospital stay and more importantly, an increase in patients' morbidity and mortality (1,5,9). Our data demonstrated the antimicrobial susceptibility patterns for the most common and clinically important Gram-positive cocci.

S. aureus was the most common isolated organisms representing 49.7% of the total isolates. Worldwide the prevalence of MRSA varies from as low as 1.8% in Switzerland to 54.4% in Portugal. Our rate of 39% is higher than the 34.2% found in the USA, 33.7% in Brazil, 11.4% in Mexico and 8.6% in Colombia (4). All *S. aureus* remained 100% susceptible to the glycopeptide vancomycin. The new antimicrobial agents, quinupristin/dalfopristin and linezolid showed excellent consistent activity against all isolates tested. Gemifloxacin was active *in vitro* against 82.5% of the MRSA isolates, and all of the 14 gemifloxacin resistant *S. aureus* were also MRSA.

Our study results demonstrated that *E. faecalis* was more prevalent than *E. faecium* (91% and 9%, respectively). Vancomycin resistant enterococci (VRE) were observed in 16% of the isolates, 19% *E faecalis* and 56.3% of the *E. faecium*. This is similar to the prevalence of VRE in the USA (17%) and much higher than that found in Latin America, Europe and Asia (19). The susceptibility of ampicillin to the tested enterococcal species was 92% (99.4% for *E. faecalis* and 20% for *E. faecium*), this is significantly higher than the susceptibility to ampicillin found in the USA and Canada (76%) and similar to that found in Latin America and Europe (19). Ampicillin demonstrated excellent *in vitro* activity against the vancomycin resistant *E. faecalis* (94.7%), but not against vancomycin resistant *E. faecium*. High-level enterococcal aminoglycoside resistance to gentamicin and streptomycin was observed in 33% and 37% of the isolates, respectively. These results are similar to those found in the USA, Latin America and Europe (19). The observation of multiple drug resistant *E. faecium* has been previously described and will continue to be a challenge for the treatment of serious enterococcal infections (7). Of the new antimicrobial agents, linezolid showed consistent activity against both, *E. faecalis* and *faecium*. Quinupristin/dalfopristin was active only against *E. faecium*. Gemifloxacin demonstrated intermediate *in vitro* activity against the enterococci.

Enterococcal VanA phenotype is typically associated with high level resistance to vancomycin and with moderate to high resistance to teicoplanin, while VanB is associated with moderate to high resistance to vancomycin but no resistance to teicoplanin (6). Although the number of isolates was small, VanB was the most common phenotype in the isolates and was detected in 79% of the *E. faecalis*. In the United States, however, VanA is the most common phenotypic resistance observed (7).

The total number of *S. pneumoniae* isolates was fairly small. There may be several possibilities to explain this finding, such as: (1) poorly taken samples, (2) inadequate handling of the specimen, and (3) laboratory misidentification. A progressive and alarming increase in PRP has been found in the United States as demonstrated by large surveillance programs (1). Our data shows that 47% of the isolates were either intermediate or highly resistant to penicillin. In the United States, the prevalence of PRP varies within geographic regions from 15% to 40%. All the pneumococcal isolates were highly susceptible to the currently available fluoroquinolones, vancomycin, gemifloxacin and linezolid, while 87% were susceptible to quinupristin/dalfopristin.

In conclusion, there are a significant number of antimicrobial resistant Gram-positive bacteria in our studied bacterial sample similar or higher than those found at other geographical areas. Methicillin resistant *S. aureus*, PRP, VRE, and HLAR were all identified, however, no glycopeptide intermediate *S. aureus* (GISA) were detected. The new antimicrobial agents quinupristin/dalfopristin, linezolid and gemifloxacin showed excellent antimicrobial activity against many of the studied pathogens. In addition, it is imperative that an islandwide antimicrobial susceptibility program is established to monitor the susceptibility trends of the most common bacterial pathogens of our region.

Resumen

Los cocos gram positivos (CGP), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *faecium* han cobrado gran importancia como agentes causantes de infecciones nosocomiales e infecciones adquiridas en la comunidad. La prevalencia de cepas multiresistentes ha aumentado significativamente durante las últimas décadas. En Puerto Rico se han llevado a cabo pocos estudios prospectivos para estudiar los patrones de susceptibilidad de estos CGP. El propósito de este estudio fue evaluar la susceptibilidad *in vitro* de cepas de CGP aisladas clínicamente, a los antibióticos usuales, así como a los nuevos agentes antimicrobianos: linezolid (LZ), quinupristin/dalfopristin (Q/D), y gemifloxacin (GM). Un total de 429 cepas fueron coleccionadas por un periodo de 5 meses en el Laboratorio de Bacteriología del Centro Médico de Puerto Rico y su susceptibilidad *in vitro* fue determinada utilizando los métodos de difusión de discos

y Etest. La distribución de los CGP es como sigue: 213 cepas de *S. aureus*, 162 de *E. faecalis*, 16 de *E. faecium* y 38 de *S. pneumoniae*. Los resultados de las pruebas de susceptibilidad demostraron que: 1) para *S. aureus*, 100% de las cepas fueron susceptibles a vancomicina (VAN), LZ y Q/D; 93% a GM; y 61% a meticilina/oxacilina; 2) para *S. pneumoniae* 100% fueron susceptibles a LZ y GM; 87% a Q/D y 53% a penicilina; 3) para E. *faecalis* 99% fueron susceptible a ampicilina, 93% a LZ; 79% a GM; 78.6% a VAN; y 0% a Q/D. Sesenta y ocho y 66% de las cepas de *E. faecalis* fueron susceptibles a gentamicina y a estreptomicina, respectivamente; 4) para *E. faecium* 100% fueron susceptibles a LZ; 94% a Q/D; 69% a GM; 37.5% a VAN y 20% a ampicilina. Para las cepas de *E. faecium* 50% y 31% fueron susceptibles a gentamicina y a estreptomicina, respectivamente. Entre los enterococos resistentes a vancomicina, vemos que 88.9% y 21% de los *E. faecalis y E. faecium* respectivamente, demostraron resistencia por el fenotipo VanA. Estos resultados demuestran que existe un alto grado de resistencia entre los CGP, incluyendo un 38% de los S. *aureus* resistentes a meticilina, cerca del 50% de los *S. pneumoniae* son resistentes a penicilina, y un número considerablemente alto de las especies de enterococos son resistentes a vancomicina. Los nuevos agentes, LZ, Q/D, y GM fueron efectivos contra *S. aureus y S. pneumoniae*, LZ y Q/D fueron activos contra *E. faecium*, mientras que para *E. faecalis*, solo LZ demostró una actividad consistente.

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