Quinupristin-dalfopristin Resistance in Gram-positive Bacteria: Experience from a Tertiary Care Referral Center in North India

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INTRODUCTION
During the last two decades, there has been a steady rise in drug resistance among Gram-positive cocci. The likely causes are multidrug-resistant staphylococci and enterococci. Drug resistance is responsible for significant increases in morbidity and mortality as well as a sharp increase in costs of both inpatient and outpatient treatments. To overcome this

ABSTRACT
During the last two decades, the resistance has increased to the routinely used antimicrobial agents in the management of serious infections caused by Gram-positive cocci. This has led to a significant increase in morbidity and mortality as well as a sharp increase in costs of both inpatient and outpatient treatments. The aim of the study was to determine the in vitro susceptibility of Gram-positive cocci to a relatively newer antimicrobial agent, quinupristin-dalfopristin, and to compare the results with other antimicrobial agents usually used in the treatment of such infections. The pus and urine samples from both outpatients and inpatients were processed following the standard protocols. The antibiotic susceptibility of various Gram-positive cocci from these samples was examined using the Clinical and Laboratory Standards Institute guidelines. Quinupristin-dalfopristin resistance was high in methicillin-resistant Staphylococcus aureus. Linezolid, the other newer reserved drug, showed uniformly promising results against all Gram-positive cocci isolated. The increasing bacterial resistance to routine antimicrobial agents is a cause of concern in management of serious Gram-positive coccal infections. (J Infect Dis Antimicrob Agents 2008;25:117-21.)

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problem, the focus has been on developing newer antimicrobial agents including quinupristin-dalfopristin and linezolid. Quinupristin-dalfopristin, the streptogramin antibiotic, originally developed to tackle serious life threatening infections caused by Gram-positive bacteria are increasingly less effective.1,2 This study was aimed to determine the in vitro susceptibility of Gram-positive cocci to quinupristin-dalfopristin, linezolid, and other commonly used antimicrobial agents.

MATERIALS AND METHODS
The study was carried out in the Department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak, Haryana State, North India. The pus and the urine samples of both inpatients and outpatients were processed. After performing Gram stains on the pus samples and wet mount preparation of the urine samples, all these samples were inoculated onto blood and MacConkey’s agar plates. After overnight incubation, the growth on the culture plates was examined and the isolates were identified following the standard protocols.3 The susceptibility patterns of all isolates of Staphylococcus aureus, coagulase-negative staphylococci (CoNS), and Enterococcus spp. isolated were recorded and analyzed over a period of one year. The antibiotic susceptibility of the various isolates obtained was carried out by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates for S. aureus and CoNS, following the Clinical and Laboratory Standards Institute (CLSI) guidelines4 and on blood agar plates for Enterococcus spp. S. aureus strain ATCC 25923 was used as the quality control in the susceptibility testing procedure. Commercially available antimicrobial discs (HIMEDIA Pvt. Ltd., Bombay) were used for performing the susceptibility testing. The respective antimicrobial concentration per disc for the antimicrobial agents tested was as follows: cephalaxin (30 μg), amoxicillin-clavulanate (30 μg), erythromycin (15 μg), doxycycline (30 μg), gentamicin (10 μg), quinupristin-dalfopristin (15 μg), linezolid (30 μg), ciprofloxacin (5 μg), gatifloxacin (5 μg), and vancomycin (30 μg).

RESULTS
A total of 806 Gram-positive cocci were recovered from the pus and the urine samples during the study period (Table 1). Of these 806 isolates, 628 (78%) isolates were S. aureus. Methicillin resistance was noted in 340 (54%) of 628 S. aureus isolates, and 405 (64%) of 628 S. aureus isolates were resistant to quinupristin-dalfopristin. Quinupristin-dalfopristin resistance was predominantly noted in methicillin-resistant S. aureus (MRSA), i.e. 295 (87%) of the isolates of MRSA were resistant to quinupristin-dalfopristin. The rate of resistance to quinupristin-dalfopristin in methicillin-susceptible S. aureus (MSSA) was 38 percent. The susceptibility rate of CoNS and Enterococcus spp. to quinupristin-dalfopristin was 65 percent and 75 percent, respectively. The susceptibility to linezolid ranged from 91 percent to 100 percent in various Gram-positive cocci. The susceptibility patterns of the isolates to the various antimicrobial agents tested are summarized in Table 1.

DISCUSSION
This study reveals a high rate of resistance to the streptogramin antibiotics (quinupristin-dalfopristin) in S. aureus, CoNS, and Enterococcus spp. Streptogramin antibiotics are derived from the bacteria, Streptomyces pristinaespiralis. This family of antimicrobials comprises several classes of antibiotics including mikamycins, pristinamycins, ostreomycins, and virginiamycins.4-7 The streptogramins are further categorized into two groups including group A (consisting of polyunsaturated cyclic macrolactone compounds e.g. virginiamycin M, pristinamycin IIA, and...
Table 1. Distribution of Gram-positive cocci isolated in accompanying with their susceptibility patterns.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Susceptibility to various antimicrobial agents*</th>
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<tbody>
<tr>
<td></td>
<td>Cephalexin</td>
</tr>
<tr>
<td>MRSA (N=340)</td>
<td></td>
</tr>
<tr>
<td>S. aureus (N=628)</td>
<td>54</td>
</tr>
<tr>
<td>MSSA (n=228)</td>
<td></td>
</tr>
<tr>
<td>CONS (N=106)</td>
<td>94</td>
</tr>
<tr>
<td>Enterococcus spp. (N=72)</td>
<td>NA</td>
</tr>
</tbody>
</table>

CoNS: coagulase-negative staphylococci, MRSA: methicillin-resistant *Staphylococcus aureus*, NA: not applicable

*All data correspond to the percentage of susceptible strains.
dalfopristin) and group B (consisting of cyclic hexadepsipeptides e.g. virginiamycin S, pristinamycin IA, and quinupristin). Quinupristin and dalfopristin are produced by modifying the structure of pristinamycin IA and pristinamycin IIA, respectively. Quinupristin-dalfopristin consists of a combination of quinupristin and dalfopristin in a weight/weight ratio of 30:70. To enable the formulation to be used as an injectable agent, the natural compounds are modified to increase their aqueous solubility. Both components inhibit the bacterial protein synthesis by interfering with different targets of 23s ribosomal RNA in the 50S subunit of the ribosome. For the isolate to be resistant, there must be a resistance to both the streptogramin A and streptogramin B components. Some of the important genes responsible for the resistance to streptogramin A component include \( \textit{erm}, \textit{vgh}, \) and \( \textit{msr} \), which encode for the enzymes responsible for the methylation of adenine residue in the 23s ribosomal RNA and a streptogramin-inactivating enzyme, lyase.

Quinupristin-dalfopristin is approved in the United States for treatment of infections caused by vancomycin-resistant strains of \( \textit{E. faecium} \) and skin and skin-structure infections caused by methicillin-susceptible strains of \( \textit{S. aureus} \) and \( \textit{Streptococcus pyogenes} \). In Europe, it is also being used in the treatment of nosocomial pneumonia and other infections caused by MRSA. The first quinupristin-dalfopristin-resistant isolate of \( \textit{S. aureus} \) was reported from France in 1975. Several studies have previously reported a diverse range of the resistance rates to quinupristin-dalfopristin ranging from 0 percent to 44 percent.

In this study, we found that 64 percent of \( \textit{S. aureus} \) isolates were resistant to quinupristin-dalfopristin. The resistance rate was exceptionally high in MRSA, a common nosocomial pathogen, with 87 percent of all MRSA isolates being resistant to quinupristin-dalfopristin. The picture was less gloomy in case of MSSA, as only 38 percent of such \( \textit{S. aureus} \) were resistant to quinupristin-dalfopristin. The resistance to linezolid was noted in 91 percent of all isolates of \( \textit{S. aureus} \), with linezolid resistance being seen only in MRSA isolates. There is paucity of published literature with regards to the susceptibility patterns of this relatively newer reserved drug in India.

This study has some limitations. According to the CLSI recommendations, the disk diffusion method for susceptibility testing in \( \textit{Enterococcus} \) spp. must be performed using Mueller-Hinton agar plate. This study uses blood agar plate for susceptibility testing in \( \textit{Enterococcus} \) spp.

Non-human sources have been increasingly suspected as reservoirs for some antimicrobial-resistant bacteria. A recent study has demonstrated that the discontinuation of the use of streptogramin (virginiamycin and avoparcin) in animal husbandry farms has lead to a reduction in antimicrobial resistance among animal isolates which could decrease human exposure to resistance genes from animal sources.

The high rate of resistance in Gram-positive cocci to quinupristin-dalfopristin should be investigated by further studies to determine the minimal inhibitory concentration (MIC) of this antimicrobial agent to the resistant isolates of \( \textit{S. aureus} \).

**CONCLUSION**

In conclusion, the rise of antimicrobial resistance is posing formidable challenges in the treatment of serious infections caused by Gram-positive cocci. This is resulting in a situation in which very few options are available for treating infections caused by multidrug-resistant Gram-positive cocci. Urgent measures to discourage the irrational and injudicious use of these agents are required to delay the development of resistance among them.
References


