Antimicrobial Susceptibility of *Streptococcus sp.* to Quinupristin-dalfopristin in China^{*}



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This study aimed to determine the in vitro activity of quinupristin-alfopristin against Streptococcus sp. isolated in China. This agent is not vet available for clinical use, but it has been tested proportion against а high of resistant Staphylococcus aureus strains. A total of 156 streptococcal isolates, which were recovered from various geographic areas and diseases, were tested using the Etest (AB Biodisk, Solna, Sweden). Quinupristin-alfopristin showed excellent activity against all of the tested streptococci isolates. These results provide useful data for the clinical use of quinupristin-alfopristin in China.

The antimicrobial resistance of streptococci remains a serious public health problem worldwide. In China, although lactam-resistant strains are rare. macrolides resistance in streptococci is common. In recent years, an increasing number of studies have reported streptococcal antibiotic resistance. In an outbreak of scarlet fever caused by group A streptococcus (GAS) Streptococcus pyogenes in China in 2011, the rate of resistance to macrolides was as high as 100%. Researchers in Hong Kong reported a novel mobile element that was designated ICE-emm12, which contains the ermB and tetM genes encoding macrolide-incomycin-treptogramin resistance and tetracycline resistance, respectively^[1-2]. Moreover, several genetic elements involved in antibiotic resistance are horizontally transferred among different Lancefield groups and within species^[3]. An outbreak of a streptococcal infection consisting of highly virulent and drug-resistant strains would be disastrous for humans. Therefore, identifying a new drug that is more therapeutic for streptococcal infections is important.

Quinupristin-alfopristin, the first semisynthetic

injectable streptogramin antibiotic, has been approved by the United States Food and Drug Administration for the treatment of adults with serious bacteremia infections involved in methicillin-susceptible Staphylococcus aureus, vancomycin-resistant Enterococcus faecium, and *Streptococcus pyogenes*^[4-10]. Quinupristin-alfopristin is not yet available for clinical use in China, but a high proportion of resistant S. aureus strains has been documented^[11-12]. Therefore, in this study, the in vitro activity of quinupristin-dalfopristin against streptococcal isolates in China was determined.

A total of 156 strains were collected from patients with different streptococcal infections, including acute glomerulonephritis, scarlet fever, and tonsillopharyngitis, as well as from healthy carriers, between January 2006 and August 2011. These strains were isolated according to β -hemolysis on trypticase soy agar containing 5% sheep's blood, and were Lancefield grouped as GAS, group B streptococcus (GBS), group C streptococcus (GCS), group F streptococcus (GFS), and group G streptococcus (GGS) using a streptococcal grouping kit (BioMe'rieux, Marcy L'Etoile, France). All of the isolates were stored at -70 °C in brain heart infusion broth containing 15% glycerol until testing. Among the 156 tested strains, 109 that tested positive for GAS were mainly recovered from patients with acute glomerulonephritis, scarlet fever, and tonsillopharyngitis. Fifteen GBS, two GCS, 26 GGS, and four GFS samples were isolated from patients with acute glomerulonephritis or tonsillopharyngitis in Guizhou Province (Table 1). Susceptibility tests were performed using the Etest (AB Biodisk, Solna, Sweden) in Mueller-Hinton agar supplemented with 5% sheep's blood in accordance with the 2011 Clinical and Laboratory Standards Institute guidelines.

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The Streptococcus pneumoniae strain ATCC 49619 was used as a quality control strain. The preliminary breakpoints of quinupristin-alfopristin are $\leq 1 \mu g/mL$ for susceptibility, 1.5 $\mu g/mL$ for intermediate susceptibility, and $\geq 2 \mu g/mL$ for resistance. All of the GAS isolates were subjected to M protein gene (*emm*) typing according to procedures described by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/M-Protei nGene_typing.htm). Typing of *emm* showed a total of 13 different types of GAS strains, including emm1, emm4, emm12, emm22, emm58, emm60, emm63, emm75, emm77, emm95, emm113, emm14, and st106m.4.

The in vitro susceptibility of gram-positive bacteria to quinupristin-dalfopristin has been investigated in several studies, which showed that this antibiotic has good in vitro activity against GAS and GBS strains recovered from the United States and Europe^[10]. Some reports in China have shown that the rate of resistance of S. aureus to quinupristin-alfopristin is as low as 0.82%, while intermediate susceptibly to quinupristin-alfopristin increased from 22% to 52% between 2000 and 2005^[11-12]. This curious phenomenon urgently requires further investigation because quinupristin-Ifopristin has never been marketed in China. Despite these findings on gram-positive bacteria, there is limited information on the susceptibility of streptococcus to quinupristin-lfopristin in China. The current study is the first to perform an investigation on susceptibility of streptococcus to quinupristin-alfopristin. In the distribution of the minimum inhibitory concentrations (MICs) quinupristin-alfopristin for 156 streptococcal strains (Table 2), all of the isolates were sensitive to quinupristin-alfopristin, with MICs of 0.125-0.75 µg/mL. A total of 148 strains had a low MIC (<0.5),

while the other eight strains had a higher MIC of 0.75 μ g/mL. No strains were resistant to quinupristin-alfopristin. Notably, among the tested GAS isolates, 103 were erythromycin-resistant, which implies that quinupristin-dalfopristin could be an alternative drug for the treatment of infections associated with such strains.

In this study, we evaluated the antimicrobial susceptibility of *Streptococcus sp.* isolates to quinupristin-alfopristin in China. This drug showed excellent activity against streptococcal isolates in China. However, one limitation of this study is the lack of invasive streptococci, which are highly resistant to quinupristin-dalfopristin. In addition, strains isolated from Beijing and Guizhou accounted for a large proportion of the tested strains, which could induce potential geographic study bias. Therefore, collection of representative strains (isolated from severe or invasive infections) from various areas of China is important to enable a more detailed investigation in the future.

Table 1. Characteristics of the Tested Strains

Lancefield Group	Origin	No of Isolates					
А	Beijing	42					
	Shenzhen	8					
	Shanghai	7					
	Guangzhou	2					
	Chongqing	1					
	Guizhou	49					
В	Guizhou	15					
С	Guizhou	2					
F	Guizhou	4					
G	Guizhou	26					

Quinupristin-anopristin for 156 Streptococcal Strains																		
Antimicrobial	MIC in µg/mL, (No of Isolates)																	
	<0.125	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32
Quinupristin- dalfopristin	-	7	65	34	9	33	8	-	-	-	-	-	-	-	-	-	-	-

Table 2. Distribution of the Minimum Inhibitory Concentrations (MICs) ofQuinupristin-alfopristin for 156 Streptococcal Strains

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REFERENCES

- Tse H, Bao JY, Davies MR, et al. Molecular characterization of the 2011 Hong Kong scarlet fever outbreak. J Infect Dis, 2012; 206, 341-51.
- You YH, Song YY, Yan XM, et al. Molecular Epidemiological Characteristics of Streptococcus pyogenes Strains Involved in an Outbreak of Scarlet Fever in China, 2011. Biomed Environ Sci, 2013; 26, 877-85.
- Beres SB, Richter EW, Nagiec MJ, et al. Molecular genetic anatomy of inter- and intraserotype variation in the human bacterial pathogen group A Streptococcus. Proc Natl Acad Sci USA, 2006; 103, 7059-64.

- Roberto M. A re-emerging class of antimicrobial agents: streptogramins (quinupristin/dalfopristin) in the management of multiresistant. Mini Rev Med Chem, 2005; 5, 1075-81.
- Grossi PA. Early appropriate therapy of Gram-positive bloodstream infections: the conservative use of new drugs. Int J Antimicrob Agents, 2009; 34 Suppl 4, S31-4.
- Hershberger E, Donabedian S, Konstantinou K, et al. Quinupristin-dalfopristin resistance in gram-positive bacteria:mechanism of resistance and epidemiology. Clin Infect Dis, 2004; 38, 92.
- 7. Bearden DT. Clinical pharmacokinetic of quinupristindalfopristin. Clin Pharmacokinet, 2004; 43, 239.
- Welte T and Pletz MW. Antimicrobial treatment of nosocomial meticillin-resistant Staphylococcus aureus (MRSA) pneumonia: current and future options. Int J Antimicrob Agents, 2010; 36, 391-400.
- Anastasiou DM, Thorne GM, Luperchio SA, et al. *In vitro* activity of daptomycin against clinical isolates with reduced susceptibilities to linezolid and quinupristin/dalfopristin. Int J Antimicrob Agents, 2006; 28, 385-8.
- 10.Soltani M, Beighton D, Philpott J, et al. Mechanisms of resistance to quinupristin-dalfopristin among isolates of Enterococcus faecium from animals, raw meat, and hospital patients in Western Europe. Antimicrob. Agents Chemother, 2000; 44, 433-6.
- 11.Peng DZ, Liu XL, Liu ZY, et al. Analysis of distribution characteristics and drug resistance of 2748 strains of pathogens isolated from burn patients. Zhonghua Shao Shang Za Zhi, 2012; 28, 87-95. (In Chinese)
- 12.Yan X, Tao X, He L, et al. Increasing resistance in multiresistant methicillin-resistant Staphylococcus aureus clones isolated from a Chinese hospital over a 5-year period. Microb Drug Resist, 2011; 17, 235-9.