In Vitro Susceptibility of Gram-Positive Cocci Isolated from Skin and Respiratory Tract to Azithromycin and Twelve Other Antimicrobial Agents

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This study was conducted to evaluate the activity of azithromycin in comparison to 12 other antibacterial agents against recent isolates obtained consecutively from patients with respiratory tract or skin infections, from January to July, 2000. A total of 717 Gram-positive cocci were analyzed in this study and the following species were studied: *Staphylococcus aureus* (n=576), β-hemolytic streptococci (n=115), and *Streptococcus pneumoniae* (n=26). Susceptibility testing was carried out by the disk diffusion method and interpreted according to NCCLS breakpoints. The activity of azithromycin was compared to erythromycin, clindamycin, chloramphenicol, ciprofloxacin, ofloxacin, oxacillin, penicillin, ceftriaxone, tetracycline, trimethoprim/ sulfamethoxazole, teicoplanin, and vancomycin. Of the 26 *S. pneumoniae* isolates recovered from the respiratory tract, 5 (19.2%) were intermediate resistant to penicillin. All of these strains were susceptible to chloramphenicol, ofloxacin, and vancomycin, and 24 (92%) were also susceptible to azithromycin, clindamycin, and erythromycin. Among the 67 β-hemolytic streptococci strains isolated from the respiratory tract, 66 (99%) were susceptible to azithromycin, erythromycin, clindamycin, and ofloxacin. All 48 β-hemolytic streptococci strains isolated from skin were susceptible to azithromycin and clindamycin, 47 (98%) were susceptible to erythromycin, and 46 (96%) were susceptible to ofloxacin. Of the 576 strains of *S. aureus*, 253 (43.9%) were isolated from the respiratory tract and 323 (56.1%) from skin. Among *S. aureus* isolates from the respiratory tract and skin, 46 (18%) and 78 (24%), respectively were resistant to oxacillin. Isolates from the respiratory tract and skin showed the same percentage of resistance (36%) to azithromycin. These *in vitro* results suggest that azithromycin can be a therapeutic option for treatment of infections caused by these bacteria since the newer macrolides have several distinct advantages over erytromycin including improved oral bioavailability, longer half-life allowing once or twice daily administration, higher tissue concentrations and less gastrointestinal adverse effects.

Key Words: Gram-positive cocci, azithromycin, respiratory tract infection, skin infection, macrolide.

Newer macrolide antimicrobials are synthesized by alteration of the erythromycin base. They are compounds with improved pharmacokinetics [1], and fewer gastrointestinal side effects [2]. In 1991 and 1992, the Food and Drug Administraton (FDA) approved two of these agents, clarithromycin and azithromycin, for clinical use.

Azithromycin is the first of the azalide antibiotics, chemically related to erythromycin but modified by the insertion of a methyl-substituted nitrogen at position 9a of the lactone ring [3]. This structural alteration has resulted in an important change in the pharmacokinetic profile and an excellent antibacterial spectrum, with activity similar to erythromycin against Gram-positive respiratory pathogens (*S. aureus*, *S. pneumoniae* and *S. pyogenes*) [4]. This structural change also makes

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the compound more stable in acid, significantly increases the serum half-life and tissue penetration, and results in increased activity against many Gram-negative organisms.

Although generally bacteriostatic, azithromycin is bactericidal against *S. pyogenes*, *S. pneumoniae* and *Haemophilus influenzae*. Resistance appears to correlate with the amount of macrolide use within a community, as evidenced by a decrease in erythromycin resistance among group A streptococci associated with a nationwide decrease in macrolide use [5]. Azithromycin was developed for oral treatment of bacterial infections of the upper and lower respiratory tract caused by organisms such as *S. pneumoniae* and *S. pyogenes*, skin and skin structure infections caused by *S. aureus*, and *S. pyogenes* [6].

Erythromycin has been considered to be the main agent for treating Gram-positive streptococcal infections in penicillin-allergic patients. However, increased resistance to erythromycin in *S. pyogenes* isolates has been reported in several parts of the world [7,8].

Since the development of penicillin resistance in *S. pneumoniae* in the 1960s, the prevalence of resistance to other beta-lactams and other classes of antimicrobial agents has increased worldwide, resulting in clinical problems in the treatment of infections caused by this microorganism, and highlighting the need for alternative therapeutic procedures [9].

Data from the Sentry Antimicrobial Surveillance Program showed that *S. aureus* was the most common etiologic agent causing skin and soft tissue infections in Latin American hospitals, with approximately, 31.6% and 30.5% (1997 and 1998, respectively) of the tested isolates showing resistance to oxacillin, and 39.7% and 46.6% showing resistance to erythromycin [10].

For effective clinical use of antibiotics, it is important to know epidemiologic data of local and regional bacterial resistance rates. The purpose of this study was to evaluate antimicrobial susceptibility of Gram-positive isolates from respiratory tract and skin to azithromycin and 12 other agents, as part of the ResistNet Brazil Antimicrobial Surveillance Program.

Materials and Methods

Five centers from the city of São Paulo (Laboratório Fleury, Laboratório de Investigação Médica - LIM 54 HC-FMUSP, Laboratório Especial de Microbiologia Clínica – LEMC-UNIFESP, Hospital Universitário - USP and Instituto de Infectologia Emílio Ribas), and 6 other centers around Brazil: Florianópolis (Laboratório Médico Santa Luzia), Salvador (Hospital Aliança), Fortaleza (Laboratório Louis Pasteur), João Pessoa (Laboratório Nucilab), Goiânia (Hospital do Câncer), and Curitiba (Hospital de Clínicas and Hospital Evangélico), were included in this project.

Bacterial strains

A total of 717 Gram-positive cocci isolated from respiratory tract and skin, consecutively collected from January to July, 2000, were analyzed. All isolates were identified at the participating center by the routine methods in use at each laboratory, according to the American Society for Microbiology (ASM) procedures.

Susceptibility testing methods

Antimicrobial susceptibility testing was performed by disk diffusion method, and results were interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) [11,12]. The testing procedures were standardized by providing common lots of disks (OXOID, Hampshire, England) and quality control strains obtained from the same manufacturer for all centers. External quality control were sent by the coordinating center to all participating centers every 2 months for identification of the microorganism and susceptibility tests.

Prior to initiating the study, each participating laboratory tested quality control organisms, and, if the results were satisfactory for all antimicrobial agents, each center could initiate the study. On each day, quality control testing was performed by each laboratory throughout the study using *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619 and *Enterococcus faecalis* ATCC 29212 .

Table 1. *In vitro* activity of azithromycin and seven other antimicrobial agents against β-hemolytic streptococci isolated from respiratory tract and skin specimens from 10 centers in Brazil - ResistNet Surveillance Program in Brazil

	Respiratory Tract (n = 67)			Skin (n = 48)		
Antimicrobial agents	**% S**	**% I**	**% R**	**% S**	**% I**	**% R**
Azithromycin	99	-	1	100	-	-
Ceftriaxone	100	-	-	100	-	-
Clindamycin	99	-	1	100	-	-
Chloramphenicol	92	4	4	88	10	2
Erythromycin	99	-	1	98	2	-
Ofloxacin	99	1	-	96	2	2
Penicillin	100	-	-	100	-	-
Vancomycin	100	-	-	100	-	-

S: Susceptible; I: Intermediate; R: Resistant.

Table 2. *In vitro* activity of azithromycin and 8 other antimicrobial agents against *S. pneumoniae* isolated from respiratory tract from 4 centers in Brazil. ResistNet Surveillance Program in Brazil

	Respiratory tract (n = 26)		
Antimicrobial agents	**% S**	**% I**	**% R**
Azithromycin	92	-	8
Clindamycin	92	-	8
Chloramphenicol	100	-	-
Erythromycin	92	-	8
Ofloxacin	100	-	-
Penicillin*	81	19	-
Tetracycline	73	12	15
Trim/sulfa	54	-	46
Vancomycin	100	-	-

S: Susceptible; I: Intermediate; R: Resistant; Trim/sulfa: trimethoprim/sulfamethoxazole; *: MIC results.

Table 3. *In vitro* activity of azithromycin and 10 other antimicrobial agents against *S. aureus* isolated from respiratory tract and skin from 10 centers in Brazil. ResistNet Surveillance Program in Brazil

	Respiratory Tract (n = 67)			Skin (n = 48)		
Antimicrobial agents	% S	% I	% R	% S	% I	% R
Amp/sulbactam	82	-	18	76	-	24
Azithromycin	64	-	36	64	-	36
Ciprofloxacin	82	3	15	79	2	19
Clindamycin	74	3	23	74	-	26
Erythromycin	59	5	36	62	4	34
Oxacillin	82	-	18	76	-	24
Penicillin	13	-	87	9	-	91
Teicoplanin	100	-	-	100	-	-
Tetracycline	75	4	21	64	4	32
Trim/sulfa	81	-	19	73	-	27
Vancomycin	100	-	-	100	-	-

S: Susceptible; I: Intermediate; R: Resistant; Trim/sulfa: trimethoprim/sulfamethoxazole.

Table 4. *In vitro* activity of azithromycin and 9 other antimicrobial agents against *S. aureus* oxacillin susceptible (OXA S) and oxacillin resistant (OXA R), isolated from respiratory tract and skin from 10 centers in Brazil. ResistNet Surveillance Program in Brazil

	Respiratory tract (n=253)						Skin (n=323)					
Antimicrobial	**OXA S (n=206)**			**Oxa R (n=47)**			**OXA S (n=249)**			**OXA R (n=74)**		
agents	**%S**	**%I**	**%R**	**%S**	**%I**	**%R**	**%S**	**%I**	**%R**	**%S**	**%I**	**%R**
Amp/sulbactam	100	-	-	-	-	100	100	-	-	-	-	100
Azithromycin	76	-	24	6	-	94	81	-	19	4	-	96
Ciprofloxacin	97	1	2	14	4	82	99	1	-	9	6	85
Clindamycin	89	2	9	13	-	87	93	-	7	9	1	90
Erythromycin	72	4	24	5	-	95	78	7	15	4	-	96
Penicillin	15	-	85	-	-	100	12	-	88	-	-	100
Teicoplanin	100	-	-	100	-	-	100	-	-	100	-	-
Tetracycline	90	1	9	17	15	68	80	-	20	17	11	72
Trim/sulfa	95	-	5	15	-	85	94	-	6	1	1	98
Vancomycin	100	-	-	100	-	-	100	-	-	100	-	-

S: Susceptible; I: Intermediate; R: Resistant; Trim/sulfa: trimethoprim/sulfamethoxazole.

Antimicrobial agents

S. aureus isolates were tested against azithromycin, erythromycin, clindamycin, ciprofloxacin, oxacillin, penicillin, tetracycline, trimethoprim-sulfamethoxazole, teicoplanin, ampicillin/sulbactam, and vancomycin.

The strains of β-hemolytic streptococci were tested against azithromycin, erythromycin, clindamycin, ofloxacin, penicillin, ceftriaxone, chloramphenicol, and vancomycin. *S. pneumoniae* strains were tested against azithromycin, erythromycin, clindamycin, oxacillin (penicillin screening resistance), chloramphenicol, tetracycline, ofloxacin, trimethoprim-sulfamethoxazole, and vancomycin. MIC for penicillin was performed when oxacillin disk diffusion results were below 20mm. The results from all centers were entered in the Whonet 5 software for further evaluation.

Results

During the 6 month period, a total of 717 Gram-positive isolates were analyzed: 576 S. aureus, 115 β-hemolytic streptococci, and 26 S. pneumoniae were included in the study. Of the total, 59.8% were isolated from outpatients, and 40.2% were isolated from inpatients. Of all isolates studied, 371 (51.7%) were isolated from skin and 346 (48.3%) were isolated from upper and lower respiratory tract.

Among β-hemolytic streptococci, 67 (58.3%) were isolated from respiratory tract, and 48 (41.7%) were isolated from skin. Azithromycin inhibited 100% of the isolates from skin and 99% of the strains isolated from respiratory tract. Erythromycin inhibited 99% and 98% of the isolates from respiratory tract and skin, respectively. Clindamycin inhibited 99% and 100% of the isolates from respiratory tract and skin, respectively. Ofloxacin inhibited 99% and 96% of the strains isolated from respiratory tract and skin, respectively. Penicillin, ceftriaxone, and vancomycin inhibited 100% of all β-hemolytic streptococci strains (Table 1).

Of the 26 *S. pneumoniae* strains isolated from respiratory tract, 54% were isolated from outpatients and 46% were from inpatients; 81% susceptible and 19% intermediate resistant to penicillin. All strains with intermediate resistance to penicillin were isolated from outpatients, except 1 strain that was isolated from tracheal aspirate of a hospitalized patient. Azithromycin, erythromycin and clindamycin inhibited 92% of the strains. Tetracycline and trimethoprim-sulfamethoxazole inhibited 73% and 54%, respectively. All strains were susceptible to ofloxacin, chloramphenicol, and vancomycin (Table 2).

Among *S. aureus* isolates, 323 were isolated from skin, 253 from respiratory tract, 24% and 18%, respectively, showed resistance to oxacillin. The resistance rate to azithromycin (36%) was identical for the skin and respiratory isolates. The resistance rates to erythromycin were 36% and 34% for isolates from respiratory tract and skin, respectively. The resistance rates for respiratory tract isolates to clindamycin, ciprofloxacin, tetracycline, gentamicin, and trimethoprim-sulfametoxazole were lower than those for skin isolates. Vancomycin and teicoplanin inhibited 100% of all *S. aureus* isolates (Table 3).

The 47 of *S. aureus* oxacillin resistant strains and the 206 oxacillin susceptible strains isolated from the respiratory tract, showed a resistance rate to azithromycin of 94% and 24%, respectively. Considering the *S. aureus* strains isolated from skin, the resistance rate to azithromycin was 96% for the oxacillin resistant strains (n=74), and 19% (n=249) for the oxacillin susceptible strains (Table 4).

Discussion

Surveillance studies are extremely important components of any action designed to control the spread of antimicrobial resistance. This study is an important guide to help clinicians choose empirical treatment for these kinds of infections. The Gram-positive cocci are important causes of nosocomial and community-acquired infections, and are sometimes resistant to multiple antimicrobial agents [13]. The number of antimicrobial agents with activity against these microorganisms is limited [14]. Most antimicrobial

regimens for initial therapy of respiratory and skin infections due to Gram-positive cocci are empiric, and usually consist of orally administered agents.

The increasing resistance of Gram-positive cocci has had an effect on the choice of therapeutic agents for these infections. It is imperative that studies be performed to determine the nature and prevalence of resistance patterns, and these results should be available to clinicians.

There are several recent studies suggesting that the overuse of macrolides might contribute to the high incidence of erythromycin resistance among *S. pneumoniae* and *S. pyogenes* [8]. Resistance to macrolides is observed in many countries, and, in recent studies, the rates of erythromycin resistance in several countries, like Spain, are about 40% for *S. pneumoniae* and 26% for *S. pyogenes* [8]. The rates of resistance to azithromycin in Taiwan are about 61%, 100%, and 99% for penicillin susceptible, intermediate, and resistant pneumococci, respectively [15].

In the present study, among β-hemolytic streptococci isolated from respiratory tract, 99% were susceptible to azithromycin and erythromycin, and, among skin isolates, 100% and 98% were susceptible to these drugs, respectively. The results of this study are in agreement with previous reports of *in vitro* susceptibility to macrolides, where the macrolides were significantly more active against β-hemolytic streptococci strains isolated in Latin America (96% to 100%) than those isolated in the United States (85.8 to 87.6%) or Canada (87.7% to 89.5%) [16].

Of the 26 *S. pneumoniae* isolated, 5 strains (19%) showed intermediate resistance to penicillin (MIC 0.094 to 1μg/mL), and, although the number of strains tested was limited, the incidence of intermediate resistance to penicillin was higher than reported in the LASER study [9] and other studies like Protek, Sentry, and Alexander surveillance studies [17,18]. In this study, resistance to macrolides and clindamycin was found in 8% of the strains. Among the five strains of *S. pneumoniae* non-susceptible to penicillin, 3 (60%) were susceptible to azithromycin. A high resistance, rate of 46% to trimethoprim-sulfamethoxazole was observed in both penicillin susceptible and intermediate strains. Other non-β-lactams agents (chloramphenicol and ofloxacin) showed excellent *in vitro* activity against all strains.

Among *S. aureus* isolated from skin and soft tissue, 24% showed resistance to oxacillin, 36% to azithromycin, 34% to erythromycin, 26% to clindamycin, and 19% to ciprofloxacin. In comparison, based on a 1997 and 1998 study of skin and soft tissue infections in Latin American hospitals, the oxacillin resistant values were 30.5%, erythromycin 39.7%, clindamycin 29.1%, and ciprofloxacin 29.1% [10]. This slightly lower resistance observed in our study may be due to the fact that most of the *S. aureus* strains were isolated from outpatients (59.8%). The resistance rates to oxacillin and other antimicrobials for *S. aureus* isolated from respiratory tract were somewhat lower when compared with the strains isolated from skin.

In conclusion, the results of the present study showed low resistance among *S. pneumoniae* and beta-hemolytic streptococci to macrolides even in the face of ever-emerging antimicrobial resistance. Azithromycin also has several distinct advantages over erythromycin including improved oral bioavailability, longer half-life allowing once daily administration, higher tissue concentrations, and less gastrointestinal adverse effects. Analysis of macrolide prescribing and resistance patterns indicates a correlation between increasing macrolide resistance and the increased use of newer, long-acting macrolides, although further study is required to investigate this correlation.

Gram-positive cocci are among the most common agents of several skin and respiratory tract infections. As we observe in this study, there is an important antimicrobial resistance among isolates of *Staphylococcus aureus*, *S. pneumoniae* and β-hemolytic streptococci, as we saw by these results. Furthermore, we demonstrated that important antimicrobial resistance exist among isolates of staphylococci, *S. pneumoniae*, and β-hemolytic streptococci. This includes oxacillin resistance among staphylococci, penicillin intermediate resistance, and trimethoprim-sulfamethoxazole resistance among *S. pneumoniae*, and some degree of macrolide resistance among staphylococci.

Surveillance studies like this one can assist in monitoring bacterial resistance profiles for local analyses as well as multicenter analysis with the use of standardized methods to ensure comparability of results from different centers in the same study and between other surveillance studies.

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