COMPARATIVE *IN VITRO* ACTIVITY OF AZITHROMYCIN AND OTHER ANTIMICROBIAL AGENTS AGAINST STAPHYLOCOCCI ISOLATES

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Abstract. In the last decades resistance and reduced susceptibility to antimicrobial agents has become a major therapeutic problem. Both *Staphylococcus aureus* and *Staphylococcus epidermidis* are frequently resistant to methilpenicillins and derivatives, including methicillin, oxacillin and nafcillin. One of the antibacterial agents which exhibits a spectrum of activity against Gram-positive bacteria is azithromycin, a macrolide with properties closely resemble those of erytromycin. Azithromycin susceptibility was obtained for 84.3% of *S. aureus* and 87.4% of *S. epidermidis* respectively. Erythromycin resistance was more prevalent in *S. aureus* strains (20.5%) than in coagulase-negative staphylococci (15%). Oxacillin resistance has low and similar resistance rates for staphylococci (between 1.6-1.8%). Meropenem had excellent activity: all strains were susceptible. The results of surveillance of resistance has not been extensive.

INTRODUCTION

An important measure for monitoring the effectiveness of antimicrobial therapy is surveillance of the *in vitro* susceptibilities of bacteria. The ability of staphylococci to develop resistance to antimicrobial agents has been recognised for decades (Ashley and Brindle, 1980).

Staphylococci cause a variety of skin, soft-tissue and invasive infections and in the last decades the increasing resistance to many antimicrobial agents has become a major therapeutic problem (Crosseley and Archer, 1997). Both *Staphylococcus aureus* and *Staphylococcus epidermidis* are frequently resistant to methilpenicillins and derivatives, including methicillin, oxacillin and nafcillin. One of the antibacterial agents which exhibits a good spectrum of activity against Gram positive bacteria is azithromycin, an azalide antibiotic that is derived from the macrolides. This agent is chemically related to erythromycin by the insertion of a methyl-substituted nitrogen into the aglycone ring (Retsema *et al*, 1987; Barry *et al*., 1989). Its activities closely resamble those of erythomycin against Gram-positive cocci but with enhanced potency against Gram-negative and anaerobic organisms (Retsema *et al.*, 1987).

The aim of this study was to investigate the *in vitro* activity of azithromycin against staphylococci, communitary isolates. Other agents (erythromycin, oxacillin and meropenem) were also included in the studies for comparative purpose.

MATERIALS AND METHODS

Bacterial strains

A total of 515 strains isolated during 2007-2008 period were included to evaluate in this study.

The strains studied, originated from nasal swabs from healthy individuals and identified by standard procedures, were the following: *Staphylococcus aureus* (n=127) and *Staphylococcus epidermidis* (n=388).

Sensitivity study

Minimal inhibitory concentrations (MICs) were determined by the agar technique according to the Clinical Laboratory Standards Institute (CLSI) recommendations (2008). An inoculum of 10^5 cfu/ml(colony forming units) was delivered by a multipoint inoculator to a series of Mueller- Hinton agar plates, which obtained the antibiotic in twofold dilutions. Incubation was for 24 h at 35^0C. The MICs were determined as the lowest concentration of antibiotic at which no visible growth or growth <3 colonies were observed.

S. aureus ATCC 25923 was used as standard reference strain for quality control.

Antimicrobial agents

Stock solutions of the following were prepared from their respective powder forms: azithromycin, erythromycin, oxacillin, and meropenem. The range of concentrations used was from 0.06 to 32 mg/l. Resistance rates are reported using the CLSI breakpoints for the fully susceptible category (moderately susceptible isolates are classified as resistant) (Table 1).

Table 1. The value of concentrations used for defining susceptible isolates

Antibiotic	S (mg/L)	R (mg/L)
Azithromycin	≤ 2	≥ 8
Erythromycin	≤ 0.5	≥ 8
Oxacillin	≤ 2	≥ 4
Meropenem	≤ 4	≥ 16

Breakpoint shown is those defining fully susceptible isolates.

RESULTS AND DISCUSSION

Between 2007-2008, a total of 515 strains of staphylococci were obtained from healthy persons from nasal swabs.
Azithromycin MIC results were compared to erythromycin, oxacillin and meropenem. In table 2 the range of MICs, MIC 50, MIC 90 and susceptibility percent are presented.

Table 2. The *in vitro* activity of tested antimicrobial agents against staphylococci strains

Organism (n)	Antibiotic	MIC (mg/L)			Susceptibility ratio
		Range	MIC 50	MIC 90	
S. aureus (127)	Azithromycin	0.25-32	0.5	8	84.3
	Erythromycin	0.125-16	0.25	2	79.5
	Oxacillin	0.25-4	0.5	1	98.4
	Meropenem	0.125-1	0.125	0.25	100
S. epidermidis (388)	Azithromycin	0.25-32	1	4	87.4
	Erythromycin	0.125-8	0.25	2	85.1
	Oxacillin	0.25-8	0.5	1	98.2
	Meropenem	0.25-2	0.5	1	100

Meropenem, a new intravenous carbapenem, approved in 1996, retained excellent potency (MIC 90, between 0.25 mg/L and 1 mg/L); 90% of strains have oxacillin MIC at 2 mg/L. For both *S. aureus* and *S. epidermidis* erythromycin was comparable in antimicrobial activity (MIC 90.2 mg/L) a value that exceed the CLSI erythromycin breakpoint (0.5 mg/L) (Table 2).
Table 3 illustrates the MIC distribution for the surveillance staphylococci strains tested against azithromycin and comparison agents as cumulative inhibition percent.

Table 3. MICs values of staphylococci strains and susceptibility, according to breakpoint defined by CLSI criteria to selected antimicrobial agents

Organism (n)	Agent	Cumulative percent of MICs (mg/L)								
		0,125	0,25	0,5	1	2	4	8	16	32
S. aureus (127)	Azithromycin		28.3	55.9	73.2	84.3	87.4	90.6	93.7	100
	Erythromycin	11	45.7	79.5	86.6	89	94.5	97.6	100	
	Oxacillin		12.6	59	84.3	98.4	100			
	Meropenem	45.7	92.1	96.9	100					
		0,125	0,25	0,5	1	2	4	8	16	32
S. epidermidis (388)	Azithromycin		23.7	44.3	54.9	87.4	90.2	92.3	96.9	100
	Erythromycin	18	24.2	85.1	89.2	91.5	95.4	100		
	Oxacillin		19.6	64.4	85.6	98.2	99.2	100		
	Meropenem		30.9	62.4 99.2	100					

Resistant MIC values are highlighted light gray.

Using the CLSI (2008) breakpoint criteria, the collection of tested staphylococci strains showed susceptibility for meropenem.

Our data show that both *S. aureus* and *S. epidermidis* strains are mostly susceptible to azithromycin (of 84.3% respectively 87.4 %(table 3).

The *in vitro* susceptibility to erythromycin was observed for 79.5% of *S. aureus* and 85% of *S. epidermidis* strains, with slowly increase in resistance by comparison with azithromycin. With regard *S. aureus* strains, erythromycin resistance was more prevalent than in coagulase-negative staphylococci. Erythromycin is active against staphylococci isolates but azithromycin is also more active against these strains.

The analysis of *in vitro* oxacillin resistance in all isolates showed low rates for both *S. aureus* and *S. epidermidis*. The results of oxacillin resistance did not significantly vary between the two strains groups (1.6%-1.8%) (figure 1). The methicillin or oxacillin resistance in staphylococci also predicts resistance to a range of different classes of antibiotics (Archer and Climo, 1994). In accordance with this observation the oxacillin-resistant staphylococci were also resistant to tested antimicrobials.

Phenotypic antibiotic susceptibility was analysed the agar dilution method and selection of the isolates as the “susceptible” and “resistant” was based on their minimum inhibitory concentration to each tested agent. According to the MIC of these isolates, the resistance to azithromycin, erythromycin and oxacillin was observed in 15.7%, 20.5% and 1.6% *S. aureus* isolates, respectively (figure 1), regarding *S. epidermidis*, the resistance was inregistred in 12.6%, 14.9% and 1.8% respectively.

The prevalence of resistance to azithromycin and oxacillin is similarly for both all strains tested. Our data showed an increase erythromycin resistance in *S. aureus* isolates.

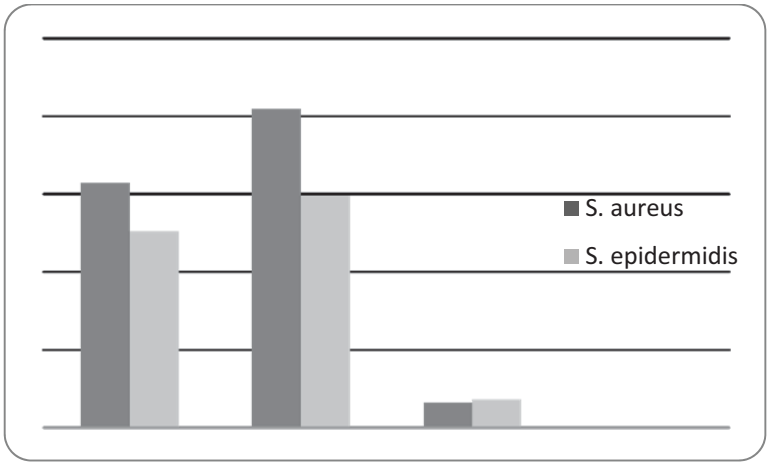

Figure 1. Resistant profile of *S. aureus* and *S. epidermidis* strains

Oxacillin was most comparable in potency with meropenem (resistance rates < 2%).

CONCLUSIONS

Meropenem was the most active of the agents tested and all isolates in the study were susceptible (MICs<2 mg/L). Our results indicate that the potency of azithromycin was slightly superior to erythromycin.

On the basis of these data and in consideration of the facts that azithromycin is usually well tolerated and the duration of treatment is generally short this agent may be recommended as possible alternatives in the treatment of staphylococcal infections by beta-lactamase positive staphylococci.

The data obtained by our surveillance study of the staphylococci strains shows that the emergence of resistance to tested agents has not been extensive.

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