

Incidence of Inducible and Constitutive Clindamycin Resistance in *Staphylococcus aureus* in an Infectious Disease Referral Hospital

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Abstract

Introduction: Clindamycin resistance may be inducible or constitutive, and the rates of inducible resistance in *S. aureus* that could produce clindamycin treatment failures.

Objective: In this study, we detect the prevalence of iMLSB phenotype among *S. aureus* isolates by double disk approximation test (D-test) in the Infectious Disease Referral Hospital in Asunción - Paraguay during 2016.

Materials and Methods: A total of 103 *S. aureus* isolated of adults patients were identified by Vitek[®] and subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method. Erythromycin-resistant isolates were tested for D-test.

Results: From 223 clinical specimens, 103 non-duplicate *S. aureus* isolates were obtained. Overall 68 isolates (66%) were MRSA. Inducible resistance was demonstrated in 15 isolates (14.5%) (iMLSB phenotype), 47 (45.6%) were D-test negative (MS) just 1 were constitutively resistant.

The inducible clindamycin resistance difference between MSSA and MRSA isolates were found to be not significant ($p = 0.25$).

Conclusion: We found no significant difference in inducible clindamycin resistant. Our study recommends routine testing of inducible clindamycin resistance at individual settings to guide optimum therapy and to avoid treatment failure in the future.

Keywords: D-test; Macrolide-Lincosamide-Streptogramin B

Introduction

It is known the prevalence of resistance of *Staphylococcus aureus* to many antimicrobial agents, especially methicillin, that result in the need for novel effective agents for the treatment of staphylococcal infections [1]. Active surveillance in our hospital has shown that in recent years inducible resistance to clindamycin has increased not only at the local level [2] but also worldwide [1,3].

When strains of *S. aureus* are resistant to erythromycin usually also are resistant to Lincosamides; phenotypically these strains can be classified into two large groups, strains with constitutive resistance, which can grow in the presence of high concentrations of antimicrobials MLS (macrolides, lincosamides and streptogramins type B) without requiring prior induction (cMLSb) and strains with inducible resistance, whose resistance to MLS antimicrobials could be induced by sub-inhibitory concentrations (0.01 to 0.1 ug/ml) of erythromycin iMLSb [1].

The incidence of these resistances groups are different by region, which makes active surveillance important for guiding local empirical treatment [3]. This cross-sectional study, which was carried out after approval by the institutional ethics committee, aims to estimate the percentage of resistance to constitutive and inducible clindamycin among clinical isolates of *S. aureus* in a Paraguayan reference infectious center.

In our hospital environment, being as a reference center for infectious diseases, clindamycin is in particular, a frequently used alternative covering skin and soft tissue and invasive infections caused by *S. aureus* [4,5].

Materials and Methods

Study design, sample collection, and identification of *Staphylococcus aureus*

The prospective study was carried out from January to December 2016 in the Department of Research at a Tertiary Care Hospital, Instituto de Medicina Tropical of Asuncion - Paraguay.

A total of 223 clinical specimens were collected, i.e. pus, blood, urine, tracheal aspirates and cerebrospinal fluid [CSF] from patients with active infections, classified in MRSA and MSSA isolated of adults patients admitted at the hospital during 2016 (Figure 1).

Microorganisms and antimicrobial susceptibility tests

The organism was identified by Vitek 2 automated antimicrobial susceptibility testing system (bioMérieux, Marcy l'Étoile, France).

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method and methicillin resistance was identified by using ceftioxin (30 µg) disc and interpreted as per CLSI guidelines [7]. Antibiotic discs used were vancomycin (15 µg), cotrimoxazole (25 µg), tetracycline (30 µg), rifampicin (5 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), and gentamicin (10 µg).

Double disk approximation test (D-test)

All isolates were subjected to inducible clindamycin resistance testing by CLSI recommended D test on Mueller Hinton agar by keeping erythromycin (15 µg) disc and clindamycin (2 µg) disc at 15 mm apart (edge to edge) [6].

Blunting of the circular zone of inhibition around clindamycin disc towards erythromycin disc indicated the presence of iMLSb resistance and was reported as resistant to clindamycin.

We interpreted the results as follows:

1. The isolate sensitive to erythromycin and clindamycin was considered susceptible phenotype,
2. Erythromycin resistant and clindamycin sensitive isolate (no D zone), MS phenotype,
3. Erythromycin resistant and clindamycin sensitive isolate (D zone present), iMLSb phenotype and
4. Erythromycin and clindamycin resistant isolate, cMLSb phenotype.

Quality control for all the antibiotics discs was done by using *S. aureus* ATCC 25923 according to standard disc diffusion QC procedure and controlled by the Laboratorio Central de Salud Pública de Paraguay (LCSP - in English as Central Laboratory of Public Health of Paraguay).

Statistical methods

Statistical analysis was performed by using Epi info version 3.3.2, and P-values of < 0.05 were considered statistically significant.

Results

The antimicrobial resistance rates of *S. aureus* in 2016 at Instituto de Medicina Tropical were 66% to oxacillin, those MRSA resistance rates were, 44% to erythromycin, 2% to cotrimoxazole, 8.8% rifampicin and 2% to gentamicin and 5.8% to ciprofloxacin (Table 1).

Antibiotic	Total	MRSA 68 (66%)	MRSA 35 (34%)	p
Vancomycin	0	0	0	NS
Cotrimoxazole	1	0	1 (2%)	NS
Tetracycline	0	0	0	NS
Rifampicin	4	1 (1.5%)	3 (8.8%)	NS
Ciprofloxacin	2	0	2 (5.8%)	NS
Erythromycin	17	2 (3%)	15 (44%)	0.0000
Gentamicin	2	1 (1.5%)	1 (2%)	NS

Table 1: Antimicrobial susceptibility of staphylococci isolated between January to December 2016 in an infectious disease referral hospital.

The percentages of strains with inducible clindamycin resistance were 14.6% and constitutive clindamycin resistance only 1% (Table 2).

	Ery (S) / Cly (S)		Ery (R) / Cly (S)		Ery (R) / Cly (S)		Ery (R) / Cly (R)	
			D test (-)		D test (+)			
	Sensitive phenotype		MS phenotype		iMLSb phenotype		cMLSb phenotype	
	n = 16	%	n = 71	%	n = 15	%	n = 1	%
MRSA 68 (66%)	8	11.8	47	69.1	12	17.6	1	1.5
MSSA 35 (34%)	8	22.9	24	68.6	3	8.6	0	0.0

Table 2: Prevalence of inducible and constitutive clindamycin resistance in staphylococcal isolates between January to December 2016 in an infectious disease referral hospital.

It was observed that percentage of inducible clindamycin resistance was higher among MRSA compared to MSSA, but this difference were not statistically significant ($p = 0.37$) (Table 3) same not statistically significant to the sensitive phenotype ($p = 0.15$) (Table 4).

	MS phenotype		iMLSb phenotype	
	n = 71	%	n = 15	%
MRSA 68 (66%)	47	66.1	12	80.0
MSSA 35 (34%)	24	33.8	3	20.0

Table 3: Comparison of D-test-positive and D-test-negative *S. aureus* isolates between January to December 2016 in an infectious disease referral hospital.

$p = 0.37$.

	Sensitive phenotype		Not showed phenotype	
	n = 16	%	n = 87	%
MRSA 68 (66%)	8	50.0	60	68.9
MSSA 35 (34%)	8	50.0	27	31.0

Table 4: Comparison according to sensitive and not showed phenotype between January to December 2016 in an infectious disease referral hospital.

$p = 0.15$.

Discussion

Accurate susceptibility data are important for appropriate therapeutic decisions. The presentation of clindamycin in both oral and parenteral formulations, 90% oral bioavailability, less expensive compared to newer drugs, good tissue penetration and may inhibit the production of certain toxins and virulence factors in *Staphylococci*, makes it Good therapeutic choice [5].

The prevalence of MRSA isolates among *S. aureus* was high (66%) in this study, which is similar to Sah., *et al.* (61.4%), Mansouri and Sadeghi (56.8%), and Chudasama., *et al.* (54.78%) [7,8].

Relationship of MRSA and MSSA with different resistant phenotypes has been studied by different authors. Incidence of MLS_B phenotypes varies significantly by geographical regions. Fiebelkorn, *et al.* [9] in their study found 34% and 29% of cMLSB and iMLSB respectively much higher than reported in our study. These differences of resistant phenotypes showed in literature can be awarded to the differences in bacterial susceptibility in different geographical regions and also due to varying antimicrobial prescribing patterns of clinicians, which may represent a reason for future studies.

Truly clindamycin-sensitive isolates, which exhibit MS phenotype, were present in 69.1% of MRSA and 68.9% of MSSA isolates in our study. This result is much higher than reported for other authors [8,10-12].

Prabhu K, *et al.* found just 10% isolates that showed inducible clindamycin resistance, and 8% showed MS phenotype, comparing with our results, those are very low, despite agreeing with our investigation as to which in MRSA inducible resistance and MS phenotype were higher to MSSA - 20%, 16% and 6%, 6%, respectively [13] against 66.1%, 80% and 33.8% and 20%.

Constitutive clindamycin resistance in our study was observed just in 1 (1%) of MRSA isolates, in different studies, the constitutive clindamycin resistance was reported varies from 1.77% to 52.3% [8,10-12] including Prabhu K that found 8% of constitutive clindamycin resistance.

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