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ORIGINAL ARTICLE

Frequency of inducible clindamycin resistance among gram-positive cocci in a tertiary hospital, Tehran, Iran

Hiva Saffar[1*], Afsaneh Rajabiani[1], Alireza Abdollahi[2], Shirin Habibi[3], Zohreh Baseri[1]

[1]Department of Pathology and Laboratory Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pathology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
[3]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: Accurate designation of antimicrobial susceptibility pattern of the infecting microorganisms is an important crucial factor in making appropriate therapeutic decisions. Macrolide, lincosamide and streptogramin B antibiotics are in a family, reserved as an alternative approach in treatment of resistant Gram positive cocci. Amongst them, clindamycin has been considered as the preferred agent due to its excellent pharmacokinetic properties. The inducible resistance to clindamycin in Gram positive staphylococci and streptococci cannot be recognized by routine broth or agar based susceptibility tests and D-zone testing is necessary. This study is conducted to evaluate the frequency of inducible clindamycin resistance in Gram positive cocci.

Materials and Methods: Using traditional culture methods, 487 isolates of staphylococcus and β-hemolytic streptococcus were evaluated. If they were resistant to erythromycin and sensitive to clindamycin in primary antibiotic susceptibility testing by Kirby-Bauer method, they were subjected to D-zone testing to detect possible inducible clindamycin resistance.

Results: Thirty three out of 172 isolates of *Staphylococcus aureus* and 50 out of 277 isolates of coagulase-negative staphylococci (CoNS) were subjected for D-zone testing. Among them 13/33 and 28/50 showed inducible clindamycin resistance, respectively. There was no significant difference in inducible clindamycin resistance regarding to methicillin susceptibility pattern. Positive D-test was observed in 17.39 and 13.33% of Group B streptococci and *Streptococcus* spp., respectively.

Conclusion: Considerable number of isolates showed inducible clindamycin resistance in our study which falsely would be reported susceptible if D-zone testing was not performed. Thus, performing D-Zone testing is necessary to avoid misleading results which may cause treatment failure.

Keywords: Staphylococci, Streptococci, Inducible clindamycin resistance, D-test

*Corresponding author: Hiva Saffar, Ph.D, Department of Pathology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.
Phone: +98 21 84902187
Fax: +98 21 84902187
E-mail: hsaffar@sina.tums.ac.ir

INTRODUCTION

Gram-positive cocci are important pathogens which can specially cause soft tissue and skin infection (1). *S. aureus* and coagulase-negative staphylococci (CoNS) are recognized as common microorganisms leading to nosocomial or commu-

nity acquired infection all over the world (2, 3). Increased incidence of methicillin resistance among staphylococci is a growing problem (1) and they are commonly reported as Multi-Drug Resistant (MDR) microorganisms (1, 2, 4). This fact has changed the trends in the usage of macrolide, lincosamide and streptogramin B (MLSB) antibiotics in the treatment of staphylococcal infections (3, 5). The MLSB antibiotics are a family, reserved as alternatives in the treatment of resistant Gram positive cocci (Staphylococci and streptococci) (1). Although they are structurally different, their mode of action is similar (1, 4). These antibiotic share common binding sites thus called MLSB phenotype (2). They inhibit bacterial protein synthesis by binding to 23S rRNA.

Amongst MLSB group, clindamycin has been considered as the preferred agent due to its excellent pharmacokinetic properties (1, 2, 4, 5) including good penetration and distribution in to the skin and other soft tissue structures (6) and acceptable oral absorption with no dosage adjustment in renal disorders (1). However regarding wide spread use of this antibiotic, increasing number of resistant isolates are being developed (1, 7). Diverse mechanisms can cause macrolide resistance (4). Firstly, it can be mediated by *msr(A)* gene encoding efflux pump and secondly by a variety of *erm* genes coding enzymes that confer inducible or constitutive resistance to MLSB agents (through methylation of the 23Sr RNA) (1, 3, 4).

Constitutive resistance can normally be detected by routine standard susceptibility testing whereas strains that demonstrate inducible resistance cannot be recognized by routine broth or agar based susceptibility tests (1, 3, 8). In such cases treatment with clindamycin may lead to clinical failure by developing constitutive resistant microorganisms (1, 5, 9). The presence of inducible clindamycin resistance can appropriately be recognized with D-zone test (9).

This study was designed to determine the frequency of inducible clindamycin resistance among staphylococci and β-hemolytic streptococci in our hospital, as a tertiary center, to highlight the necessity of performing D-test in routine practice in order to avoid reporting false susceptible results. Also, we aimed to evaluate any possible correlation between the frequency of inducible clindamycin resistance and susceptibility pattern to methicillin in staphylococci.

MATERIALS AND METHODS

Bacterial isolates. Total number of 487 consecutive non duplicate isolates of staphylococci and β-hemolytic streptococci Group B and spp. were recovered from various clinical specimens during March to September 2014 at Microbiology Laboratory of Shariati Hospital, Tehran, Iran.

Identification of microorganisms. During this period, 172, 277, 23 and 15 isolates of *S. aureus,* coagulase-negative staphylococci and β-hemolytic Group B streptococci and spp. were identified, respectively. Identification was done based on colony morphology on 5% Blood agar, gram staining and further conventional biochemical tests (10).

Antimicrobial susceptibility test. Staphylococci and β-hemolytic streptococci isolates were evaluated for the pattern of erythromycin susceptibility using Kirby-Bauer disk diffusion method on Muller Hinton agar with or without blood supplement. All erythromycin resistant and clindamycin sensitive isolates were subjected to D-zone test using erythromycin (15 µg; Rosco Diagnostica, Denmark) and clindamycin (2 µg; Rosco Diagnostica, Denmark) according to Clinical and Laboratory Standards Institute (CLSI) (11).

D-zone test. Briefly, erythromycin disks were placed at a distance of 15 mm and 12 mm (edge to edge) from clindamycin disk (for staphylococci and streptococci, respectively) on plates inoculated with the bacterial suspension with adjusted turbidity to McFarland standard 0.5 followed by overnight incubation at 37 °C. The results were interpreted as follows (1); MS phenotype, sensitive to clindamycin with circular zone of inhibition around the disk; iMLSB (inducible resistance) phenotype, sensitive to clindamycin with a D-shaped zone of inhibition around clindamycin disk; cMLSB (constitutive resistance) phenotype, resistant to clindamycin with a circular shape of inhibition.

Methicillin Susceptibility (MS) Pattern. Resistance to methicillin in staphylococci was detected by disk diffusion method using Cefoxitin disk (30 µg;

Rosco Diagnostica, Denmark) according to CLSI (11). Routine quality control of the disks was performed by *S. aureus* ATCC25923. Complementary quality control for D-test also was done with selected in-house strain that was D-test positive.

Statistical analysis. Statistical analysis was performed using SPSS software (v. 19). Parametric quantitative variables were compared using the independent sample t-test. Comparison of non-parametric quantitative variables was performed by Mann-Whitney U test while chi-square and Fisher's exact test was utilized for analyzing qualitative data. Differences were considered significant at $P<0$ .05.

RESULTS

Total number of 487 microorganisms from different clinical samples was subjected to the study including 172 (35.3%) *S. aureus,* 277 (56.9%) coagulase-negative staphylococcus isolates, 23 (4.7%) streptococcus Group B and 15 (3.1%) *Streptococcus* spp.

Eighty six out of 172 (50%) *S. aureus* isolates and 174/277 (62.8%) CoN staphylococci were categorized as methicillin-resistant. Erythromycin resistance was detected in 100/172 (58.1%) of *S. aureus* isolates and 214/277 (77.25%) CoN staphylococci.

The frequency of susceptibility pattern to erythromycin as well as different patterns of susceptibility to clindamycin in both *S. aureus* and CoN staphylococci are summarized in Table 1.

We did not observe any significant difference between inducible clindamycin resistance and methicillin susceptibility pattern. Data have are shown in Table 2.

We also performed D-test in β-hemolytic streptococci (Group B and other *Streptococcus* spp.). The results are summarized in Table 3.

We also determined the frequency of inducible clindamycin resistance in different hospital wards and clinical samples. Medical wards were categorized as emergency (general, oncology, obstetrics), internal medicine (nephrology, respiratory, general,

Table 1. Frequency of different patterns of susceptibility to erythromycin and clindamycin in staphylococcus isolates

Susceptibility pattern	Resistance to Erythromycin (%)			Sensitive to Erythromycin (%)	
Microorganism	iMLSB (to Clindamycin)	cMLSB (to Clindamycin)	MS (to Clindamycin)	Sensitive to Clindamycin	Resistant to Clindamycin
S. aureus	13/172 (7.56)	67/172 (38.95)	20/172 (11.63)	58/172 (33.72)	14/172 (8.14)
CoN Staphylococci	28/277 (10.11)	164/277 (59.21)	22/277 (7.94)	57/277 (20.58)	6/277 (2.16)
Total	314/449 (69.93)			135/449 (30.07)	

Table 2. Frequency of inducible clindamycin resistance in staphylococci regarding to methicillin susceptibility

Methicillin Susceptibility	No. MRSA*	No. MSSA**	No. MR-CoN staphylococci***	No. MS-CoN staphylococci****
D-test				
Negative	79	80	155	94
Positive	7	6	19	9
Total	86	86	174	103

* Methicillin-resistant *S. aureus*

** Methicillin-susceptible *S. aureus*

*** Methicillin-resistant CoN staphylococci

**** Methicillin-susceptible CoN staphylococci

renal transplant, endocrinology, gastrointestinal, heart and CCU), Surgical wards (general, orthopedics, urology, neurosurgery, gynecology, head and neck), Intensive Care Units (general, NICU, neurosurgery) and hematology/oncology/bone marrow transplant. Data are summarized in Tables 4 and 5, respectively. Considering the number of submitted samples, the most frequent inducible resistant microorganisms were retrieved from internal medicine wards. In regard to the number of samples, urine specimens revealed the most frequency.

DISCUSSION

Accurate designation of antimicrobial susceptibility pattern of the infecting microorganisms is an important crucial factor in making appropriate therapeutic decisions (2, 12). Regarding the emergence of resistance in Gram positive cocci, especially staphylococci, various antibiotics have been considered as alternative therapeutic options. The macrolide, lincosamide and streptogramin B (MLSB) family including clindamycin as one of the best preferred agents serves as one of these alternatives (2). Clindamycin has good oral bioavailability and is helpful for outpatient therapy or as oral agent can be followed after intravenous therapy (2). Clindamycin also has good penetration into skin or soft tissues or may be able to inhibit production of some toxins or virulence factors by staphylococci and is cost effective as well (4-6, 13). The widespread use of this family of antibiotics has led to the development

Table 3. Frequency of susceptibility patterns towards erythromycin and clindamycin in streptococci

Susceptibility patterns	Resistant to Erythromycin (%)			Sensitive to Erythromycin (%)	
Microorganisms	iMLSB (to Clindamycin)	cMLSB (to Clindamycin)	MS (to Clindamycin)	Sensitive to Clindamycin	Resistance to Clindamycin
Group B Streptococcus	4/23 (17.40)	2/23 (8.69)	2/23 (8.69)	14/23(60.88)	1/23(4.34)
***Streptococcus* spp.**	2/15 (13.33)	3/15 (20.00)	1/15 (6.67)	6/15(40.00)	3/15 (20.00)
Total	14/38 (36.84)			24/38 (63.16)	

Table 4. Frequency of inducible clindamycin resistance regarding to different hospital wards

Hospital Wards / Isolate	Internal Medicine (%)	Surgical Wards (%)	ICU (%)	Emergency Dept. (%)	Hematology/ Oncology and Transplant (%)	Out Patients (%)	Total
S. aureus	3/35 (8.57)	1/24 (4.16)	2/31 (6.45)	2/36 (5.54)	4/40 (10)	1/6 (16.6)	13/172
CoN staphylococci	9/49 (18.36)	1/41 (2.43)	4/51 (7.84)	7/53 (13.2)	7/70 (10)	0/13 (0)	28/277
Group B streptococci	1/4 (25)	1/5 (20)	0/2 (0)	1/2 (50)	0/4 (0)	1/6 (16.6)	4/23
***Streptococcus* spp.**	0/2 (0)	0/2 (0)	1/2 (50)	0/1 (0)	0/4 (0)	1/5 (20)	2/15

Table 5. Frequency of inducible clindamycin resistance regarding to different clinical specimens

Samples / Isolates	Urine (%)	Respiratory samples (%)	Wounds/Abscess (%)	Blood (%)	Body fluids (%)	Other samples (%)	Total
S. aureus	2/12 (16.6)	0/13 (0)	1/24 (4.16)	10/102 (9.80)	0/20 (0)	0/1 (0)	13/172
CoN Staphylococcus	6/32 (18.75)	2/30 (6.66)	3/36 (8.33%)	16/146 (10.95)	1/27 (3.70)	0/6 (0)	28/277
Group B Streptococcus	4/20 (20)	0/0 (0)	0/0 (0)	0/2 (0)	0/1 (0)	0/0 (0)	4/23
***Streptococcus* spp.**	1/7 (14.28)	0/0 (0)	1/3 (33.3)	0/4 (0)	0/1 (0)	0/0 (0)	2/15

of resistant strains (7, 14). The common mechanism of resistance is via *erm* gene encoding for enzymes confer constitutive or inducible resistance to MLS_B family (7, 15-17).

Routine *in vitro* susceptibility tests may fail to detect inducible resistance to clindamycin when erythromycin and clindamycin disks are not placed adjacent to each other thus false susceptible report may result in treatment failures (2, 4, 7, 12). The prevalence of inducible MLSB resistance varies in different geographic locations and is believed that depends on patient population, hospital characteristics and geographic area (3).

According to our findings, the frequency of erythromycin resistance was 69.93% among staphylococci (58.1% in *S. aureus* isolates and 77.25% in CoN staphylococci) which is higher than the prevalence rates reported by others (1, 4, 5, 18). Our data revealed the similar incidence rate of inducible clindamycin resistance among methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin sensitive *Staphylococcus aureus* (MSSA) isolates (7/86 and 6/86 strains, respectively). Although this finding is the same as published reports in other parts of the world (4, 19) but higher rate of inducible resistance among MRSA have also been reported (1, 5, 18, 20, 21) and even controversial results have been observed (3, 22, 23). Among coagulase negative staphylococci, 28/214 (13%) of erythromycin resistant isolates showed inducible clindamycin resistance phenotype which is close to the 17% incidence rate reported by others (1).

The frequency of methicillin resistance in our study was 50% and 62.8% in *Staphylococcus aureus* and CoN staphylococci, respectively. In a study by Dibah et al. (24) the frequency of MRSA isolates in Ardabil (North West of Iran) was 46.3% and most of the specimens were isolated from ICU. In another national study by Pourmand et al. (25) the frequency of MRSA was 50% using both cefoxitin disk diffusion method and PCR assay. Both of these studies approximately supported our findings. In another study conducted in a burn center in Ahvaz, higher frequency of methicillin resistance in *Staphylococcus aureus* isolates was reported (60%) while the rate of methicillin resistance among CoN staphylococcus strains was 63% (26) which was quite similar to the present study.

For *Streptococcous agalactiae,* the frequency of resistance towards erythromycin was about 34.78% which is near to the 40% frequency rate reported by Hraoui et al. (27). In our study the rate of inducible and constitutive clindamycin resistance among erythromycin resistant microorganisms was 17.40 and 8.69%, respectively while Hraoui et al. (27) reported 10 and 78.7% rates, respectively (27).

CONCLUSION

The emergence of antibiotic resistance, especially methicillin resistance in Gram positive cocci, considers clindamycin as an acceptable alternative treatment option. The frequency varies greatly in different geographic regions. According to our findings, considerable number of bacterial isolates in our center showed iMBL pattern. Because this type of resistance cannot be recognized in routine tests, CLSI recommends D-zone testing as a simple test which should be performed to avoid false susceptible results leading to treatment failure.

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