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Prevalence of Inducible Clindamycin Resistance among Community and Hospital Acquired Staphylococci Isolates

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The resistance to antimicrobial agents among staphylococci is a major concern worldwide. This study was undertaken to find out the presence of inducible clindamycin ($iMLS_B$) resistance among hospital and community associated staphylococci in our geographical area. A total of 560 staphylococci isolates from various clinical samples were studied. Inducible clindamycin resistance was detected by "D-test" using erythromycin and clindamycin discs as per CLSI guidelines. Three hundred seventy four (66.79%) isolates were hospital acquired and 186(33.21%) community associated. The overall prevalence of $iMLS_B$ was 122(21.78%). Community associated staphylococci revealed significantly lower prevalence of $iMLS_B$ (14.51% versus 25.40%) and higher rate of constitutive $cMLS_B$ (9.67% versus 2.67%) resistance compared to hospital acquired. $iMLS_B$ resistance was predominant among MRSA 70.11% and least among MSCONS 4.2%. Majority of $iMLS_B$ isolates were sensitive to gatifloxacin 87.70%, amikacin 80.32% and resistant to ampicillin 99.18%, ciprofloxacin 68.03%. The occurrence of $iMLS_B$ resistance in hospital as well as community set up raises concern of clindamycin treatment failure. It is essential to include "D-test" to detect inducible clindamycin resistance in routine antimicrobial susceptibility testing for the optimum treatment of patients.

Key words: Clindamycin, Community, D-test, Hospital, Staphylococci.

Staphylococcus aureus and coagulase-negative staphylococci (CONS) are recognized to be causing nosocomial and community-acquired infections in every region of the world.[1] Skin and soft- tissue infections (SSTIs) are a common manifestation of Staphylococcal disease in many community outbreaks.[2] Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives available to treat staphylococcal infections. The Macrolide-Lincosamide-Streptogramin B (MLS_B) family of antibiotics serves as one such alternative.[3] The good oral absorption of clindamycin makes it attractive option for use in outpatients or as follow-up treatment.[4] Expression of inducible resistance to clindamycin could limit the effectiveness of this drug.

Present study was aimed to find out the percentage of inducible clindamycin resistance among hospital and community associated staphylococci isolates in our geographical area. Also to know the difference in the antibiotic resistance pattern among these isolates.

MATERIALS AND METHODS

Detailed history is obtained from patients attending out patient department (OPD) regarding prior hospitalization and antibiotic intake. Isolates were designated as hospital acquired if the source

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patient had following risk factors: 72hour or more duration of hospital stay, residence in a long-term care facility, post operative wound, presence of a permanent indwelling catheter or percutaneous device, history of hospitalization, dialysis or surgery within one year. Isolate obtained from patient visiting OPD for the first time and without any of the above mentioned risk factors was considered community acquired.

Clinical samples were processed as per standard procedure. Staphylococci were identified by conventional method.[5] Methicillin resistance was detected by using cefoxitin disk (30µg). Antimicrobial susceptibility testing done by Kirby Bauer disc diffusion method as per CLSI guidelines.[6]

Inducible Clindamycin resistance was detected by Disk diffusion induction test "D Test".[6,7] Mueller Hinton Agar plate was inoculated with staphylococcal bacterial suspension with 0.5 McFarland turbidity. Erythromycin (15 µg) disk was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disk. Following overnight incubation at 37°C, isolates showing resistance to erythromycin while being sensitive to clindamycin and giving D shaped flattening of zone around clindamycin in the area between the two discs, were labeled as inducible $iMLS_B$ phenotype.[8] Further isolates showing small colonies growing near clindamycin disk in otherwise clear zone were labeled as D+ $iMLS_B$ phenotypes.[9] Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥ 21mm) were labeled as MS phenotypes. Isolates resistance to both erythromycin and clindamycin (Zone size ≤14mm) were labeled as constitutive $cMLS_B$ phenotype.[8]

Quality control (QC) for the erythromycin and clindamycin disc was performed with *Staphylococcus aureus* ATCC 25923 according to the standard disc diffusion procedure. Additional QC was performed with separate in-house selected *Staphylococcus aureus* strains that demonstrated positive and negative D- test reactions. [10]

Statistical analysis done by using Chi-square test.

RESULTS

A total of 560 staphylococci were isolated from pus, wound swab, aspirates, blood, cerebrospinal fluid and urine sample. Of these 374(66.7%) were hospital acquired and 186(33.2%) community associated. Majority 434 (77.50%) of isolates were *Staphylococcus aureus* and 126(22.50%) isolates were coagulase negative staphylococci. Methicillin resistance detected by using cefoxitin disk revealed, 87 (15.53%) isolates were methicillin resistant *Staphylococcus aureus* (MRSA), 347 (61.96%) methicillin sensitive *Staphylococcus aureus* (MSSA), 31 (5.53%) methicillin resistant coagulase negative staphylococci (MRCONS) and 95 (16.96 %) methicillin sensitive coagulase negative staphylococci (MSCONS).

A total 398 (71.07%) isolates were sensitive to both erythromycin and clindamycin. Inducible MLS_B detected by using D-test, revealed 122 (21.78%) isolates were of $iMLS_B$, 30 (5.35%) $cMLS_B$ and 10 (1.78%) MS phenotype. Majority

Table 1. Frequency of MLS_B phenotypes among different Staphylococci strains.

MLS_B phenotypes	MRSA n=87	MSSA n=347	MRCONS n=31	MSCONS n=95	Total
$iMLS_B$	61 (70.1%)	52 (14.9%)	05(16.1%)	04(4.2%)	122(21.7%)
$cMLS_B$	09(10.3%)	05(1.4%)	12(38.7%)	04(4.2%)	30(5.35%)
MS	00	5 (1.4%)	02(6.4%)	03(3.15%)	10(1.78%)

$iMLS_B$ - Inducible resistance, $cMLS_B$-Constitutive resistance.
MS -Resistance to erythromycin and sensitive to clindamycin.
MRSA- Methicillin resistant *Staphylococcus aureus*.
MSSA-Methicillin sensitive *Staphylococcus aureus*
MRCONS- Methicillin resistant coagulase negative staphylococci
MSCONS- Methicillin sensitive coagulase negative staphylococci

Table 2. Comparison of incidence of MLS_B phenotypes among Hospital acquired and community associated isolates

MLS_B phenotypes	CA (n=186)	HA (n=374)	Total (n=560)	P value
$iMLS_B$	27(14.51%)	95(25.4%)	122(21.7%)	< 0.01
$cMLS_B$	18(9.67%)	10 (2.67%)	28 (5%)	<0.001
MS	5 (2.68%)	5 (1.33%)	10 (1.78%)	>0.05

CA-Community acquired. HA-Hospital acquired.

Table 3. Antibiotic resistance pattern of different MLS_B Phenotypes

Antibiotics	iMLS B (n=122)		cMLS B (n=30)		MS (n=10)	
	No	%	No	%	No	%
Ampicillin	121	(99.18)	30	(100)	10	(100)
Amoxyclav	101	(82.78)	30	(100)	6	(60)
Tetracycline	59	(48.36)	19	(63.33)	4	(40)
Gentamicin	68	(55.73)	18	(60)	3	(30)
Amikacin	24	(19.67)	15	(50)	0	(0)
Ciprofloxacin	83	(68.03)	22	(73.33)	0	(0)
Gatifloxacin	15	(12.29)	9	(30)	0	(0)
Cotrimoxazole	71	(58.19)	22	(73.33)	2	(20)

of $iMLS_B$ strains were isolated from 102 (83.60%) pus samples. Distribution of MLS_B phenotypes and comparison of MLS_B phenotypes among hospital acquired and community associated isolates shown in Table 1 and 2 respectively. Antibiotic resistance pattern of MLS_B phenotypes shown in Table 3. All the isolates 560 (100%) were sensitive to vancomycin and linezolid.

DISCUSSION

Staphylococcal strains have shown a disconcerting propensity to develop resistance to antimicrobial agents and has become a challenge for the clinicians as well as infection control programme.[11] Resistance to antimicrobial agents is a major concern worldwide and is exemplified by the global spread of MRSA[12] and development of resistance to Macrolide, Lincosamide, Streptogramin B (MLS_B) group of antibiotics.

The resistance to MLS_B antibiotics can be mediated by msrA gene coding for efflux mechanism or target site modification by erm gene which can be expressed either constitutively $cMLS_B$ or inducibly $iMLS_B$.[13] Clinically bacterial strains exhibiting $iMLS_B$ have a high rate of spontaneous mutation to constitutive resistance and use of non inducer antibiotics such as clindamycin can lead to selection of constitutive mutants at frequencies of 10^{-7}cfu. leading to treatment failure.[14]

A total of 560 (12.54%) Staphylococcal strains were isolated from 4465 samples received during the study period of one year. Staphylococcal infection was more pronounced among hospitalized patients374 (66.80%). Predominant isolates were MSSA (61.96%). Least type was MRCONS 31 (5.53%). Predominance of MSSA was also reported by Angel MR et al [4] and Ajantha G S *et al.*[15]

Among CONS only 31 (24.60%) were methicillin resistant. Other studies have observed MRCONS ranging from 20.80 to 39.4%.[1,16] indicating lower range of prevalence of MRCONS in our area during this study period.

Three hundred and ninety eight (71.07%) isolates were sensitive to both erythromycin and clindamycin. MSSA isolates exhibited higher

susceptibility to erythromycin and clindamycin 285 (82.13%) and 290 (83.57%) respectively, compared to other isolates.

Erythromycin resistant isolates were 162 (28.92%). The overall incidence of $iMLS_B$ in the present study 21.78% is in agreement with Yilmaz G et al report.[1] Different investigators from India and other countries have reported incidence ranging from 11.8 to 29.8% .[3,7,16-21] Favorable factor is incidence of $cMLS_B$ resistance is very low 5.35% compared to other studies.[1,3,11,16] We did not observe any D+ isolate in our study.

Community associated staphylococci revealed significantly lower prevalence of iMLSB 27(14.51%) resistance compared to hospital acquired 95(25.4%). These findings correlate with the study by Patel, Waites *et al*. [2] Contrary to this cMLSB 10(9.67%) resistance was significantly higher among community associated staphylococci compared to hospital acquired 10(2.67%).

Fifty percentage of $iMLS_B$ were found to be among MRSA, least among MSCONS 3.23%. The incidence of $iMLS_B$ and $cMLS_B$ is higher among MRSA (70.11% and 10.34%) compared to MSSA (14.98% and 1.44%) respectively. None of the MRSA isolates were of MS phenotype. Few studies have reported MS phenotypes among MRSA ranging from 5.2 to 24.3%. [8,10,21,22] Incidence of $iMLS_B$ and $cMLS_B$ was higher among MRCONS (16.12% and 38.70%) compared to MSCONS (4.21% each). Similar observation is reported by other studies. [1,16]

Among the 122 (21.78%) $iMLS_B$ majority were sensitive to gatifloxacin 87.7% and amikacin 80.32%. More than 50% $iMLS_B$ strains were resistance to ampicillin (99.18%), amoxyclav (82.78%), gentamicin (55.73%), ciprofloxacin (68.03%) and cotrimoxazole (58.19%) i.e. routinely used drugs for empirical treatment of skin and soft tissue infection and respiratory tract infection. Gupta V, Datta P et al, reported least sensitivity to cotrimoxazole and ciprofloxacin.[10] Whereas, Pal, Sharma *et al.,* observed majority of $iMLS_B$ phenotypes 78.78% were sensitive to ciprofloxacin.[16] Isolates exhibiting $cMLS_B$, 70% were sensitive to gatifloxacin , 50% to amikacin, 36.6% to tetracycline. All (100%) the $cMLS_B$ strains were resistance to ampicillin and amoxyclav. Compared to MS phenotypes significantly higher resistant rate was seen among $iMLS_B$ towards ciprofloxacin and cotrimoxazole (p value <0.005 and <0.001). Compared to $iMLS_B$ significantly higher resistance rate was exhibited by $cMLS_B$ phenotypes towards amoxyclav, amikacin and gatifloxacin (P value <0.025,<0.001and <0.025 respectively).

All the isolates were sensitive to vancomycin and linezolid. Currently vancomycin resistance *Staphylococcus aureus* (VRSA) is not widespread. [10,16,21,23]

Overall 75.30% of erythromycin resistant, 23.01% of clindamycin sensitive isolates were shown to have $iMLS_B$ resistance by D-test. Strains with $iMLS_B$ demonstrate in vitro resistance to erythromycin while appearing susceptible to lincosamide and type B Sterptogramin. In vitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution and disk diffusion testing with erythromycin and clindamycin disks in non-adjacent positions.

These observation suggest that without D-test all these isolates 122 (23.01%) with $iMLS_B$ resistance would have been misidentified as clindamycin susceptible. In the present study only 10 (6.20%) erythromycin resistance isolates showed true clindamycin susceptibility(MS phenotypes). Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infection. D-test is necessary to correctly discriminate between $iMLS_B$ resistance and true susceptibility.[16]

The different patterns of resistance phenotypes observed in various studies are because $iMLS_B$ resistance varies by geographical region, age group, methicillin susceptibility and even from hospital to hospital.[16] Hence it should be determined in individual settings. Periodic surveillance of the prevalence of $iMLS_B$ isolates in the community and effective policy for the control of antimicrobial usage is required to monitor and to prevent the spread of these strains.

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