Antimicrobial profile of inducible clindamycin resistant strains of staphylococcus species

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Abstract

Introduction: The appearance of continuous resistant to multiple drugs among Staphylococci is a global burden due to its ability to cause severe infections. The selective use of drugs is necessary to overwhelm the situation. Taking in account present study was carried out to rule out true susceptibility of clindamycin towards staphylococcus species and its antimicrobial profile for judicial use of the drugs.

Material and Methods: All the clinical samples received in the Department of microbiology were screened for Staphylococci as per standard guidelines which were further subjected to Antimicrobial susceptibility testing and D-test to detect MLSb phenotypes.

Results: A total of 421 Staphylococcus species, 359(85.3%) were Staphylococcus aureus and 62(14.8%) were Coagulase negative Staphylococci; among them 42(10%) were Staphylococcus epidermidis & 20(4.8%) were Staphylococcus saprophyticus. D-test for S.aureus shows that 173(48.2%) inducible Clindamycin resistant, 113(31.5%) strains were constitutive MLSb phenotypes and 58(16.2%) strains shown to have MSb phenotypes. Among CoNS; among Staphylococcus epidermidis and S.saprophyticus 9.5% & 5% were Inducible Clindamycin resistance, 38.1% & 85% were constitutive MLSb phenotypes and 28.6% & 10% were MSb phenotypes respectively. All the isolates were sensitive to Linezolid, Vancomycin and Ceftaroline.

Conclusion: Inducible clindamycin resistant strains of Staphylococci found to be among half of the strains, indicating that true susceptibility of clindamycin should be rule out on routine basis for proper institution of the therapy.

Keywords: Staphylococci, MLSb phenotypes, AST.

Introduction

A notorious pathogen, which is gram positive cocci arranged in clusters, is making the condition worsen day by day with acquisition of multidrug resistance. Specifically, Staphylococcus aureus have left use very few therapeutic alternatives to treat such infection. Staphylococcus aureus causes variety of infection range from minor skin and soft tissue infection to life threatening condition like endocarditis, septicemia, toxic shock syndrome, Osteomyelitis etc. Emergence of methicillin resistant strains of Staphylococcus aureus is one of the major concerns. First resistance to this organism was noted in year of 1930 for sulphonamide, which was tackled by benzyl penicillin in 1941. The continuous uses of penicillin cause selection of resistant strain by the production of beta-lactamasase enzyme. Introduction of synthetic penicillin like methicillin and cloxacillin were seems to be control of, but in the year of 1962, methicillin resistant Staphylococcus species has started to emerge, that have evolved resistance to all penicillin group of drugs, newer synthetic penicillins and cephalosporins. Methicillin resistant strains of Staphylococcus aureus is mediated by the production of low affinity Penicillin binding protein 2a encoded by mecAgenes.

The macroline-lincosamide-streptogramin B (MLSb) group of antibiotics found to be good therapeutic alternative to treat MRSA, with Clindamycin being preferred agent due to its excellent pharmacokinetic properties. In recent year, resistant to MLSb antibiotics has started to emerge, additionally inducible resistance to Clindamycin under the influence of erm genes lead to therapeutic failure.

The MLSb group of drugs interact with 30s ribosomal subunit to inhibit protein synthesis of bacterial genes. The erythromycin (Macrolide) resistant strains of staphylococci enhance the production of methylase enzyme encoded by erm genes which modifies the target site and might predict the resistance to other group of drugs (Clindamycin). The target site modification either expressed inducible or constitutively, and these strains are difficult to detect as they appear as erythromycin resistant and Clindamycin sensitive in-vitro. In such case, in-vivo therapy with Clindamycin, erm genes mutant may be express constitutively resulting in therapeutic failure. Second mechanism of resistance to MLSb group antibiotics is presence of efflux pump encoded by msrA genes which leads to resistance to Macrolide and Streptogramin-B but not to Lincosamide known as MSb phenotypes. The genotypic detection of erm genes can be done, but it is costly and inconvenient in resource constraint settings, CLSI recommend phenotypic D-test which is simple, reliable, inexpensive, can be perform on routine basis.
The present study was carried out to detect inducible clindamycin resistant strains of staphylococcus species by using simple D-test and their antimicrobial susceptibility profile to find out the resistance pattern at our area.

**Material and Methods**

Present study was carried out in the Department of microbiology. After the clearance of RAC and IEC, all the clinical samples received from in-patients and out-patients were analysed for the isolation of pathogen. A total of 421 Staphylococcus species were isolated as per standard guidelines. All the isolates were further antimicrobial susceptibility testing by using Kirby-Bauer disc diffusion method as per CLSI guidelines. Those isolates which were resistant to erythromycin and sensitive to clindamycin were evaluated to detect MLSb phenotypes by using D-test.13,14 Briefly; Erythromycin (15μg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2μg) disc on a MHA plate previously inoculated with 0.5 McFarland bacterial suspensions, incubated at 37°C for 24 hours and result was interpreted as per CLSI guidelines as follows:15

1. **MSb phenotype:** Staphylococcus species exhibiting resistance to erythromycin (Zone size ≤ 13 mm) and sensitive to clindamycin (Zone size ≥ 21 mm) and giving circular zone of inhibition around clindamycin disc.

2. **Inducible MLSb phenotype:** Staphylococcus species exhibiting resistance to erythromycin (Zone size ≤ 13 mm) and sensitive to clindamycin (Zone size ≥ 21 mm) and giving D shaped zone of inhibition around clindamycin disc.

3. **Constitutive MLSb phenotype:** Staphylococcus species exhibiting resistance to both erythromycin (Zone size ≤ 13 mm) and clindamycin (Zone size ≤ 14 mm) with giving circular shape of zone of inhibition if any around clindamycin disc.

**Statistical Analysis**

It was done by using IBM SPSS (20 version) software. Frequencies & percentages were calculated for all the parameters. Non Parametric test was run by selecting one sample, in which automatically compares observed data to hypothesized using the Chi-Square test.

**Observation and Result**

A total of 421 Staphylococcus species isolated from various clinical samples, out of which 359 were *Staphylococcus aureus* and 62 were CoNS. All the isolates were subjected to detection of methicillin resistance and MLSb phenotypes.

| Methicillin resistance | S. aureus | CoNS |
|------------------------|-----------|------|
| MRSA                   | 280       | 44   |
| MSSA                   | 79        | 18   |
| Total                  | 359       | 62   |

| Type of resistance | MRSA (n=280) | MSSA (n=79) |
|-------------------|--------------|-------------|
| Frequency         | Percent      | Frequency   | Percent  |
| ER-sensitive      | 0            | 0           | 15       | 19      |
| iMLSb phenotype   | 101          | 36.1        | 12       | 15.2    |
| cMLSb phenotype   | 162          | 57.9        | 11       | 13.9    |
| MSb phenotype     | 17           | 6           | 41       | 51.9    |
| Total             | 280          | 100         | 79       | 100     |

**Table 1:** Methicillin resistant strains of *Staphylococcus* species

**Table 2:** Frequency of MLSb phenotype among *Staphylococcus aureus*

**Table 3:** Distribution of MLSb phenotype among MRSA and MSSA isolates
Table 4: Frequency of MLSb phenotypes among CoNS

| CoNS           | Phenotypes | Frequency | Percent | Valid Percent | Cumulative Percent |
|----------------|------------|-----------|---------|---------------|--------------------|
| S.epidermidis  | ER-Sensitive | 10         | 23.8    | 23.8          | 23.8               |
|                | cMLSb      | 16         | 38.1    | 38.1          | 61.9               |
|                | iMLSb      | 4          | 9.5     | 9.5           | 71.4               |
|                | MSb        | 12         | 28.6    | 28.6          | 100.0              |
|                | Total      | 42         | 100.0   | 100.0         | 100.0              |
| S.saprophyticus| ER-Sensitive | 0          | 0       | 0             | 0                  |
|                | iMLSb      | 1          | 5.0     | 5.0           | 5.0                |
|                | cMLBb      | 17         | 85.0    | 85.0          | 90.0               |
|                | MSb        | 2          | 10.0    | 10.0          | 100.0              |
|                | Total      | 20         | 100.0   | 100.0         | 100.0              |

One-sample Chi-square test

Test statistics: 7.143
Degree of freedom: 3
Asymptomatic p-value (2-sided test): 0.067

Table 5: Distribution of MLSb phenotype among MRCNSS and MSCNSS

| Type of strain | Type of resistance |
|----------------|--------------------|
| MRCNSS (n=44)  | iMLSb 4  cMLSb 31  MSb 0  Total 38 |
| MSCNSS (n=18)  | iMLSb 0  cMLSb 0  MSb 11  Total 13 |
| Total          | iMLSb 06  cMLSb 31  MSb 14  Total 51 |

Table 6: Antibiotic sensitivity pattern of MLSbphenotypes Staphylococcal aureus

| Antibiotics    | iMLSb(n=113) | cMLSb(n=173) | MSb(n=58) |
|----------------|--------------|--------------|-----------|
| S (%) | R (%) | S (%) | R (%) | S (%) | R (%) |
| Erythromycin   | 00 | 113(100) | 00 | 173(100) | 00 | 58(100) |
| Clindamycin    | 113(100) | 00 | 00 | 173(100) | 58(100) | 00 |
| Cefoxitin      | 12(10.6) | 101(89.4) | 11(6.4) | 162(93.6) | 41(70.7) | 17(29.3) |
| Penicillin     | 00 | 113(100) | 00 | 173(100) | 00 | 58(100) |
| Trimetho-sulfa | 05(4.5) | 108(95.5) | 67(38.8) | 106(61.2) | 39(67.2) | 19(32.8) |
| Ceftaroline    | 113(100) | 00 | 173(100) | 00 | 58(100) | 00 |
| Linezolid      | 113(100) | 00 | 173(100) | 00 | 58(100) | 00 |
| Tetracycline   | 16(14.2) | 97(85.8) | 00 | 173(100) | 41(70.7) | 17(29.3) |
| Vancomycin     | 113(100) | 00 | 173(100) | 00 | 58(100) | 00 |
| Rifampin       | 113(100) | 00 | 173(100) | 00 | 55(94.8) | 03(5.2) |
| Chloramphenicol| 16(14.2) | 97(85.8) | 27(15.6) | 146(84.4) | 06(10.4) | 52(89.6) |
| Ofloxacin      | 00 | 113(100) | 16(9.3) | 157(90.7) | 45(77.6) | 13(22.4) |
| Gentamycin     | 15(13.3) | 98(86.7) | 16(9.3) | 157(90.7) | 38(65.5) | 20(34.5) |

Table 7: Antibiotic sensitivity pattern of erythromycin resistant CONS

| Antimicrobial agents | S.epidermidis=42 | S.saprophyticus=20 |
|---------------------|-------------------|---------------------|
|                     | S | %  | R | %  | S | %  | R | %  |
| Erythromycin        | 10 | 23.8| 32 | 76.2| 01 | 5  | 19 | 95 |
| Clindamycin         | 26 | 61.9| 16 | 38.1| 05 | 25 | 15 | 75 |
| Cefoxitin           | 18 | 42.8| 24 | 57.2| 00 | 0  | 20 | 100|
| Penicillin          | 00 | 0  | 42 | 100| 00 | 0  | 20 | 100|
| Trimethoprim-sulfa  | 07 | 16.6| 35 | 83.4| 08 | 40 | 12 | 60 |
| Ceftaroline         | 42 | 100 | 0  | 0  | 0  | 20 | 100| 0  |
| Linezolid           | 42 | 100 | 0  | 0  | 0  | 20 | 100| 0  |
| Tetracycline        | 22 | 52.4| 20 | 47.6| 04 | 20 | 16 | 80 |
| Vancomycin          | 42 | 100 | 0  | 0  | 20 | 100| 0  |
| Rifampin            | 40 | 95.2| 02 | 4.8 | 19 | 95 | 01 | 5  |
| Chloramphenicol     | 21 | 50  | 21 | 50 | 16 | 80 | 04 | 20 |
| Ofloxacin           | 06 | 14.3| 36 | 85.7| 16 | 80 | 04 | 20 |
| Gentamycin          | 19 | 45.2| 23 | 54.8| 13 | 65 | 07 | 35 |
Discussion
A total of 421 Staphylococcus species were isolated from different clinical specimens. Out of which, 359(85.3%) were Staphylococcus aureus and 62(14.8%) were Coagulase negative Staphylococci; among them 42(10%) were Staphylococcus epidermidis& 20(4.8%) were Staphylococcus saprophyticus.

Inducible Clindamycin resistant strains were found out among erythromycin resistant strains of Staphylococcus species by using D-test. The test was performed by placing erythromycin and Clindamycin disc at 15 mm distance from edge to edge. The Inter-disc distance of 15 mm has been found to satisfactory by Ajanta G.S. etal16 and Fiebelkorn K.R et al.17 In our study same protocol of keeping inter-disc distance of 15 mm was followed.

In present study, out of 359 Staphylococcus aureus isolates, 344(95.8%) were erythromycin resistant, these were further subjected to D-test. The D-test revealed MLSb phenotypes among Staphylococcus aureus, 113(31.5%) strains were D-test positive indicating inducible Clindamycin resistant strains of Staphylococcus aureus (iMLSb phenotype), 173(48.2%) strains were constitutive MLSb phenotypes and 58(16.2%) strains shown to have MSb phenotypes. Similar studies were conducted by Sunil Hatkar.et al10 (iMLSb phenotypes 26.13%, cMLSb phenotypes 58.52%, and MSb phenotypes15.34%), VeenaManjunathetal18 (iMLSb phenotypes 33.33%, cMLSb phenotypes 18.75%, and MSb phenotypes 47.9%) which is in concordance with present study.

Prevalence of MLSb phenotypes among MRSA and MSSA were analysed and it was observed that inducible and constitutive Clindamycin resistant strains of Staphylococcus aureus were higher amongst MRSA isolates (36.1% & 57.9% respectively) as compared to MSSA isolates (15.2% & 13.9% respectively). In a study of VeenaManjunathetal,18 percentages of inducible Clindamycin resistance were higher among MRSA as compared to MSSA (57.63% and 16.22% respectively).

The coagulase negative staphylococci also screened for Inducible Clindamycin resistant strains. Among Staphylococcus epidermidis and S.saprophyticus 9.5% & 5% were Inducible Clindamycin resistance, 38.1% & 85% were constitutive MLSb phenotypes and 28.6%& 10% were MSb phenotypes.

Antimicrobial susceptibility of inducible Clindamycin resistant strains of Staphylococcus aureus (erythromycin resistant & Clindamycin sensitive) were analysed by using Kirby Bauer’s disc diffusion method as per CLSI guidelines. It was observed that penicillin, Ofloxacin, were 100% resistant, followed by Trimethoprim-sulfamethoxazole 108(95.5%), Cefoxitin 101(89.4%), Gentamicyn 98(86.7%) and 97(85.8%) were resistant to Tetracycline & Chloramphenicol while Linezolid, Vancomycin, Ceftrarline, Rifampin were 100% sensitive.

Conclusion
Emergence of multidrug resistant strains of Staphylococcus species is an alarming and clinicians should aware about it. Use of Clindamycin without knowing the inducible resistance may lead to therapeutic failure. In present study, prevalence of inducible resistance was significant and we can conclude that the detection of inducible Clindamycin resistance on routine basis is mandatory for judicial use of the drug and proper institution of the therapy.

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