Original Article

Profile of antimicrobial resistance mechanisms in clinical isolates of Staphylococci with special reference to inducible clindamycin resistance

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Abstract :

The presence of multidrug resistance Staphylococci and possession of different mechanisms of drug resistance induced us to undertake the study to assess the frequency of different resistance mechanisms and especially the presence of inducible clindamycin resistance. A total of 206 Staphylococcal isolates comprising of 142 (68.93%) Coagulase positive Staphylococci and 64 (31.07%) coagulase negative Staphylococci (CONS) were tested against antibiotic susceptibility by disk diffusion technique. The Beta-lactamase production, methicillin resistance and inducible clindamycin resistance were also determined. All the Staphylococci were sensitive to vancomycin(100%), followed by amikacin(97.08%), gentamycin(94.17%), rifampicin(93.20%), amoxyclav(76.69%), clindamycin(76.69%), pristinamycin(69.90%), cephalexin(68.93%), ciprofloxacin(68.93%), and with low sensitivity to erythromycin(58.25%) and ampicillin(1.94%). Beta-lactamase production was seen in 196 (95.15%) of 206 Staphylococcal strains. While 32(22.53%) out of 142 were methicillin resistant Staphylococcus aureus (MRSA) and 14(21.87%) out of 64 were methicillin resistant coagulase negative Staphylococci. Inducible clindamycin resistance was seen in 32 (15.53%) of isolates (12 D and 4 D^+ phenotype). All the three resistance mechanisms were co-existent in 6 (2.91%) of Staphylococci while two mechanisms were simultaneously observed in 64 (31.06%) isolates and single mechanism was observed in 128 (62.14%) strains. The results indicate that the Staphylococcal isolates in this region are fairly resistant strains, Beta-lactamase production is very common, MRSA strains are also frequently encountered and inducible clindamycin resistance can be detected. The D-test is simple and useful for its detection and also for determination of its phenotypes. It is important to notice simultaneous occurrence of these mechanisms of resistance. Hence, periodic assessment of all these factors is necessary for control of resistant pathogens.

Keywords : Beta-lactamase, D-Test, Inducible clindamycin resistance, Methicillin resistant Staphylococcus aureus.

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Introduction:

Staphylococcus is one of the major notorious nosocomial pathogen and the increasing and indiscriminate use of antibiotics is leading to the introduction of more and more drug resistant strains. The beta lactamase production, methicillin resistance and inducible clindamycin resistance are some of the important issues related to the drug resistance in Staphylococci which decide the choice of antibiotics for treatment as well as eradication of strains. The frequency, type of resistance and the different mechanisms that operate in these isolates vary from place to place (1-3). The beta lactamase production and methicillin resistance has been studied frequently (4-5). In case of inducible clindamycin (iMLSb) resistance, 14-15 membered macrolides, lincosamides and streptogramin b induces the activation of the erm gene (6) and confers resistance against them. However, erythromycin (a macrolide) is a more potent inducer of this gene than any other antibiotic in MLSb group, and it can induce this resistance in-vitro as well, unlike clindamycin (a lincosamide), which is a weak inducer and fails to induce resistance in-vitro (1, 6-7). Hence, the routine lab tests fail to detect the presence of inducible clindamycin resistance and gives false results which may lead to clinical failure (8-9). The data in this regards is rather fragmentary (6). Therefore, it is always desirable to generate local data and update it regularly for proper treatment and effective eradication strategies for such pathogens.

The present study was designed to study the pattern of antimicrobial resistance in clinical isolates of Staphylococci. The frequency of occurrence of common mechanisms viz., beta lactamase production, methicillin resistance and inducible clindamycin resistance were also evaluated. A special emphasis was given to study the different phenotypes of clindamycin resistance as obtained by D test. Further, the sensitivity of these isolates to rifampicin and pristinamycin, which are recommended drugs for empirical treatment of MRSA, was also assessed.

Material and Methods:

A total of 206 isolates of Staphylococci obtained from various clinical specimens of patients admitted in various

the city were included in the study. Each isolate of Staphylococcus was identified and characterized to be Staphylococcus aureus or Coagulase negative Staphylococcus (CONS) in the laboratory by morphology, culture characteristics & standard biochemical tests (10).

Antimicrobial Susceptibility Testing: All the strains were then tested against 12 different antibiotics (Hi-Media): amikacin (30μg), ampicilin (10 μg), amoxycillin+clavulinic acid (30 μg), cefuroxime (30 μg), cephalexin (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamycin (10 μg), pristinamycin (15 μg), rifampicin (5 μg), and vancomycin (30 μg) by Kirby-Bauer Method (11) as per the CLSI guidelines (12).

Beta-Lactamase Test: All strains were tested for Beta-Lactamase production by Iodometric method (10). Here 0.1ml of penicillin solution (6000μg/ml) was inoculated with a loopful culture of Staphylococcus and incubated at 37^{0}C for an hour. Then 2 drops of freshly prepared 1% starch solution was added to each tube and mixed well. This was followed by the addition of Iodine solution, which gives blue coloration to the solution. If the blue colour decolorized within 10 minutes the strain was declared Beta-Lactamase positive and if the colour persisted the strain was Beta-Lactamase negative.

Detection of Methicillin Resistance: The methicillin resistance was determined in all the strains using oxacillin discs (2μg) by standard disk diffusion method as per the CLSI standards (12). The strains which showed zone diameter less than 10 mm were the Methicillin resistant strains.

D-Zone Test: All the strains were subjected to D-Zone Test to ascertain the presence of inducible clindamycin resistance (1). The procedure in brief is as follows. The erythromycin (15μg) disc was placed at a distance of 15mm, centre to centre, from clindamycin (2μg) disc on a Mueller-Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. It was then subjected to overnight incubation at 37^{0}C, where the flattening of the zone on the inner side around clindamycin, giving it an appearance of D, indicated inducible clindamycin resistance (1). After incubation different phenotypes were observed and interpreted (Refer to Table 2 for detailed description of phenotypes).

Results:

Out of 206 Staphylococcal isolates 142 (68.93%) were coagulase positive and 64 (31.07%) were coagulase negative. Their antimicrobial susceptibility profile is shown in Table 1. The Beta lactamase production was observed in 196 (95.15%) of Staphylococcal isolates of which 136 (95.77%) Coagulase positive Staphylococci and 60 (93.75%) Coagulase negative Staphylococci.

Out of 142 coagulase positive Staphylococci, 32 (22.53%) were methicillin resistant (MRSA) and of the 64 coagulase negative Staphylococci 14 (21.87%) had methicillin resistance (MRCONS).

Inducible clindamycin resistance as determined by D-test was seen in 32 (15.53%) (24 plus 8, D & D + phenotypes respectively) of all the isolates. All the 32 D-Test positive isolates were Coagulase positive Staphylococci. Of the total 96 erythromycin resistant strains of Staphylococci 26 (27.08%) strains were truly sensitive to clindamycin, 38 (39.58%) showed constitutive MLSb resistance and 32 (33.33%) had inducible resistance. The resistant phenotypes as observed by D-test (Figure 1) are shown in Table 2.

Table 1: Antimicrobial susceptibility of coagulase positive & coagulase negative Staphylococci

Sr. No.	Antibiotics	Coagulase Positive (Sensitive) (n-142)	Coagulase Negative (Sensitive) (n-64)	Total Sensitive (n-206)
1	Vancomycin	142 (100%)	64 (100%)	206 (100%)
2	Amikacin	138 (97.18%)	62 (96.87%)	200 (97.08%)
3	Gentamicin	134 (94.36%)	60 (93.75%)	194 (94.17%)
4	Rifampicin	132 (92.95%)	60 (93.75%)	192 (93.20%)
5	Pristinamycin	120 (84.50%)	24 (37.5%)	144 (69.90%)
6	Cefuroxime	112 (78.87%)	24 (37.5%)	136 (66.01%)
7	Amoxyclav	110 (77.46%)	48 (75%)	158 (76.69%)
8	Cephalexin	98 (69.01%)	44 (68.75%)	142 (68.93%)
9	Clindamycin	106 (74.64%)	52 (81.25%)	158 (76.69%)
10	Ciprofloxacin	84 (59.15%)	38 (59.37%)	142 (68.93%)
11	Erythromycin	68 (47.88%)	52 (81.25%)	120 (58.25%)
12	Ampicillin	4 (2.8%)	0 (0%)	4 (1.94%)

The occurrence and co-existence of Inducible clindamycin resistance, methicillin resistance and Beta lactamase production is shown in Table 3.

Table 3: Individual and simultaneous occurrence of different mechanisms of resistance in Staphylococcal isolates

Mechanism Only one test positive	No. of isolates (C +ve) n=142	No. of isolates (C -ve) n=64	Total n=206
BL	80 (55.55%)	46 (71.87%)	126 (61.17%)
MR	0 (0.0%)	0 (0.0%)	0 (0.0%)
D-Test	2 (1.40%)	0 (0.0%)	2 (0.97%)
(A) Total	82 (57.76%)	46 (71.87%)	128 (62.14%)
Any Two tests positive			
BL + MR	26 (18.31%)	14 (21.88%)	40 (19.41%)
BL + D-Test	24 (16.90%)	0 (0.0%)	24 (11.65%)
MR + D-Test	0 (0.0%)	0 (0.0%)	0 (0.0%)
(B) Total	50 (35.21%)	14 (21.88%)	64 (31.06%)
All Three tests positive			
(C) BL + MR + D-Test	6 (4.22%)	0 (0.0%)	6 (2.91%)
Total (A+B+C)	138 (97.18%)	60 (93.75%)	198 (96.12%)

BL: Beta Lactamase, MR: Methicillin Resistant, C+ve: Coagulase Positive, C-ve: Coagulase Negative.

Table 2: Different phenotypes of iMLSb resistance

Induction Test Phenotype	No. of strains	Resistance Phenotype	Clindamycin (CLI) results	Erythromycin (ERY) results	Induction Test Description
D	24	Inducible MLS_B	S	R	Blunted, D-shaped clear zone around CLI disc proximal to ERY disc.
D^+	08	Inducible MLS_B	S	R	Blunted, D-shaped clear zone around CLI disc proximal to ERY disc and small colonies growing to CLI disc in otherwise clear zone
Neg	26	MS_B	S	R	Clear zone around CLI disc.
HD	00	Constitutive MLS_B	R	R	Two zones of growth appear around CLI disc. One zone is a light, hazy growth extending from the CLI disc to the second zone where the growth is much heavier. The inner heavy zone is blunted proximal to the ERY disc as in phenotype D.
R	38	Constitutive MLS_B	R	R	No hazy zone. Growth up to CLI and ERY discs.
Total	96			R	All ERY Resistant
S	110	No resistance	S	S	Clear, susceptible zone diameters.
Grand Total	206				

Discussion:

Staphylococci are associated with various infections and their propensity to acquire resistance to various drugs induced us to study the profile and common mechanisms of drug resistance amongst Staphylococcal isolated from various specimens.

The antimicrobial susceptibility profile in the present study indicates common occurrence of multidrug resistance amongst the both Coagulase positive and Coagulase negative Staphylococci. The frequency of the resistance varies from place to place (1-3). All the strains in the study were uniformly sensitive to vancomycin; but resistance to other antimicrobial agents was variable. All the isolates were particularly more resistant to more frequently and empirically used drugs viz. ampicillin, erythromycin and ciprofloxacin. Interestingly, there was not much difference in susceptibility to various antibiotics between Coagulase positive Staphylococci and CONS. CONS many times being the part of commensal flora might be having repeated exposure to different antibiotics and would have acquired the resistance. All the isolates in the present study were from the patients admitted in the hospitals, hence it is again expected that they are likely to be more drug resistant.

One of the common mechanisms of drug resistance in Staphylococci is by production of Beta-Lactamase enzyme. In the present study the frequency of beta lactamase production was very high, both for Coagulase positive as well as coagulase negative staphylococci. Similar high prevalence has been reported by others (13).

Occurrence of MRSA is yet another important aspect of drug resistance in staphylococci. In the present study, 32 (22.53%) of S. aureus isolates were MRSA. The prevalence of MRSA varies greatly from place to place ranging between 20 and 55 per cent (14-15). Our prevalence rate of MRSA is in accordance with the prevalence rate reported from this subcontinent (14). In our study MRCONS were also detected, nevertheless, the genetic determinant in them is different from that of MRSA (16).

Clindamycin, which is a lincosamide, serves to be choice of drug in case of skin, soft tissue and bone infections (6). However, widespread use of MLSb (Macrolide, Lincosamide and Streptogramin b) antibiotics is leading to resistance development against it as well. There are two different type genes conferring the resistance against these groups of antibiotics by different mechanims. (i) msrA gene mediating drug resistance by efflux mechanism, and (ii) erm gene (ermA and ermC) conferring MLSb resistance by target site modification, which may be Constitutive MLSb (cMLSb) resistance or Inducible MLSb (iMLSb) resistance (1,7-9).
Inducible clindamycin resistance, as studied by D-test, in the present study was observed in 32 (15.53%) of all the 206 Staphylococcal isolates. All these 32 isolates were Coagulase positive Staphylococci. Again the prevalence of inducible clindamycin resistance varies greatly (1, 8, 17). The sensitivity to clindamycin was consistent in 110

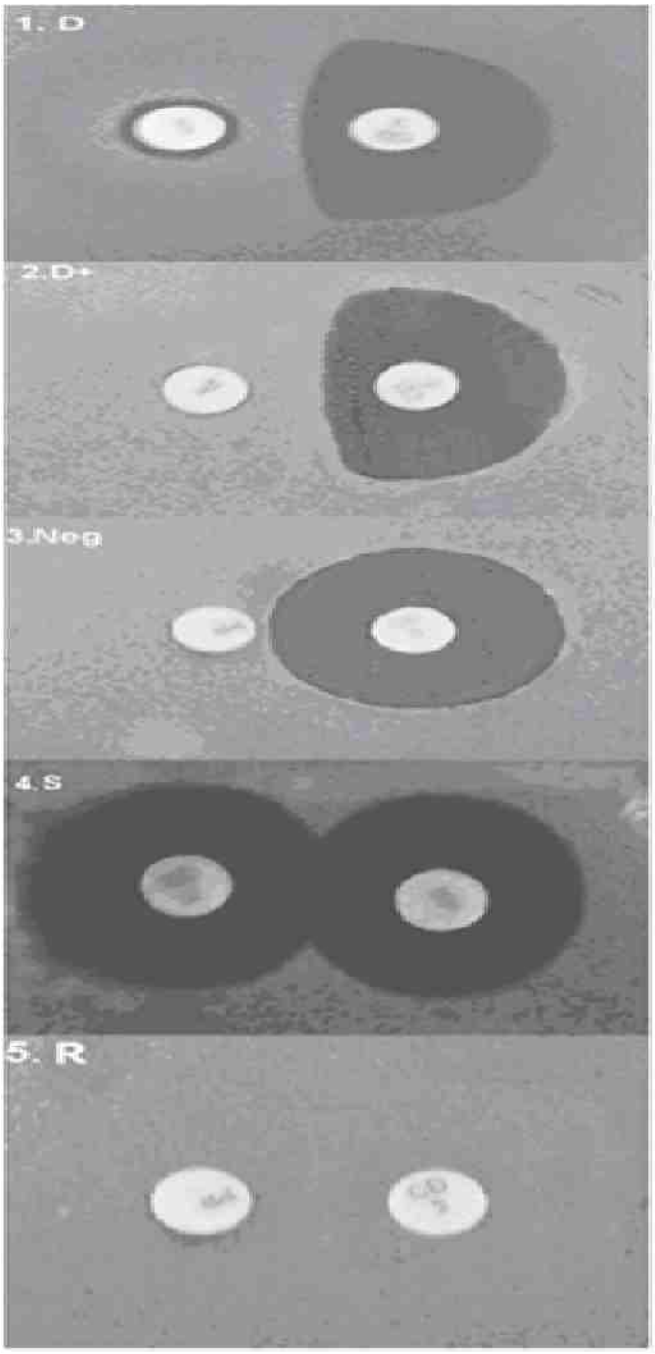

Figure 1: D-Test (1) D: Blunted, D-shaped clear zone around CLI disc proximal to ERY disc.
(2) D^+: Blunted, D-shaped clear zone around CLI disc proximal to ERY disc and small colonies growing to CLI disc in otherwise clear zone.
(3) Neg: Clear zone around CLI disc.
(4) S: Clear, susceptible zone diameters.
(5) R: No hazy zone. Growth up to CLI and ERY discs

zerythromycin sensitive strains. Out of 96 erythromycin resistant strains, clindamycin showed constitutive resistance in as many as 39.58 per cent strains while out of remaining strains only 27.08 per cent were true sensitive and almost one third of erythromycin resistance strains revealed inducible clindamycin resistance. Hence, it is indeed very important to determine inducible clindamycin resistance in Staphylococcal isolates that are resistant to erythromycin, otherwise, patient may receive clindamycin unnecessarily without any actual therapeutic benefit.

However, a standard modification of regular disk diffusion test called as D-Test or D-Zone Test is devised by CLSI, is capable detecting the prevalence of inducible MLSb resistance, even in moderately equipped laboratories. This test involves placement of erythromycin and clindamycin disk at a distance of 15mm, from centre to centre, on Mueller-Hinton Agar and incubated at 37°C for 18 to 24 hrs. As the antibiotic diffuses through the media erythromycin induces the erm gene activation, which confers resistance to the organism against both erythromycin and clindamycin. However, this induction is detectable only upto the region of erythromycin diffusion in the media. This causes the blunting of the zone around clindamycin giving it an appearance of a "D" (8, 12).

The D-test used in this study is simple, easy to perform, economical and suitable for any moderately equipped laboratory. This method is found to be quite simple and useful to discriminate between phenotypes (Figure 1) by us as well as others (1, 18).

The three mechanisms of resistance studied in the present study revealed them to be present in as many as 96.12 % of Staphylococcal isolates, either singly or together. All these three mechanisms can confer resistance simultaneously to many antibiotics. In our study, in 4.22 % of Staphylococcus aureus strains all the three mechanisms were simultaneously existent making them potentially more problematic strains to treat. Although the number is very tiny at present, but the existence of such strains by themselves should be cause of concern. The association of different mechanisms has occasionally been described earlier (8).

The rifampicin & pristinamycin are empirically administered drugs for such type of multidrug resistant staphylococci. In the present study, it is observed that the sensitivity to rifampicin is good however strains showed substantial resistance to pristinamycin and hence this drug should be cautiously used for empirical treatment in this region. Pristinamycin resistance has also been observed by others (19-20).

Thus, the results indicate that there is high prevalence of multi drug resistant Staphylococci in this region, which include Beta lactamase producing strains with very high frequency, MRSA as well as MLSb strains. This further confirms the observation that various factors may operate simultaneously for induction of drug resistance in bacteria.

Hence it becomes necessary to isolate the organism from the clinical specimens and study its antimicrobial susceptibility

pattern. It is further essential to evaluate the different factors and means by which it acquires the antimicrobial resistance to choose the appropriate antimicrobial agent for therapy and formulate the policy for eradication of drug resistant problematic strains of Staphylococci. The generation of such data further helps to formulate the antibiotic policy and also the control measures.

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