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Research Paper

D- Test and Treatment of MRSA

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Abstract
Staphylococcus aureus especially Methicillin Resistant Staphylococcus aureus (MRSA) is challenging as a hospital pathogen, as it is not only resistant to Methicillin but to most of the first line antibiotics used for treatment. This usually limits the choice for treatment to Clindamycin, Vancomycin and Linezolid. The latter two being the second line antibiotics need to be kept as reserve drugs. Clindamycin is tolerable, cheaper, available as oral preparations, has good tissue penetration, hence a good choice for skin and bone infections. On routine antibiotic testing by Disc-Diffusion Clindamycin may appear sensitive, but resistance is expressed after induction with Erythromycin, leading to treatment failure, making Clindamycin unpopular in the treatment of MRSA. These strains can be picked out by adopting a simple method, D-test. A total of 1086 Staphylococcus aureus strains isolated from various clinical specimens were tested for antibiotic sensitivity by Kirby Bauer disc diffusion method, Methicillin resistance was detected using Cefoxitin (30µg) disc, and D-test was done on those isolates which were erythromycin resistant. Among the 1086 isolates, 402 were MRSA, out of which 271 (67.4%) were truly sensitive to Clindamycin (after testing for inducible resistance). Only 32.6% need to be treated with Linezolid or Vancomycin. Hence by doing D-test routinely, majority of isolates could be treated with Clindamycin without fear of treatment failure and inadvertent use of Linezolid could be avoided.

Keywords: *Methicillin Resistant Staphylococcus aureus, Clindamycin, Inducible resistance, D-test, Linezolid.*

Introduction

Staphylococcus aureus causes a spectrum of clinical diseases ranging from superficial skin infections to deep seated abscesses and various systemic pyogenic infections like otitis media and endocarditis [(1)].

The usual first line antibiotics used for treating Staphylococcal infections and hence the panel for antibiotic sensitivity testing include penicillins, first generation cephalosporin (cephalexin), β-lactamase resistant penicillin, erythromycin (macrolides), clindamycin (lincosamides), cotrimoxazole, and gentamicin.

The ability of *Staphylococcus aureus* to develop resistance to penicillin and other antibiotics enhance their importance as a human pathogen especially in the hospital environment. [(2).]

The mechanism of penicillin resistance is either plasmid mediated (β-lactamase production) or chromosomally mediated[(1)] (altered penicillin binding proteins- PBP2a, mecA gene). Isolates showing chromosomally mediated penicillin

resistance (MRSA) can be detected by using oxacillin / cefoxitin discs.

MRSA can be treated with macrolides, aminoglycosides, lincosamides, glycopeptides and linezolid. Usually, MRSA being resistant to aminoglycosides and macrolides, the list of antibiotics for treatment of MRSA is narrowed down to lincosamides, glycopeptides (vancomycin) and linezolid.

.Resistance to macrolides and lincosamides is known to be mediated by two mechanisms [3] -

i) due to *msrA* gene which mediates an efflux pump that pumps out erythromycin. Such strains will be resistant to erythromycin, but sensitive to clindamycin.

ii) Resistance to macrolides and lincosamide usually is due to an *erm* gene (MLS resistance). Strains with erm A/B/C are typically resistant to erythromycin; but when initially tested, may appear to be sensitive to clindamycin. In such isolates, clindamycin resistance is expressed after induction with erythromycin.

Hence, on routine antibiotic sensitivity testing by Kirby Bauer method, strains with erm genes which appear sensitive to clindamycin, are reported as sensitive but show inducible resistance resulting in treatment failure. These strains could be detected by adopting a simple method called D-test in which erythromycin and clindamycin discs are placed adjacent.

Three different phenotypes can be noted after doing the D-test [4]

a. MS phenotype: Isolates exhibiting resistance to erythromycin while sensitive to clindamycin. (negative D test)

b. Inducible MLS_B phenotype: Isolates showing resistance to erythromycin and sensitivity to clindamycin but giving a D-shaped zone of inhibition around clindamycin.(positive D test)

c. Constitutive MLS_B phenotype: Isolates that show resistance to both erythromycin and clindamycin.

Materials and methods

A total of 1086 *Staphylococcus aureus* strains isolated from various clinical specimens over a period of three years in a tertiary care centre was included in the study. These isolates were tested for antibiotic sensitivity by Kirby Bauer disc diffusion method, and methicillin resistance detected as per CLSI guidelines.

Those isolates which were erythromycin resistant, were further subjected to D-test. Erythromycin (15µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2µg) on a Mueller Hinton agar plate inoculated with *Staphylococcus aureus*. Following overnight incubation, flattening of zone (D- shaped) around clindamycin indicated inducible clindamycin resistance (CLSI guidelines). Fig 1

Results

Among the 1086 *Staphylococcus aureus* isolates, 402 (37%) were MRSA.

Table -1 Distribution of MRSA isolates after D- test

Year	2014	2015	2016		
Phenotype	No: of MRSA isolates	No: of MRSA isolates	No: of MRSA isolates	Total number	Percentage
ER and CD sensitive	15	14	14	43	10.7%
ER resistant and CD sensitive (D-test negative- MS phenotype)	98	51	79	228	56.7%
ER resistant and CD resistant (Constitutive CD resistance)	16	8	8	32	8%
ER resistant and CD D- test positive (Inducible MLS_B phenotype)	14	28	57	99	24.6%
Total	143	101	158	402	100%

In the above study, out of the 402 MRSA isolates, 271 (67.4%) were truly sensitive to clindamycin (43 were sensitive to both cindamycin and erythromycin; 228 were only resistant to erythromycin- D test negative).

Of the remaining 131 MRSA isolates, 75% expressed inducible clindamycin resistance shown by positive D test.

Discussion

Staphylococcus aureus especially *MRSA* is challenging as a hospital pathogen, as it is not only resistant to methicillin but to most of the first line antibiotics used for treatment. This usually limits the choice for treatment to clindamycin, vancomycin and linezolid. The latter two being the second line antibiotics need to be kept as reserve drugs.

In this study, most of the MRSA isolates, 89.3% are resistant to erythromycin.

Vancomycin, which is commonly prescribed to treat infections by MRSA is unpopular for its high cost & toxicity.

Linezolid is another valuable drug useful for MRSA. Its high bioavailability, post antibiotic effect, ease of switching to oral therapy and the fact that it can be used in patients of all ages and in patients with liver disease and poor kidney function, and its increased effectiveness over glycopeptides makes this a precious drug in the treatment of resistant *Staphylococcal* and other Gram positive bacterial infections. [(5)]

Clindamycin is an alternative to treat MRSA, because it is tolerable, available as oral preparations, has good tissue penetration and hence is a good choice for skin and bone infections[(6)]

But before clindamycin is to be reported as sensitive, D-test is to be done to avoid treatment failure, by confirming that the isolate is truly sensitive to clindamycin and will not develop inducible resistance. This will enable us to conserve linezolid which is a wonderful drug to treat more severe infections due to MRSA when all other drugs are resistant.

In the present study, only 131 isolates (32.6%) which are clindamycin resistant (both constitutive and inducible) needed to be treated with linezolid or vancomycin.

Hence, we may consider application of the D-test in routine antibiotic sensitivity testing by placing erythromycin and clindamycin 15 mm apart, so that re-testing for inducible resistance and thereby the delay in reporting can be avoided. It may be mentioned in the sensitivity report - "tested for inducible clindamycin resistance "to build up confidence in the treating physician.

The same principle holds good for *Coagulase negative Staphylococci*, which is commonly isolated from blood cultures of new borns where it needs to be treated.

Conclusion

By introducing D test (effective, simple, and cheap method) and its reporting in routine testing,

a) Clindamycin reported as sensitive after performing D-test can be used for treating MRSA.
b) If D-test is positive, clindamycin can be reported as resistant to minimize treatment failure.
c) Indiscriminate use of linezolid can be avoided and it can be reserved for multidrug resistant strains in critically ill patients with severe infections.

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