

INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCI ISOLATED FROM CLINICAL SAMPLES IN AN URBAN HOSPITAL OF DHAKA CITY

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Abstract

Inducible clindamycin resistance was determined in 200 clinical isolates of staphylococci from pus (53.5%) and wound swab (46.5%). The study was done from July 2009 to June 2010, in the Department of Microbiology, BIHS Hospital Dhaka. Inducible clindamycin resistance was demonstrated by placing an erythromycin disc (15 µg) 15 mm apart from the edge of a clindamycin (2 µg) disc in Mueller Hinton agar. When the clindamycin inhibited zone becomes D-shaped the organism was regarded as positive for inducible resistance (D- test positive). Out of 200 staphylococci, 20% had inducible clindamycin resistance, 5% had constitutive clindamycin resistance and remaining 75% was clindamycin sensitive. In case of methicillin resistant *Staphylococcus aureus* (MRSA), 48% had inducible clindamycin resistance while 11.5% was constitutively resistant to clindamycin and remainder were clindamycin sensitive. All clindamycin resistant strains were 100% sensitive to vancomycin and linezolid followed by gentamycin (42%) and tetracycline (42.3%). The findings demonstrated that a substantial proportion of staphylococci in our tertiary care hospital had inducible resistance to clindamycin.

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**Key words:** *Staphylococcus aureus*, Inducible clindamycin resistance, Constitutive clindamycin resistance, D-test.

Introduction

Multidrug resistance is an ever increasing problem in staphylococci which is responsible for nosocomial as well as community acquired infections.<sup>1</sup> Methicillin resistant *Staphylococcus aureus* (MRSA) poses special threat to treatment because these are resistant to most common drugs.<sup>2,3</sup> So, newer drugs are needed to treat infections with MRSA.

In some recent studies clindamycin has been used successfully to treat different infections due to staphylococcus including MRSA. But some strains of staphylococci found sensitive to clindamycin by *in-vitro* susceptibility test exhibit resistance to clindamycin *in-vivo* leading to treatment failure.<sup>4,5</sup> The inducible clindamycin resistance by staphylococci cannot be detected by routine antibiotic susceptibility test (AST). This potential resistance to clindamycin can be induced in AST by placing a 15µg erythromycin

disc close to a 2µg clindamycin disc (15 mm apart edge to edge). Flattening of the clindamycin inhibition zone adjacent to erythromycin disc indicates that the strain has inducible resistance to clindamycin and the test is known as D-test.<sup>6,7</sup>

The prevalence of positive D-test has been reported as 21.9% in all staphylococcal strains, 24.4% in MRSA and 14.8% in MSSA (methicillin sensitive *Staphylococcus aureus*) in India.<sup>5</sup> The rate in methicillin sensitive and resistant coagulase negative *Staphylococcus* (CoNS) are 25.7% and 19.9% respectively.<sup>5</sup>

This study was designed to determine the prevalence of inducible clindamycin resistance among the clinical isolates of *Staphylococcus* sp as there is no available data regarding this in Bangladesh.

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## Materials and Methods

The study was conducted over a period of one year from July 2009 to June 2010 at the Department of Microbiology, BIHS hospital, Mirpur, Dhaka. Clinical specimens such as pus and wound swab were cultured and *Staphylococcus* was identified following standard procedure.<sup>7,8</sup>

Antibiotic susceptibility tests were performed by the standard disc diffusion methods.<sup>6</sup> Methicillin resistance was detected, based on CLSI recommendations, using a 1 µg oxacillin disc. *Staphylococcus aureus* ATCC 25923 was used as control organism for the disc diffusion method.<sup>6</sup>

The erythromycin-clindamycin double disc susceptibility test (D-test) was performed as per CLSI guideline 2004.<sup>6</sup> An erythromycin disc (15 µg) was placed 15mm apart from the edge of a clindamycin (2 µg) disc in Mueller Hinton agar media. When the clindamycin zone became D-shaped, the organism was regarded as positive for inducible resistance to clindamycin (D-test positive, Fig-1).<sup>6,7</sup>

## Results

A total of 200 *Staphylococcus* were isolated from specimens of pus (53.5%) and wound swab (46.5%) during the study period. Among the 200 strains, 87 (43.5%) were MRSA, 70 (35.0%) were MSSA and 43 (21.5%) were CoNS. Table-1 shows different types of clindamycin resistance among isolated staphylococci strains. It is to be noted that 44 (22%) strains had inducible clindamycin resistance of which 42 (95.5%) were MRSA. Table-2 shows the antibiogram of MRSA having inducible clindamycin resistance. It was noted that antibiogram of other staphylococcal strains had no significant difference in the pattern of antibiogram from that of clindamycin resistant staphylococci strains.

## Discussion

Clindamycin, though not a new drug and is used for other purposes, can be used for the treatment of MRSA and multiple resistant staphylococci. It is a lincosamide drug having good tissue penetration and is well tolerated even in kidney diseases.<sup>4</sup>

But, in some recent studies<sup>6,7</sup> it has been shown that some strains found sensitive by disc diffusion



**Fig-1.** D-test positive isolate showing flattening of zone of inhibition of clindamycin towards to erythromycin disc

antimicrobial susceptibility tests, but are resistant in clinical practice causing treatment failure. This potential resistance can be induced (hence called inducible resistance) in AST by placing clindamycin (2 µg) disc and erythromycin disc (15 µg) side by side. Flattening of the clindamycin inhibited zone adjacent to erythromycin disc indicates inducible resistance. As the flattening of the clindamycin inhibited zone looks like D, the test is called D-test.<sup>6,7</sup>

This study has been conducted to see the prevalence of inducible clindamycin resistance among clinical isolates of staphylococci and to study the antibiogram of clindamycin resistant strains. In this study, 22% (44 out of 200) staphylococci had inducible clindamycin resistance, 5% had constitutive clindamycin resistance. In our study, 48.3% (42 out of 87) MRSA strains had inducible clindamycin resistance. Mallick *et al.*<sup>9</sup> in

**Table-1:** Rate of isolation of inducible clindamycin resistance among isolated staphylococci strains (n=200)

| Types of Staphylococcus | Total    | Positive for inducible clindamycin resistance n (%) | Negative for inducible clindamycin resistance n (%) | Susceptible to Clindamycin n (%) |
|-------------------------|----------|---|---|----------------------------------|
| MRSA                    | 87(43.5) | 42(48.3)  | 10(11.5)  | 35 (40.2)                        |
| MSSA                    | 70(35.0) | 0(0.0)  | 0 (0)   | 70 (100)                         |
| CoNS                    | 43(21.5) | 2 (4.8)   | 0 (0)   | 41 (95.3)                        |
| Total                   | 200      | 44 (22.0)   | 10 (5.0)  | 146 (73.0)                       |

Note: Figure in Parenthesis indicates Percentage, MRSA = Methicillin Resistant *Staphylococcus aureus*, MSSA = Methicillin Sensitive *Staphylococcus aureus*, CoNS = Coagulase negative *Staphylococci*

**Table-2:** Antibiotic susceptibility profile of Clindamycin resistant MRSA (n=52)

| Antibiotics   | Susceptible |            |
|---------------|-------------|------------|
|               | Number      | Percentage |
| Ciprofloxacin | 15          | 28.8       |
| Vancomycin    | 52          | 100        |
| Linezolid     | 52          | 100        |
| Gentamycin    | 22          | 42.3       |
| Tetracycline  | 22          | 42.3       |

India found 36% of MRSA had inducible clindamycin resistance. However, authors from different parts of India reported that 30-64% of their MRSA strains had inducible clindamycin resistance.<sup>5,10,11</sup>

The incidence of constitutive clindamycin resistance is variable in different studies. Angel *et al.* and Gadepalli *et al.* did not find any constitutive resistant strains in their studies.<sup>10,11</sup> Others found constitutive clindamycin resistance in 3.8%- 44.2% of their MRSA isolates.<sup>9,12,5</sup> However, incidence of constitutive clindamycin resistance in our study was 5% in MRSA strains which is much nearer to that of Mallick *et al.*<sup>9</sup>

In this study, neither inducible nor constitutive clindamycin resistance was found in MSSA strains. Mallick *et al.* also did not find any inducible resistance, but found constitutive clindamycin resistance in 1.1% of MSSA strains.<sup>9</sup> However, others from India reported both inducible (14.8%) and constitutive (4.5%) clindamycin resistance in MSSA strains.<sup>5</sup>

In our study, inducible clindamycin resistance was found in 4.8% of coagulase negative *staphylococci* (CoNS). However, no constitutive clindamycin resistance was found in these strains. Yilmaz *et al.* reported both inducible (24.3%) and constitutive (31.5%) clindamycin resistance in CoNS.<sup>5</sup>

In the present study, all of the MRSA strains having clindamycin resistance (both inducible and constitutive) were susceptible to vancomycin and linezolid followed by gentamycin (42.3%) and tetracycline (42.3%). Therefore, D-test may be done routinely while performing AST of staphylococci with clindamycin.

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