International Journal for Development of Science and Technology (IJDST)

Vol. 1, Issue, 8, March 2021 PP.261-269

www.ijdst.com

ISSN: 2456-8503

INDUCIBLE CLINDAMYCIN RESISTANCE ALONG WITH D-TEST IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES

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Abstract

Staphylococcus aureus (S.aureus) is commonly isolated pathogen from clinical specimen with increasing trend of antimicrobial resistance. This has leads to resume interest in the usage of antibiotics to treat *Staphylococcus aureus* (*S.aureus*) infections. The main aim of this study was to isolate *S. aureus* from different 150 clinical samples such as blood, urine, pus, sputum and vaginal swab and to determine their susceptibility patterns by standard biochemical techniques and their antibiotic susceptibility tested by standard disk diffusion method. Detection of inducible Clindamycin resistance was performed by D-test on a Mueller Hinton agar plate with a lawn culture of the isolates. The isolates of Staphylococcal revealed the resistance zone to erythromycin at ≤ 12mm and a D shaped, cleared zone of inhibition round the disc of clindamycin was delegated as the inducible clindamycin resistance. MRSA between clindamycin resistances *S.aureus* was noted for inducible was 10. On conclusion, observation of D-effect among some isolates provokes the necessities for development of new strategies.

Keywords: *Staphylococcus aureus*, MRSA, Inducible clindamycin resistance

Introduction

Staphylococcus aureus is one of the old pathogen well known is still most of the common cause of infection of pyogenic in humans. *S.aurues* is a skin normal flora that can enter the body by abrasion, burns, cuts, intravenous catheter, surgical incision and cracks and caused pyogenic infections. *S.aureus* is the regular gram positive pyogenic bacteria which accountable for various disorders that ranged from severe from skin and soft tissues infection to life threatening issues like sepsis, endocartitis and pneumonia (2). In the framework of increased methicillin resistant *S.aureus* infections, the clindamycin group of antibiotics that are essentially varied with similar mechanism of action served as one of the good substitute.

The antibiotics hinder the bacterial pathogen protein synthesis in susceptible microbes by reversible binds to the 23S ribosomal nucleic acid receptors 50S

ribosomal ribonucleic acid. Between these, clindamycin is the favour agent because of its good effects of pharmacokinetics obtainable in both oral and intravenous production with 90% of bioavailability, lower cost, good perforation of tissues and extensive abscesses accumulation, not hinder by higher bacterial pathogen burdens at the site of infection and also able to inhibits the some toxin production and virulence factors in Staphylococci (1). But, the random use of antibiotics has leads to high in resistance between isolates of Staphylococcus.

The antimicrobial resistance has been noted that one of the paramount microbial threat of the 21 century. The multi drug resistance too many of the antibiotics used in the infections produced by staphylococci is an increased issues. The methicillin resistance emergence between *S.aureus* strains leads to problems in the infection therapy. Antibiotics resistance has results in larger hospitals staying of patients results in social burden also psychological stress. So the surveillance on the susceptibility antimicrobial pattern of *S.aureus* is the utmost important in the understanding in modern and emerging resistance trends also in the hospital and community management obtained infections.

Clindamycin is the rare drug for either anaerobic infection or gram positive with the exception of acute streptococcal fasciitis or cellulitis, anaerobic infection of lungs and diabetic foods. The reviewers classified clindamycin as the choice of agents for pelvic and abdominal infection though safe and cheap regimens make to that are preferred. Yet, clindamycin remain a valuable drug for allergy cases and used against some clear defined syndromes. It is particularly suitable for out patients however it should be used occasionally in cases due to the correlation of *C.difficile* colonization and diarrhea.

Clindamycin is used in suspected patient's toxic shock syndrome, frequently combined with bactericidal agents like vancomycin. The ratio for the detain is a assumed synergy among vancomcin that caused he bacterial death by cell wall breakdown and clindamycin that is important inhibitors of synthesis of toxins (Shantala et al.2011). Both the in vivo and in vitro analysis showed that clindamycin reduced the exotoxins production by staphylococci, it may also induced modification in the bacterial structure surface that make them to more sensitive to immune system attack of phagocytosis and opsonization.

Clindamycin has been proved to reduce the risk of premature births in women diagnoses with bacterial vaginosis at early pregnancy to third of untreated risk of women. The combined quinine and clinamycin is the standard therapy for serious babesiosis. Clindamycin used to cure toxoplasmosis and combined with primaquine is effectual in treatment of mild to moderate *Pneumocystis jirovecii* pneumonia. Clindamycin applied either in skin or taken orally by mouth may used in hidradenitis suppurativa.

The *S.aurues* infections produced are usually treated by different antibiotics. Clindamycin is one of the most potent antibiotics usually used in the medical management for the effectual treatment of infection produced by Staphylococcus sp. it showed a wider range of usage of hospital and community acquired Staphylococcal infections (3). To determine the proportion of S, the current investigation was conducted. *aureus* having inducible clindamycin resistance in clinical samples using D-test.

Materials and methods

This present study was conducted in the PSG Hospital, Coimbatore from August 2021 to September 2022. Totally 150 *Staphyloccocus aureus* isolates were isolated and identified from clinical samplings such as blood, urine, pus, sputum and vaginal swab includes in this study. All the specimens were collected from in and out cases who are attended PSG Hospital, Coimbatore. *Staphyloccocus aureus* isolated from the samples that were selected for this study. For isolation of Staphylococcus species the gram staining was used in this study.

The isolates were first identified by standard biochemical techniques (6) and then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using the drugs such as amoxyclavulanic acid (30µg), cefotaxime(30µg), linezolid(30 µg), cephelexin(30µg), vancomycin(30µg), doxycycline (30µg), cotrimoxazole(25µg), penicillin(10u), oxacillin(1µg) and ciprofloxacin(5µg) as per CLSI guidelines (5).

Clindamycin Inducible resistance was tested by using 'D test' as per CLSI guidelines (7). In brief, 15 µg of erythromycin disc was placed at a distance of 15 to 20 mm edge to edge from 2 µg of clindamycin disc on a Mueller–Hinton agar plate, before inoculated with 0.5 McFarland standard bacterial suspensions. It was possible to determine the inducible clindamycin resistance by continuing the overnight incubation at 37°C and observing

the flattening of the D-shaped zone around clindamycin in the area between the two discs.

Results

Among 150 patients, bacterial species were isolated by selective culture medium and standard biochemical test was identified as *Staphylococcus aureus*. All clinical samples showed *Staphylococcus aureus* (SA) were primary isolates. Then the *S.aureus* was identified by biochemical tests incudes catalase, coagulase,tube test, indole test,sugar fermentation test, methyl red, Voges proskauer test, urease and O/F test. From the biochemical tests the positive isolates were found in catalase test, coagulase test, methyl red test, sugar fermentation test and indole test. The VP and O/F test showed negative results (Table-1).

The Antibiotic susceptibility test was accomplished on Muller Hinton agar plate through Kirby Bauer disk diffusion method using CLSI guidelines. This present work the more isolates of *S.aureus* was sensitive 100% (150) to vancomycin and 99% (149) to linezoid. 76% (115) sensitive to doxycycline, 66% (100) sensitive to oxacillin, 65.3% (98) sensitive to cotrimoxazole, 63.6% (95) sensitive to amoxyclavulanic acid, 53.3% (80) sensitive to cefotaxime, 46% (68) sensitive to ciprofloxacin .The lower level of resistance were seen in penicillin.

The resistance of *S.aureus* isolate pattern. Out of 150 *S.aureus* isolates 100% (150) were penicillin resistant, 54.6% (82) were ciprofloxacin resistant, 36.6% (55) were resistant to amoxyclavulanic acid, 23.3% (35) were resistant to cephalexin, 34.6% (52) were cotrimoxazole resistant, 20.6% (70) were cefotaxime resistant, 23.3 % (35) were resistant to doxycycline, 33.3% (50) were resistant to oxacillin, 23.3% (35) were Cephelexin resistant and 1% (2) were linezoid resistant. Very minimum resistance was noted in vancomycin (Table-3).

The isolates of Staphylococcal revealed the resistance zone to erythromycin at ≤ 12mm and a D shaped, cleared zone of inhibition round the disc of clindamycin was delegated as the inducible clindamycin resistance (Table-2). The clindamycin resistance for *S.aureus* was noted for inducible was 10 respectively.

Discussion

Staphylococcus aureus has appeared a main source of infection of nosocomial for silent some times. Clindamycin is a beneficial drug in the treatment of soft tissues and skin infections. It also used in the allergy of penicillin for the cases. It is

the hopeful therapeutic options in the drug resistance era. The expensive antibiotics such as vancomycin can be quiet acute illness. The erythromycin resistant staphylococcal isolates will be conflated as clindamycin sensitive to D test (8). To prevent prescribed clindamycin that exhibits inducible clindamycin resistance, D test must do continuously.

Clindamycin drug that is used for therapy of both methicillin resistant and susceptible infections of staphylococcus. Best oral adsorption makes a chief option in therapy of out patients as followed after the therapy of intravenous. Clindamycin is also the substitute antibiotics for the penicillin allergic cases. Since the mechanism of iMLSB resistance is not suggested using standard susceptibility tests and its prevalence differ in geographical areas and from hospital to hospital, the D- test become an essential part of continuous antimicrobial susceptibility tests for clinical *S.aureus* all isolates (9).The false report to patients is infected with MRSA will caused fatal significance because of insufficient therapy also false labeling of the cases infected with MRSA and MSSA leads to unwanted use of expensive drugs such as vancomycin (13).

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In our study found that the higher percentage of methicillin resistant of *S.aureus* isolates was 36%. Between them 20% isolates were tested positive for inducible clindamycin resistance through D-test, while the isolates were negative for D-test. The findings are compatible with the before studies (14) and these results suggested that the D-test has not been executed, one-third of methicillin-resistant isolates would have not been identified as clindamycin sensitive resulting in failure of therapies.

Several research works have found a very higher frequency of inducible resistance MRSA. On the conflicting, some studies have showed high percentage of inducible resistance in MSSA than MRSA

(10). The exact susceptibility data are chief for suitable treatment decisions. The *S.aureus* macrolide resistance pattern differs in various regions.

Our study highlights an inducible clindamycin resistance higher prevalence among the Staphylococcal isolates. Hence, it is mandatory to perform D test for clinical isolates on the routine basis to avoid treatment failure with clinical specimens. For the emergence of MRSA, only a few alternatives are available to treat such infections of *S.aureus*. The MLSB family of antibiotics is one such difereent and Clindamycin is favoured. Clinical microbiology labs should give inducible Clindamycin resistance in *Staphylococcus aureus* and D-test can be used as easy, supplemental and dependable techniques to Delineate constitutive and inducible Clindamycin resistance in routine clinical labs.

The *S.aureus* infections increased is the result of microorganism capacity to adjust the environment modification and its ability to outspread. MRSA is a threat not only to immunocompromised persons however also to common public. Also exposure of drug resistance between MRSA is a mainly concerned. So the methicillin resistance detection in *S.aureus* is most salient for the therapy of cases and to stop its spreading.

The drugs includes clindamycin are required to stem the acute MRSA effect. The usage of clindamycin prevents cost, intravenous glycopeptides for MRSA treatment. Clindamycin is a therapy options in children. It is used particularly in penicillin allergic persons. It has better oral bioavailability. So it can be used to outpatient treatment by clinicians as well as convert after the intravenous antibiotics in hospitalized cases. The clindamycin resistance pattern to MRSA differs in various areas. When the clindamycin is known for treatment, the resistance likes constitutive or inducible that exists to detect.

D-test is completely needed for microbial laboratories. This is due to prevent mistake of clindamycin resistance by clear described inducible clindamycin resistance from constitutie clindamycin resistance. Also it is easy, less expensive and dependable. So the D test is recommended for the continuous antibiotic susceptibility test to determine the inducible and constitutive clindamycin resistance and thus prevent the treatments failures. Therefore, this work was done.

Depends on the rate of prescription will not be same in various regions. There is no considerable data regards to clindamycin prescription from India. It is retaining as a reserve drug is commonly advocated in serious MRSA patients infections depends on the results of antimicrobial susceptibilities. Also, by clindamycin usage, the vancomycin usage can be stopped (12). But, the inducible resistance expression to clindamycin could limit the drug effectiveness (11). Therefore, the clinical laboratories should found inducible clindamycin resistance in *S.aureus* and D-test can be used as a easy, reliable and auxiliary technique to delineate inducible clindamycin resistance in continuous clinical laboratory.

Conclusion

Our study highlights an inducible clindamycin resistance higher prevalence among the Staphylococcal isolates. Hence, it is mandatory to perform D test for clinical isolates on the routine basis to avoid treatment failure with clinical specimens. The *S.aureus* infections increased is the result of microorganism capacity to adjust the environment modification and its ability to outspread. So the methicillin resistance detection in *S.aureus* is most salient for the therapy of cases and to stop its spreading.

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Table-1: Biochemical characteristic of the bacterial culture isolated

Catalase test	Coagulase test	Sugar fermentation test	MR test	VP test	O/F test	Indole test
+	+	+	+	-	-	+

Table-2: Prevalence of MRSA among *staphylococcus aureus* isolates

Total no. of isolates	MRSA	iClindamycin resistance
150	36%	10%

Table-3: Antibiotic sensitivity pattern of clindamycin resistance and sensitive

Drugs	Sensitive	Resistance
Vancomycin	100% (150)	-
Linezolid	99% (148)	1% (2)
Doxycycline	76% (115)	23.3% (35)
Oxacillin	66% (100)	33.3% (50)
Cotrimoxazole	65.3% (98)	34.6% (52)
Amoxyclavulanic Acid	63.6% (95)	36.6% (55)
Cefotaxime	53.3% (80)	20.6% (70)
Ciprofloxacin	46% (68)	54.6% (82)
Cephelexin	76.6% (115)	23.3% (35)
Penicillin	-	100% (150)