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DETECTION OF CONSTITUTIVE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCI FROM A TERTIARY CARE HOSPITAL

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Abstract
The resistance to antimicrobial agents among *Staphylococci* is an increasing problem. This has leads to resume interest in the usage of antibiotics to treat *Staphylococcus aureus* (*S.aurues*) 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 constitutive Clindamycin resistance was performed by D-test on a Mueller Hinton agar plate with a lawn culture of the isolates. 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
S.aureus commonly acts as a human commensal microbiota commensal, it also an expedient microbe, being a usual cause of infections of skin such as food poisoning, abscesses and respiratory tract infection sinusitis. Microbial strains frequently caused infections by promote virulence factors like powerful protein toxins and expression for the cell surface protein which binds and the antibodies inactivation (1). *S.aureus* is the caused microbes for death correlated with resistance of antimicrobials and the antibiotic resistant strains emergence like methicillin resistant *S.aureus* is a global issue in clinical therapy. In spite of most of the research and growth, no vaccine for *S.aureus* has been accepted (2).

Methicillin Resistant *Staphylococcus aureus* (MRSA) is an example of a 'superbug' or *Staphylococcus aureus* bacterial strain that has developed resistance to an antibiotic methicillin is part of a class called as the beta-lactams. The antibiotic beta-lactam is efficient to kill various Gram positive and negative microorganisms by impairing the cell's capacity to produce peptidoglycan, an essential component for cell stability and structure. Bacteria become resistant grow enzymes called as beta-lactamases and penicillinases which are effective to destroying the ring structure of beta-lactam or functional group of antibiotics (3).

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. Clindamycin is an antibiotic medicine for the therapy of bacterial infection includeing osteomyelitis or infection in joints, pelvic inflammatory disease, strep throat, severe otitis media, endocarditis. Also it used to cure acne and methicillin resistant *staphylococcus aureus*(4).

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Clindamycin is a semi synthetic derivative of lincomycin, a natural antibiotic formed by the *Streptomyces lincolnensis* actinobacterium. It is produced by lincomycin 7 (s) chloro substitution of the 7(R) hydroxyl group. The clindamycin was first announced by 5thinterscience conference on antimicrobial agents and chemotherapy on 1966. It has been on the market since 1968. Clindamycin is yellow or white. It is vastly water soluble. The topically used clindamycin phosphate is a phosphate ester of clindamycin prodrug (15).

The increased frequency of infections of staphylococcal between patients and modifying treatment samples in antimicrobial resistance have caused to renewal interest in the clindamycin treatment used to cure like infections. Clindamycin regularly used to cure bone and skin infections due to its cost, tissue penetration, tolerability and oral form and it accumulates in no renal and abscesses adjustments are required. Much absorption makes a main choice in outcases treatment or follow up after intravenous treatment. Clindamycin is a chief substitute for the therapy of both susceptible and methicillin staphylococcal infections. In this study, we conductedto estimate the prevalence of constitutive clindamycin resistance.

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. For isolation of Staphylococcus species the gram staining and biochemical studies were conducted.

The antibiotic drugs such as amoxyclavulanic acid (30µg), cefotaxime(30µg), linezolid(30 µg), cephelexin(30µg), vancomycin(30µg), doxycycline (30µg), cotrimoxazole(25µg), penicillin(10µg), oxacillin(1µg) and ciprofloxacin(5µg) were dispensed into the inoculated agar plate surface through sterile forceps. Every disc was pressed down to ensured the full contact with the surface of agar. Then the plates were inverted fro incubation as moisture growth produced interference in test interpretation. The tubes incubated for 24 hours at 37ºC that the zone of inhibition was measured by measuring zone scale and interpreted as per the CLSI standards (5). The transmitted light was used to analyze the growth of light of isolates of methicillin resistant.

Clindamycin constitutive resistance was tested by using ‘D test’ as per CLSI guidelines (6). 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. Continuing overnight incubation at 37°C, flattening of D shaped zone around clindamycin in the region among the two discs, showed the constitutive clindamycin resistance.

Results

Among 150 patients, bacterial species were isolated by selective culture medium and standard biochemical test was identified as *Staphylococcus aureus* (SA). Out of150 samples received, 49 (32.6%) were female and 101 were (67.3) were male were positive to *S.aureus* culture, 65 were from the age groups 21 to 40, 40 from the age groups 41 to 60, 25 were from the age group 61-80 and 20 from the age group 0 to 20, the isolates taken were 0 to 20 age groups were showed between 13.3%, 20 to 40 age groups were showed at 43.3%, 41 to 60 age groups showed 26.6% and 61 to 80 age groups at 16.6% respectively.The pus samples constituted 127 (84.6%), urine 6%(09), blood 3.3%(05), sputum 2.6%(04), vaginal swab 2% (03) and synovial body fluids 1.3% (02) (Table-1).

The detection MSSA and MRSA between different clinical specimens, among 150 isolates n = 54 (36%) were MRSA and n = 96 (64%) were MSSA (Table-2). In this study methicillin resistance is

highly predominant than the methicillin resistance. Out of 150 *S.aureus* isolates 39% were isolated from infection of wound, 8% from cutaneous ulcer,7% from abscess, 7.4% from cellulitis, 7.3% from suppurative otitis media, 6% from pyoderma, 5.5% from urinary tract infection, 4% from osteomyelitis, 3.5% from burns, 3% from septicemia, 2.3% from pneumonia,1.8 % from gangrene, 1.5 % from vaginal infection 1.2% from necrotizing fascitis, and 1% from septic arthritis.

The MRSA isolated from wound infection at 40.20%, cutaneous ulcer at 11.50%, abscess at 9.52%, cellulitis at 7.4%, pyoderma at 7.50%, osteomyelitis at 5.11% and urinary tract infection at 3.5%. Gangrene, septicemia, burns, suppurative otitis media and necrotizing fascitis constituted 1.90% of each MRSA. It is inferred from the derived data that infection of wound constituted more percentage of MRSA.

In this study, the antibiotic susceptibility test of *S.aureus* represents that out of 150 *S.aureus* 64% MSSA was more predominate compared to MRSA (36%). The isolates of *S.aureus* was sensitive to clindamycin were 44% followed by constitutive clindamycin resistance 24% and inducible clindamycin resistance 20%. Out of 150 *S.aureus*isolates, D test was positive in n= 17 of the 36% MRSA (Table-3) and n=10 of the 64% of MSSA (Table-4) that denotes constitutive clindamycin resistance.

Discussion

The antimicrobial resistance in *S.aureus* has one of the ever raising issues between the both of inpatients and outpatients of health care provisions. Clindamysin a lincosamide has been desirable viewpoint of MRSA and MSSA skin infections and soft tissue. It is obtainable in parenteral and oral forms, 90% of oral bioavailability, low cost compared to new drugs, better tissues penetration and capability to inhibit the some toxin and virulence constituent production in Staphylococci. But clindamycin resistance is highly differing and resistance phenotypes incidence differ by geographic areas and among hospitals. These types of isolates had higher level of impulsive variation at the action of therapeutics that enables them to grow resistance to clindmycin (7). Thus the actual therapy options against infection of *S.aureus* have become inducible and constitutive clindamycin resistance in Eastern sides of India. So this work is taken up to anaylse and to detail the clindamycin resistant phenotypes present tertiary care hospital.

Out of 150 *S.aureus* isolates, we observed that the 36% in MRSA and 64% in MSSA. Many studies have showed the high levels of constitutive strains of *S.aureus*. Das et al. (7) observed 21.8% isolates of constitutive and 15.4% of inducible clindamycin resistance. Mokta et al (8) found MLSBi 13.7% and MLSBc strains 17.1% also in another study by Mittal et al (9) found MLSBi strains at 23.2% and MLSBc strains at only 6.1% between *S. aureus*isolates.In variance *to several studies they showed that* MRSA MLSBcin very much reduced. Study done at Tehran has determined *S.aureus*of MLSBc strains at 38.9% and MLSBi strains at 7.5% however it was lesser than in coagulase negative staphylococci that observed 59.2% and 10.1% respectively (10).

In this study we observed that statistically significant constitutive clindamycin resistance strains in MRSA and MSSA. Many studies showed across the country have found that constitutive and inducible MLSB strains are seen more in MRSA than MSSA strains (11). Therefore MLSBi strains does not detected by automatic susceptibility test or E-test, simply and easy performed, low cost and reproducible test like D-test can be including as a part of continuous antibiotic susceptibility test.

The D-test (double disk approximation test) involved in the antibiotic disk placement in the proximity of the disc contains clindamycin. The antibiotics diffused by the agar, resistant to the clindamycin are persuading the results in blunting or flattening of the zone of clindamycin disk giving an S shape to the zone. The clinical laboratorical standard institute (CLSI) suggested D test that is phenotypic screening technique for inducible clinamycin resistance. So all the antibiotics

resistant *S.aureus* should be examined for inducible and constitutive clindamycin resistant to stop clindamycin therapy failures and to detail the resistant phenotypes prevalence that are differ extensively (14). This work was pursuing to analyze the constitutive and inducible clindamycin resistant in *S.aureus* isolates from different clinical samples at a tertiary care hospital in Coimbatore, Tamil nadu, S.India.

Wound/pus samples accounts for mostly n=150 which *S.aureus* has been isolated and increased number showed constitutive as n= 84.6% clindamycin resistance.

MS phenotype in this study was established to be 13% between *S.aureus*. The isolates of our study showed that susceptibility to vancomycin and linezolid has been reported in many studies. Same as our study, the antibiotics resistance has ranged from 1% to 100%. Sensitivity of *S.aureus* includes MRSA showed in another study as 90.2% sensitive to tetracycline and 48.4% to co-trimoxazole which in contrast our study showed only 28.5% and 22.3% respectively (12). Some reports of lower susceptibility of emergence of *S.aureus* are more recently resistant to antibiotics an additional concern. Suggestions to usage of antibiotics for MRSA as reserve drugs required to emphasize in hospitals (13).

Conclusion

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 alternative and Clindamycin is preferred. Clinical microbiology laboratories should report inducible Clindamycin resistance in *Staphylococcus aureus* and D-test can be used as an easy method to Delineate constitutive Clindamycin resistance in routine clinical laboratories.

This study concludes that the comprised larger population in various locations with quick tests would be helpful in MRSA computing prevalence. The increased prevalence of MRSA develops high emphasis on the requirement to grow larger stringent regulations and policies for the usage of antibiotics in the human health care system. The strict adherence of hand hygienic and judicious usage of antibiotics will highly decrease the MRSA incidence. The indiscriminate awareness of antibiotics usage and the prevention options should be introduced to combat the epidemic spreading of the drug resistance bacteria in India.

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Table-1:*S.aureus* isolation from different clinical specimens

Samples	Total no of *S.aureus*	Isolates n=150
Pus	127	84.6%
Urine	06	6%
Blood	05	3.3%
Sputum	04	2.6%
Vaginal swab	03	2%
Synovial fluid	02	1.3%

Table-2: Antibiotic susceptibility pattern of *Staphylococcus aureus*

MRSA	MSSA	iClindamycin resistance	cClindamycin resistance
36%	64%	10%	24%

Table-3: MRSA isolates among clindamycin resistance *S.aureus*

MRSA	
INDUCIBLE CL RESISTANCE	10

CONSTITUTIVE CL RESISTANCE	**13**
PHENOTYPE	**5**
SENSITIVE	**8**
TOTAL	**36**

CL- Clindamycin

Table-4: MSSA isolates among clindamycin resistance *S.aureus*

MSSA	
INDUCIBLE CL RESISTANCE	**11**
CONSTITUTIVE CL RESISTANCE	**08**
PHENOTYPE	**10**
SENSITIVE	**35**
TOTAL	**64**

CL- Clindamycin