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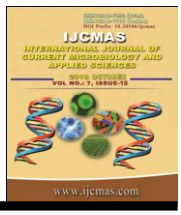

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Inducible Clindamycin Resistant Strains of *Staphylococcus aureus* - Associated Skin and Soft Tissue Infection

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A B S T R A C T

Staphylococcus aureus causes skin and soft tissue infections (SSTIs), if not treated with proper antimicrobials can leads to serious form of infection. Multi-drug resistant strains left us very few therapeutic alternatives, in such cases clindamycin can be a good option with true sensitivity. The clinical samples exhibiting skin and soft tissue infection received in the department of microbiology further subjected to isolation of *Staphylococcus aureus*. Isolated strains further subjected to D-test to detect inducible clindamycin resistant strains as per standard guidelines. Antimicrobial susceptibility testing performed for all the isolates as per CLSI guidelines. A total of 176 *Staphylococcus aureus*, 82 were isolated from skin and soft tissue infection. Out of 82 strains, 23(28%) were inducible clindamycin resistant strains of *Staphylococcus aureus*, 49(59.8%) were constitutive resistant, and 10(12.2%) were MS phenotype. All the isolates were sensitive to vancomycin, followed by doxycyclin 62(75.6%), Amikacin 60(73.2%) and 57(69.5%) were sensitive to linezolid. Detection of inducible clindamycin resistant strains of *Staphylococcus aureus* on routine basis is crucial for judicial use of the drug and proper institution of the therapy especially in skin and soft tissue infections.

Introduction

Staphylococcus aureus forms normal flora of human anterior nares, nasopharynx, perineal area, skin, and various mucosal surfaces. The anterior nares are considered to be primary colonization site and approximately 30% of healthy people carry the bacteria in their anterior nares (Nilsson and Ripa, 2006).

Skin and soft tissue infections caused by *Staphylococcus aureus* are frequent worldwide (Layer *et al.*, 2006). Generally, it causes skin infections such as folliculitis, furuncle, carbuncles, impetigo, mastitis, and various wound infections. The *Staphylococcus aureus* skin and soft tissue infections frequently begin as minor boils or abscesses and may progress to deep infections that spread from skin to cause bacteremia to involve bones, joints, deep organs, scalded skin syndrome in neonates, toxic shock syndrome, and food poisoning (Layer *et al.*, 2006). The severe form of infection can be avoided by giving early treatment like incision and draining the lesion with proper antimicrobial therapy.

In recent studies carried out worldwide reported that the most of the skin and soft tissue infection are community acquired and methicillin resistant strains of *Staphylococcus aureus* (MRSA) are one of the major concerns. Evaluation of demographic data as well as history of antimicrobial therapy is most important to overcome the situation. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties (Fiebelkorn *et al.*, 2003; Saxena *et al.*, 2012). However, widespread use of MLS_B antibiotics hassled to an increase in number of staphylococcal strains acquiring resistance to MLS_b (Yilmaz *et al.*, 2007). Clindamycin is used in the treatment of skin and soft-tissue infection, caused by the *Staphylococcal* species and good oral absorption makes this drug an important option in outpatient therapy. It has wide distribution in inflamed tissue except for the CNS and dosage adjustment will not require even in severe hepatic or renal dysfunctions (Buck, 2008). Clindamycin is also used as an alternative for patients who are allergic to penicillin (Fiebelkorn *et al.*, 2003). However, treatment of an infection using clindamycin or any non-inducer macrolide, caused by a strain carrying inducible *erm* gene, can lead to clinical failure due to development of resistance (Weisblum, 1995; Kloos, 1999). Three unrelated groups of antimicrobial agents share the same ribosomal binding site in the bacterial cell- macrolide, lincosamide and streptograminB. Therefore, it is possible that to one group of antibiotics (macrolides) might predict resistance to the other groups. Resistance to erythromycin is used as an indicator of possible resistance to clindamycin. Most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibely (inducible MLS_B phenotype) (Kumar *et al.*, 2012; Reddy and Suresh, 2017).

The strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive in-vitro when not placed adjacent to each other. In such case, in-vivo therapy with clindamycin may select constitutive *erm* mutants leading to clinical therapeutic failure (Deotale *et al.*, 2010). In case of another mechanism of resistance, *Staphylococcus aureus* can also develop isolated macrolide resistance based on the presence of an efflux pump, encoded by *msr*A gene leads to resistance to macrolide and type B streptogramins but not to lincosamide. These isolates known as MS phenotype also shown in vitro resistance to erythromycin and sensitive to clindamycin same as in inducible resistance phenotype, but clindamycin therapy can be safely given in infections with this phenotype and there is no risk of clinical failure (Roberts *et al.*, 1999).

Clinical and Laboratory standard Institute (CLSI) recommends the double disk diffusion test (D-test) to detect the presence of phenotypic inducible clindamycin resistance (Wayne, 2007). Reporting *Staphylococcus aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On other hand, negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option (Perez *et al.*, 2007).

Materials and Methods

After the permission from ethical committee, present study conducted at Department of Microbiology at tertiary care hospital. A total of 176 *Staphylococcus aureus* were isolated from various clinical specimens. The

Staphylococcus aureus were isolated by standard techniques like colony morphology, gram staining, catalase test, and coagulase test. All the isolates subjected to antimicrobial susceptibility testing susceptibility testing by Kirby Bauer's disc diffusion method as per CLSI guidelines. The erythromycin resistant strains of *Staphylococcus aureus* exhibiting skin and soft tissue infection were further subjected to D-test to detect inducible clindamycin resistant strains and routine antimicrobial susceptibility testing as per CLSI guidelines (Wayne, 2007). Clinical history is taken from patient's case paper form to correlate SSTIs.

Antimicrobial susceptibility testing

Kirby Bauer disc diffusion method

A well isolated colonies of coagulase positive isolates was taken and suspended in peptone water and incubated at 37^0C for 4 hours, the bacterial suspension were compared with 0.5 McFarland turbidity standard, comparison was corrected by using addition of peptone water or further incubation. The bacterial suspension was inoculated on Mueller Hinton agar plates, appropriate antibiotic disc was put and incubated at 37^0C for 24 hours as per CLSI guidelines (Wayne, 2007; Deotale *et al.,* 2010).

D-test

D-test was performed on erythromycin resistant strains of *Staphylococcus aureus* to rule out inducible clindamycin resistant strains of staphylococci as per standard guidelines and interpreted as three MLSb phenotypes (Wayne, 2007; Deotale *et al.,* 2010).

Statistical analysis

Results were analyzed by SPPS 20.0 version software, by using one-sample Chi-square test, one-sample Binomial test and p-value >0.05 were considered as statistically significant.

Results and Discussion

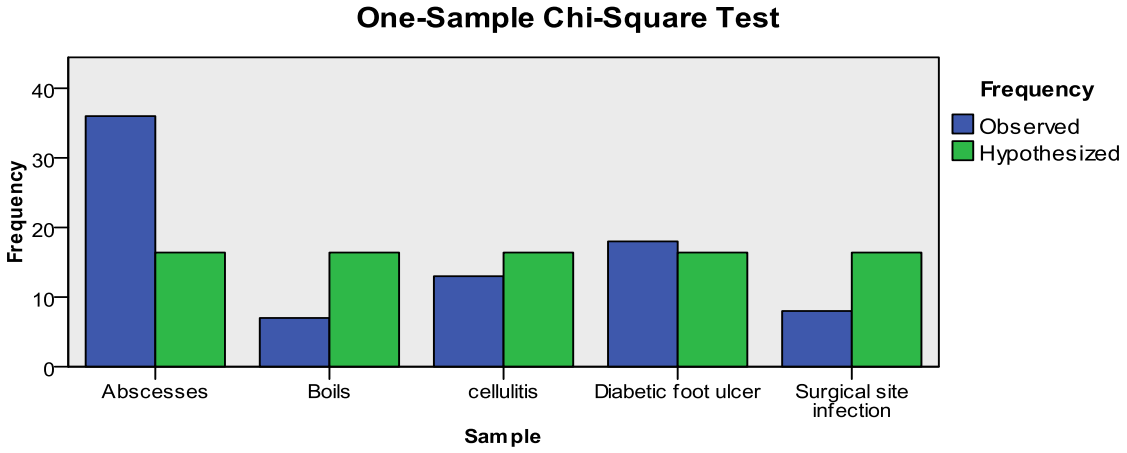
A total 176 *Staphylococcus aureus* were isolated from various clinical samples, out of which 82(47.15%) were recovered from skin and soft tissue infections exhibiting erythromycin resistant strains. *Staphylococcus aureus* is an important pathogen causing Skin and soft tissue infections, toxin mediated infections, and urinary tract infection. The determination of antimicrobial susceptibility of a clinical isolates is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and emergence of multidrug resistant organisms. There are many options available for treatment of MRSA and MSSA infections, with clindamycin being one of the good alternatives (Fiebelkorn *et al.,* 2003). However, clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both in-vitro and in-vivo during clindamycin therapy (Yilmaz *et al.,* 2007). Reporting *Staphylococcus aureus* as susceptible to clindamycin without checking for inducible resistance may result in inappropriate clindamycin therapy especially in skin and soft tissue infections.

In the present study, among the erythromycin resistant *Staphylococcus aureus* (n=82) exhibiting skin and soft tissue infections, male were 39(47.6) and female were 43(52.4%), and most of the patients were in the young active age group i.e. - 21-30 years and 41-50 years, so that there was no significant gender difference in the study group. Horieh saderi *et al.,* (2009) reported, out of 244 *Staphylococcus aureus*, 134 were male and 110 were female.

Out of 176 erythromycin resistant *Staphylococcus aureus*, 82 (47.15%) of *Staphylococcus aureus* were isolated from skin and soft tissue infections. Out of which 39 (43.9%) were from abscesses, 18 (22.6%) from diabetic foot ulcer, 13 (15.9%) from cellulitis, 8 (9.8%) from surgical site infections and 7 (8.5%) were from boils specimen. Similar observation noted by Horieh saderi *et al.*, (2009), out of 244 *Staphylococcus aureus*, 106 were isolated from wound/SST infections. B Shrestha *et al.*, (2009) reported skin infection isolates contributed a major part 72.5% of infection by *Staphylococcus aureus* followed by lower respiratory tract infection 11.41% and urinary tract infection 8.7%. Kumari *et al.*, (2008) reported 64% *Staphylococcus aureus* from pus/SSTIs samples followed by 20% from blood, 4.8 % device associated and 3.2% from urine.

Methicillin resistance detected by phenotypic method using cefoxitin (30mcg) disc diffusion method. Recent studies indicated that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic method like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA (Skov *et al.*, 2006). Result of cefoxitin disc diffusion test is in accordance with PCR for *mec*A gene. Thus, the test can be alternative to PCR for detection of MRSA (Anand *et al.*, 2009) (Table 1–5).

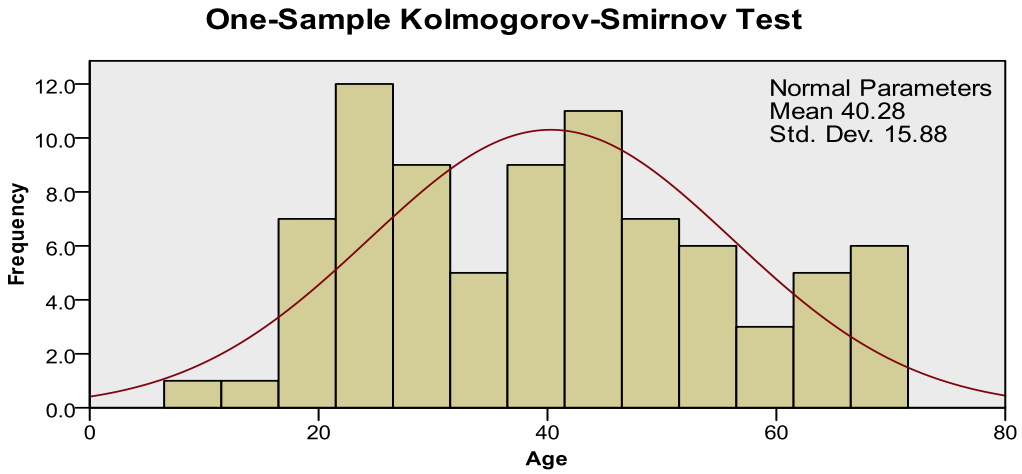
Graph.1 Distribution of SSTIs among clinical samples



Total N	82
Test Statistic	33.976
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

1. There are 0 cells (0%) with expected values less than 5. The minimum expected value is 16.400.

Graph.2 Age-wise distribution of SSTIs



Total N		82
Most Extreme Differences	Absolute	.107
	Positive	.107
	Negative	-.068
Test Statistic		.971
Asymptotic Sig. (2-sided test)		.303

Table.1 Distribution of *Staphylococcus aureus* among clinical samples (n=176)

Type of specimen	Number of samples	Percentage
Pus swab from SSTIs	82	47.15 %
Blood	28	15.90 %
Sputum	26	14.77 %
Endotracheal secretion	14	7.95 %
Urine	14	7.95 %
Vaginal swab	04	2.27 %
Pleural fluid	02	1.13 %
Serous fluid	01	0.56 %
Throat swab	02	1.13 %
AC tapping	01	0.56 %
Ear swab	02	1.13 %
TOTAL	**176**	**100 %**

Table.2 Demographic data pertaining SSTIs (n=82)

Source		Frequency	Percent	Cumulative Percent
Gender	Female	43	52.4	52.4
	Male	39	47.6	100.0
OPD/IPD	IPD	65	79.3	79.3
	OPD	17	20.7	100.0
Distribution of SSTIs	Abscesses	36	43.9	43.9
	Boils	7	8.5	52.4
	Cellulitis	13	15.9	68.3
	Diabetic foot ulcer	18	22.0	90.2
	Surgical site infection	8	9.8	100.0

Table.3 Antimicrobial susceptibility of *Staphylococcus aureus* exhibiting SSTIs

Antimicrobial agents	Sensitive		Resistant	
	Frequency	Percent	Frequency	Percent
Penicillin	2	24	80	97.6
Cefoxitin(MIC)	19	23.2	63	76.8
Erythromycin	00	00	82	100
Clindamycin	31	37.8	51	62.2
Trimethoprim/Sulfamethoxazole	22	26.8	60	73.2
Doxycycline	62	75.6	20	24.4
Levofloxacin	47	57.3	35	42.7
Amikacin	60	73.2	22	26.8
Linezolid	57	69.5	25	30.5
Vancomycin	82	100	00	00

Table.4 Inducible clindamycin resistant strains of *S. aureus* exhibiting SSTIs

Inducible Result					
Type of resistance		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	cMLSb Phenotype	49	59.8	59.8	59.8
	iMLSb Phenotype	23	28.0	28.0	87.8
	MSb Phenotype	10	12.2	12.2	100.0
	Total	82	100.0	100.0	

Table.5 Methicillin resistant strains of *S. aureus* exhibiting SSTIs

Methicillin					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	MRSA	63	76.8	76.8	76.8
	MSSA	19	23.2	23.2	100.0
	Total	82	100.0	100.0	

In the present study, cefoxitin disc sensitivity revealed that 63 (76.8 %) isolates were MRSA and 19 (23.2) were MSSA indicating predominant isolates were MRSA. Horieh Saderi *et al.,* (2009) reported, 54.5 % isolates were MRSA and 45.5 % were MSSA. Nuran Delialioglu *et al.,* (2005) reported 55.65 % isolates were MRSA and 44.34 % were MSSA.

The D-test revealed that the percentage of MLS_B phenotype among *Staphylococcus aureus* isolated from skin and soft tissue infection specimens, 23 (28%) isolates tested positive for $iMLS_B$ resistance, 49 (59.8 %) were shown to have $cMLS_B$ and 10 (12.2%) were MS phenotype.

These observation suggest that D-test had not been performed, nearly 23 (28%) of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive. Reporting these strains as sensitive to clindamycin without performing D-test would have resulted in therapeutic failure.

Similar observation were noted by Deotale *et al.,* (2010) 36 (14.5%) higher incidence $iMLS_B$ resistance, while Yilmiz *et al.,* (2007) 175 (19.8%) and Nuran Delialioglu *et al.,* (2005) 18(7.8%) have indicated lower incidence. Our observation is in concordance with the report by Deotale *et al.,* (2010).

Out of 82 erythromycin resistant *Staphylococcus aureus* exhibiting SSTIs, 82 (100%) were sensitive to Vancomycin followed by Doxycyclin 62 (75.6%), Amikacin 60 (73.2%), linezolid 57 (69.5%). Majority of the isolates were resistant to penicillin 80 (97.6 %).

In present study, all though we did not studied the prevalence of clindamycin resistance in our area, from the current study; we can conclude that there is high percentage of clindamycin resistance amongst the staphylococcal isolates that shows erythromycin resistance.

Staphylococcal skin and soft tissue infection treatment is important avoid subsequent secondary infection and emergence of MRSA strains are one of the major concern. The best therapeutic alternative, clindamycin is can be the sight of proper institution of the therapy. Hence, detection of inducible clindamycin resistant (true sensitivity) on routine basis is mandatory for judicial use of the drug.

References

Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA. Indian journal of medical microbiology. 2009 Jan 1; 27(1):27.

Buck ML. Use of clindamycin in pediatric infections. Pediatric pharm. 2008; 14:2.

Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis. 2005 Apr 1; 58(2):104-6.

Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian journal of medical microbiology. 2010 Apr 1; 28(2):124.

Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. Journal of clinical microbiology. 2003 Oct 1; 41(10):4740-4.

Kloos WE. Staphylococcus and micrococcus. Manual of clinical microbiology. 1999: 264-82.

Kumar S, Bandyopadhyay M, Bhattacharya K, Bandyopadhyay MK, Banerjee P, Pal N, Mondal S, Ghosh T. Inducible clindamycin resistance in staphylococcus isolates from a tertiary care hospital in Eastern India. Annals of Tropical Medicine and Public Health. 2012 Sep 1; 5(5):468.

Kumari N, Mohapatra TM, Singh YI. Prevalence of methicillin-resistant

Staphylococcus aureus (MRSA) in a tertiary-care hospital in Eastern Nepal. JNMA J Nepal Med Assoc. 2008 Apr 1; 47(170): 53-6.

Layer F, Ghebremedhin B, Moder KA, König W, König B. Comparative study using various methods for identification of Staphylococcus species in clinical specimens. Journal of clinical microbiology. 2006 Aug 1; 44(8):2824-30.

Nilsson P, and Ripa T. *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. Journal of clinical microbiology. 2006 Sep 1; 44(9):3334-9.

Perez LR, Caierão J, Antunes AL, d'Azevedo PA. Use of the D test method to detect inducible clindamycin resistance in coagulase negative staphylococci (CoNS). Brazilian Journal of Infectious Diseases. 2007 Apr; 11(2):186-8.

Reddy PS, and Suresh R. Phenotypic detection of Inducible Clindamycin resistance among the clinical isolates of *Staphylococcus aureus* by using the lower limit of inter disk space. Journal of Microbiology and Biotechnology Research. 2017 Mar 18; 2(2):258-64.

Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrobial agents and chemotherapy. 1999 Dec 1; 43(12):2823-30.

Saderi H, Owlia P, Eslami M. Prevalence of Macrolide-Lincosamide-Streptogramin B (MLSB) resistance in *S. aureus* isolated from patients in Tehran, Iran. Iranian journal of pathology. 2009 Sep 1; 4(4):161-6.

Saxena S, Singh T, Dutta R. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* at a tertiary care hospital: implications for clinical therapy. The Journal of communicable diseases. 2012 Jun; 44(2):97-102.

Shrestha B, Pokhrel B, Mohapatra T. Study of nosocomial isolates of *Staphylococcus aureus* with special reference to methicillin resistant *S. aureus* in a tertiary care hospital in Nepal. Nepal Med Coll J. 2009 Jun; 11(2):123-6.

Skov R, Smyth R, Larsen AR, Bolmstrom A, Karlsson A, Mills K, Frimodt-Moller N, Kahlmeter G. Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. Journal of clinical microbiology. 2006 Dec 1; 44(12):4395-9.

Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2007, vol-2(1).

Weisblum B. Insights into erythromycin action from studies of its activity as inducer of resistance. Antimicrobial agents and chemotherapy. 1995 Apr; 39(4):797.

Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. Journal of medical microbiology. 2007 Mar 1; 56(3): 342-5.

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