ORIGINAL RESEARCH PAPER

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CHARACTERIZATION OF MLS_B PHENOTYPES AMONG STAPHYLOCOCCUS AUREUS ISOLATES: A SIMPLE TWO DISK TEST THAT WORKS

KEY WORDS: *Staphylococcus aureus*, MRSA, MLS_B phenotype, ICR, D-zone

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ABSTRACT

Introduction: *Staphylococcus aureus* is among the most common pathogens causing a wide array of infections in humans. Macrolides, streptogramins and licosamide B are part of the primary choices for the treatment of *S. aureus* infections and they are used as alternate drugs in treatment of MRSA as well. But resistance to these agents may hamper in vivo therapy. **Objective:** To identify MLS_B resistance phenotypes in Staphylococcus aureus isolates from various clinical samples and to identify an association between MRSA and ICR positivity. **Material and methods:** Over a period of 6 months, *Staphylococcus aureus* was isolated from various clinical samples using conventional microbiological techniques. Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion; MRSA was detected using cefoxitin (30 μg); and, the D-zone was detected by approximating erythromycin (15 μg) and clindamycin (2 μg) disks. All the 3 tests were performed and interpreted as per CLSI guidelines. **Results:** A total of 80 *S. aureus* strains were isolated from multiple clinical specimens. MRSA were 21.25%; iMLSB, cMLSB and MS phenotypes were identified in 18.75%, 21.25% and 26.25% of *S. aureus* respectively; a significant ($p<0.049$) association was found between MRSA and ICR positivity. **Conclusion:** A simple disc diffusion test with erythromycin and clindamycin which can easily identify MLSB phenotypic resistance patterns in *Staphylococcus aureus* is useful and accurate even today. The correct utilization of macrolides, licosamindes and streptogramin B in clinical practice, especially in MRSA strains can be aided by this modest test.

INTRODUCTION

Emergence of methicillin resistant *Staphylococcus aureus* (MRSA) isolates which are resistant to multiple classes of antibiotics has increasingly led to the widespread use of drugs belonging to the macrolide-licosamide-streptogramin B (MLS_B) group for the treatment of infections caused by these pathogens. Among the group, clindamycin is the preferred antibiotic. But, constitutive or inducible resistance mechanisms due to the *erm* gene poses a significant threat of treatment failure (Seifi, Kahani, Askari, Mahdipour and Nederi, 2012). Hence, exact identification of clindamycin susceptibility is a necessity. This study was undertaken to identify the MLS_B phenotypes in *Staphylococcus aureus* isolates and to find an association between MRSA and inducible clindamycin resistance (ICR).

MATERIALS AND METHODS

A prospective study was conducted in a tertiary care hospital of Udaipur for 6 months. The Microbiology Laboratory received 4795 samples during the study period. Using standard biochemical techniques (Kloos and Banerman, 1999), isolates were identified as *Staphylococcus aureus*. Antimicrobial susceptibility testing was performed on Mueller Hinton agar plates by Kirby Bauer disk diffusion method using penicillin-G (10 units), cefoxitin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), rifampicin (5 μg), tetracycline (30 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), linezolid (15 μg), quinupristin-dalfopristin (15 μg) and nitrofurantoin (30μg) disks as per Clinical Laboratory Standards Institute (CLSI) guidelines 2019. Vancomycin (30 μg) was interpreted as per CLSI 2006.

Isolates with cefoxitin zone diameters > 22 mm were grouped as MRSA. The 'D test' for ICR was performed as outlined in the CLSI guidelines 2019. In short, on an inoculated Mueller Hinton agar plate with 0.5 McFarland standard bacterial suspension of isolate, erythromycin (15 μg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 μg). After overnight incubation at 37°C, flattening of zone (D-zone) on the clindamycin side in the area between the two antibiotic disks indicated inducible clindamycin resistance.

RESULTS

A total of 80 *Staphylococcus aureus* were identified from various samples (Table 1) and included in this study.

Table 1: Distribution pattern of *S. aureus* isolates in various clinical samples (n=80)

Type of Sample	Number of isolates	Percentage
Pus including swabs*	41	51.25%
Blood	24	30%
Urine	6	7.5%
Respiratory specimens#	5	6.25%
Tissue	2	2.5%
Body fluids‡	2	2.5%
Total	**80**	**100%**

*Swabs- wound swab, ear swab, conjunctival swab, vaginal swab.
#Respiratory specimens- sputum, endotracheal aspirate, bronchial aspirate.
‡Body fluids- pleural fluid, ascitic fluid, joint fluid.

The isolates were found more in male patients, 51 (63.75%), as compared to female patients, 29 (36.25%) and more in indoor patients, 66 (82.5%), than outdoor patients, 14 (17.5%). The resistance pattern of both methicillin sensitive *Staphylococcus aureus* (MSSA) and MRSA strains are represented in Table 2.

TABLE 2: Resistance pattern of MSSA (n=63) and MRSA (n=17) isolates

Antibiotics	MSSA	MRSA
Penicillin G	61 (96.82%)	17 (100%)
Gentamicin	1 (1.58%)	9 (52.95%)
Ciprofloxacin	51 (80.95%)	16 (94.11%)
Levofloxacin	49 (77.77%)	16 (94.11%)
Erythromycin	37 (58.73%)	16 (94.11%)
Clindamycin	22 (34.92%)	13 (76.47%)

Rifampicin	8 (12.69%)	4 (23.52%)
Tetracycline	6 (9.52%)	2 (11.76%)
Trimethoprim-sulphamethoxazole	35 (55.55%)	13 (76.47%)
Chloramphenicol	3 (4.76%)	2 (11.76%)
Linezolid	3 (4.76%)	3 (17.65%)
Quinupristin-dalfopristin	17 (26.98%)	7 (41.17%)
Vancomycin	8 (12.69%)	3 (17.65%)
Nitrofurantoin (only in urine)	0 (0.0%)	0 (0.0%)

Cefoxitin resistance was seen in 17 isolates. The phenotypic characterization of the *S. aureus* isolates based on their susceptibility to erythromycin and clindamycin are denoted in Table 3.

TABLE 3: Phenotypic characterization of *S. aureus* isolates (n=80)

Phenotype	MSSA(%) (n=63)	MRSA (%) (n=17)	Total (%) (n=80)
(ER-R, CL-S, D-test positive) (iMLS$_B$ phenotype)	9 (60%)	6 (40%)	15 (18.75%)
(ER-R, CL-R) (cMLS$_B$ phenotype)	10 (58.82%)	7 (41.17%)	17 (21.25%)
(ER-R, CL-S, D-test negative) (MS phenotype)	18 (85.71%)	3 (14.28%)	21 (26.25%)
(ER-S, CL-S) (Sensitive phenotype)	26 (96.29%)	1 (3.70%)	27 (33.75%)

ER-Erythromycin, CL-Clindamycin, S-Sensitive, R-Resistant, iMLS$_B$-Inducible Macrolide Lincosamide Streptogramin B phenotype, cMLS$_B$-Constitutive Macrolides Lincosamide Streptogramin B phenotype, MS-Macrolide Streptogramin phenotype.

A significant association ($p<0.049$) was found between MRSA and ICR positivity.

DISCUSSION

Staphylococcus aureus is an important nosocomial and community-acquired pathogen worldwide, which can cause both superficial and deep pyogenic infections as well as several toxin-mediated illnesses (Munjal and Mudey, 2018). In this study, 80 *Staphylococcus aureus* isolates were included. As expected, maximum numbers of isolates were from pus samples (51.25%) and least were from sputum samples (7.5%). This finding corresponds with study by authors Tyagi and Oberoi (2016) and Thapa *et al* (2021) who also isolated *S. aureus* from pus and other pyogenic samples in 63% and 39.2% respectively.

Once susceptible to penicillin, these bacteria rapidly developed resistance to it and methicillin was introduced for its treatment (Patil *et al*, 2014). Emergence of methicillin resistance in *S. aureus* has left us with very few therapeutic alternatives available to treat infections caused by these bugs (Chaudhary *et al*, 2015). MRSA are naturally selected to be resistant to multiple drugs of the β-lactam antibiotic group. Resistance is developed as a result of beta lactamase enzyme production or presence of *mec* A genes (Patil *et al*, 2014). We isolated 21.25% of MRSA during the study period corroborating the findings of other authors (Mokta *et al*, 2015, Prabhu *et al*, 2011, Adhikari *et al*, 2017). Resistance was the highest to penicillin G (97.5%) followed by resistance to quinolones (81.25%), erythromycin (66.25%) and trimethoprim-sulphamethoxazole (60%). The staphylococcal isolates were most sensitive to chloramphenicol (93.75%) and subsequently to linezolid (92.5%), tetracycline (90%) and vancomycin (86.25%).

In recent times, clindamycin has become an excellent drug for treating staphylococcal infections particularly of the skin and soft tissues and as an alternative in penicillin-allergic patients (Grace, 2013). Also, clindamycin has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics (Supriyarajvi, Tina and Sharma, 2015, Chaudhary *et al*, 2015, Prabhu *et al*, 2011). However, target site modification by *erm* genes is a hindrance in treating resistant strains. This modification is common to MLS$_B$ family which are structurally unrelated groups of antibiotics but have the same mode of action, i.e., binding to the 50s ribosomal subunit of the bacteria.

The *erm* gene in the MLS$_B$ family is expressed in one of two forms – constitutive (cMLS$_B$) or inducible (iMLS$_B$). *S. aureus* isolates harboring constitutive resistance mechanisms demonstrate resistance to both erythromycin and clindamycin individually when tested in vitro. On the other hand, inducible variants demonstrate erythromycin resistance but clindamycin susceptible when tested individually in vitro. Despite this "supposed" susceptible result to clindamycin, treatment failure may ensue clinically if clindamycin is used. To identify the inducible property, both the drugs need to be approximated and looked for the D-zone blunting on the clindamycin side (Tyagi *et al*, 2016). A third phenotype can also be appreciated in a few strains where in erythromycin is resistant and clindamycin is susceptible without a D-zone, the MS phenotype, macrolide and streptogramin B resistant but lincosamide susceptible (Prabhu *et al*, 2011).

Two thirds of the isolates of the study (66.25%) were resistant to erythromycin. Among them, constitutive resistance was seen in 17 (21.25%), a D-zone was noted in 15 (18.75%) and true clindamycin sensitive strains were 21 (26.25%). These results were consistent with studies by authors Patil *et al* (2014) and Juyal *et al* (2013). Dissimilar reports were observed in studies of Supriyarajvi *et al* (2015) and Naik, Peerapur and Sandhya (2017). However, one common link in all these research findings was the occurrence of ICR commonly in MRSA strains than in MSSA strains. A positive association ($p<0.049$) between methicillin resistant *Staphylococcus aureus* and inducible clindamycin resistance was evident from our study results.

CONCLUSION

Spontaneous rise of constitutive clindamycin resistant mutants both in vivo and in vitro can be attributed to an inducible clindamycin phenotype in some strains (Seifi et al, 2012). Therefore, reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in more harm than good to the patient. A simple laboratory test incorporated into the routine antimicrobial susceptibility testing panel can identify this phenotype correctly.

REFERENCES

1. Adhikari, R. P., Shrestha, S., Barakoti, A., Amatya, R. (2017). Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infectious Diseases, 17(1), 483.
2. Chaudhary, A. B., Shah, K. K., Parmar, R. J., Kavthia, G. U., & Goswami, Y. S. (2015). Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. International Journal of Applied Research, 1(9), 922-924.
3. Clinical and Laboratory Standard Institute. (2006). CLSI Document M100. Performance standards for antimicrobial susceptibility testing. Wayne.
4. Clinical and Laboratory Standard Institute. (2019). CLSI Document M100. Performance standards for antimicrobial susceptibility testing. Wayne.
5. Grace, F. (2013). D-Test for detection of antimicrobial susceptibility in methicillin resistant Staphylococcus aureus (MRSA). IOSR Journal of Pharmacy and Biological Sciences, 7, 32-35.
6. Juyal, D., Shamanth, A. S., Pal, S., Munesh K. Sharma, M. K., Prakash, R., & Sharma, N. (2013). The prevalence of inducible clindamycin resistance among staphylococci in a tertiary care hospital – A study from the Garhwal Hills in Uttarakhand, India. Journal of Clinical and Diagnostic Research, 7(1), 61-65.
7. Kloos, W. E., & Banerman, T. L. (1999). Staphylococcus and Micrococcus. In: Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolken, R. H (7th ed.). Manual of clinical microbiology (pp. 264-283), Washington DC: ASM press.
8. Mokta, K. K., Verma, S., Chauhan, D., Ganju, S. A., Singh, D., Kanga, A., Kumari, A., & Mehta, V. (2015). Inducible clindamycin resistance among clinical isolates of Staphylococcus aureus from sub Himalayan region of India. Journal of Clinical and Diagnostic Research, 9(8), DC 20-23.
9. Munjal, R., & Mudey, G. (2018). Nasal carriage of Staphylococcus aureus among undergraduate medical students: Prevalence and antibiogram including methicillin-resistant, inducible clindamycin resistance, and high-level mupirocin resistance. Journal of Datta Meghe Institute of Medical Sciences University, 13, 91-94.

10. Naik, V. R., Peerapur, B. V., & Sandhya, P. (2017). A retrospective study for detection of inducible resistance to clindamycin in Staphylococcus aureus isolates using D-test at RIMS Teaching Hospital, Raichur. International Journal of Current Microbiology and Applied Sciences, 6(10), 1537-1542.
11. Patil, N. R., Mali, U. S., Kulkarni, S. A., Mali, S., Mane, V., & Ghorpade, M. V. (2014). Detection of inducible clindamycin resistance among clinical isolates of Staphylococcus aureus in a tertiary care hospital. International Journal of Current Microbiology and Applied Sciences, 3(9), 689-694.
12. Prabhu, K., Rao, S., Rao, V. (2011). Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Clinical Samples. Journal of Laboratory Physicians, 3(1), 25-7.
13. Seifi, N., Kahani, N., Askari, E., Mahdipour, S., & Naderi, NM. (2012). Inducible clindamycin resistance in Staphylococcus aureus isolates recovered from Mashhad, Iran. Iranian Journal of Microbiology, 4(2), 82-86.
14. Supriyarajvi, Gupta, A., Tina, G., & Sharma, B. P. (2015). Detection of inducible clindamycin resistance among staphylococcal isolates from various clinical specimens in a tertiary care institute in north west region of Rajasthan. International Journal of Current Microbiology and Applied Sciences, 4(10), 741-749.
15. Thapa, D., Pyakurel, S., Thapa, S., Lamsal, S., Chaudhari, M., Adhikari, N., & Shrestha, D. (2021). Staphylococcus aureus with inducible clindamycin resistance and methicillin resistance in a tertiary hospital in Nepal. Tropical Medicine and Health, 49(1), 99.
16. Tyagi, S., & Oberoi, A. (2016). Inducible clindamycin resistance in Staphylococcus aureus isolates and its correlation with multidrug resistance: a study from North India. Journal of Evolution in Medical and Dental Science, 59(6), 749-752.