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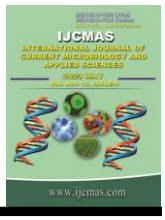

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Study of Antibiotic Resistance Pattern in Methicillin-Resistant *Staphylococcus aureus* with Special Reference to Newer Antibiotics in a Tertiary Care Hospital in Haryana, India

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A B S T R A C T

Staphylococcus aureus (S. aureus) is a leading source of nosocomial infections, such as ventilator-associated pneumonia, surgical site infections and bloodstream infections. This study was conducted in the Department of Microbiology Pt. B.D. Sharma University of Health Sciences, Rohtak on 200 isolates of *S. aureus*. Results: The rate of isolation of *S. aureus* was maximum from pus (77.0%) followed by blood (23%). The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was observed in 55% of *S. aureus* isolates. MRSA was significantly more among Pus samples. As per D-Zone test, inducible clindamycin resistance was found among 20 (18.2%) isolates. As per vancomycin E-test, vancomycin was found to be sensitive in 106 (96.4%) and intermediate sensitive in 4 (3.6%) isolates. Linezolid and daptomycin were found to be sensitive in all (100.0%) isolates. The present study concluded that *S. aureus* is a pervasive pathogen in both our hospital and in community settings, with constantly changing trends in virulence, resistance and epidemiology.

Introduction

Infections are one of the leading causes of disease and death in the human population globally. *S. aureus* is a leading source of nosocomial infections, such as ventilator-associated pneumonia (VAP) and surgical site infections (SSI), as well as community-acquired illnesses such as bloodstream infections (BSI), skin and wound infections, osteomyelitis, and endocarditis (Tiwari *et al.*, 2009). Antimicrobial resistance is a worldwide public health issue that severely restricts infection prevention and treatment and threatens to undo medical progress. *S. aureus* has a remarkable capacity to rapidly adapt to each new antibiotic by developing a resistance mechanism, beginning with penicillin and progressing to the most recent linezolid and daptomycin (WHO, 2014). MRSA is a prevalent concern in hospitals, sports facilities, clinics, and the general public. MRSA strains linked with hospitals

are known as hospital-acquired MRSA (HA-MRSA) and are the leading cause of hospital-acquired infections. Septic shock (56%), pneumonia (32%), endocarditis (19%), bacteremia (10%), and cellulitis (6%), are the most often reported invasive MRSA-related diseases. Strains connected with the population are known as community-acquired MRSA (CA-MRSA), and they may even be found in persons who are asymptomatic carriers.(Deleo *et al.,* 2010) MRSA has led to renewed interest in uses of macrolides, lincosamides, streptogramin B (MLSB) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.(Ajantha *et al.,* 2008) The glycopeptide antibiotic vancomycin and the lipopeptide antibiotic daptomycin are used to treat severe MRSA infections. Linezolid, the first oxazolidinone, introduced in 2000, has been approved for treatment of infections caused by various gram-positive bacteria including MRSA and Vancomycin resistant enterococci (VRE) (Zurenko *et al.,* 1997).

Materials and Methods

A prospective study was carried out in the department of Microbiology, Pt. B. D. Sharma PGIMS Rohtak. Approximately 200 *S. aureus* strains isolated from pus and blood samples received from various indoor and outdoor patients were included in the study over a period of one year.

Identification of the isolates was cofirmed by colony morphology, gram staining, catalase test, tube coagulase test and mannitol fermentation test following standard microbiological procedures.(Gajdacs *et al.,* 2017) Detection of methicillin resistance done by cefoxitin disc diffusion test, zone diameter ≤21mm was reported as MRSA. Detection of inducible clindamycin resistance was done by D-zone test.

The Isolates that turned out to be erythromycin resistant were further subjected to double disc diffusion approximation test (D-zone test) as per CLSI guidelines for inducible clindamycin resistance. Epsilometer test (E-test) was performed for determining Minimum Inhibitory Concentration (MIC) for vancomycin, linezolid and daptomycin.

To determine MIC of daptomycin, MHA was supplemented with 25mg/L calcium due to its dependence on calcium. Plates were incubated at 35ºC±2ºC for 16-20 hours before reading results (CLSI, 2020; Collee *et al.,* 1996).

Statistical analysis

SPSS version 25.0 analyzed the Excel data when it was loaded. Quantitative (numerical variables) data was given as mean and standard deviation, whereas qualitative (categorical variables) data was provided as frequency and percentage.

The student t-test was used to compare the two groups' mean values, while the chi-square test analyzed their frequency differences. If p0.05, it was statistically significant.

Results and Discussion

Table 2 shows that MRSA was significantly more among Pus samples whereas MSSA was significantly more among blood samples.

Table 3 shows that majority of the subjects belonged to Skin (24.5%), followed by Orthopaedics (21.8%), Surgery (19.1%), Paediatrics (10.9%), Obg (10.0%), ENT (6.4%), Sports medicine (2.7%), ICU (1.8%), Dental & PICU (0.9% each).

Table 4 shows as per D-Zone Test, Ery-S, CL-S was found among 64 (58.2%) cases, Ery-R, CL-R (Constitutive MLS_B) was found among 12 (10.9%) cases, Ery-R, CL-S (D-test positive, i MLS_B) was found among 20 (18.2%) cases and Ery-R, CL-S (D-test negative, MS) was found among 14 (12.7%) cases.

Table 5 shows that 106 isolates were sensitive to vancomycin. 0.9% MRSA isolates had vancomycin MIC value of 0.25 and 4.5 % of MRSA had MIC

value of 0.5. 5.5% of isolates had MIC value of 0.75 and 60% had MIC value of 1.00. 25.5% had MIC value of 1.50. Only 4 isolates had MIC value > 2. Two isolates had MIC value 4.00 and 2 isolates MIC value of 6.00.

Table 6 shows that all isolates were sensitive to linezolid. 25.5% MRSA isolates had linezolid MIC value of 1.00 and 64.5 % of MRSA had MIC value of 1.5. 9.1% of isolates had MIC value of 2.00 and only 1 isolate had MIC value of 3.00.

Table 7 shows that all isolates were sensitive to daptomycin. 29.1% MRSA isolates had daptomycin MIC value of 0.75 and 61.8 % of MRSA had MIC value of 0.5. 4.5% of isolates had MIC value of 0.38 and 4.5% of isolates had MIC value of 0.25.

In present study, MRSA was significantly more among pus samples (59.1%) whereas MSSA was significantly more among blood samples (58.7%). Sapkota *et al.,* (2019) found that 78.95% MRSA was isolated from pus and wound infections. In our study, majority of the specimens belonged to Skin (24.5%), followed by Orthopaedics (21.8%), Surgery (19.1%), Paediatrics (10.9%), OBG (10.0%), ENT (6.4%), Sports medicine (2.7%), ICU (1.8%), Dental & PICU (0.9% each). Chaudhary *et al.,* (2022) discovered that 40% were from sepsis patients, 18.36% from unexplained pyrexia, 12.56% from surgical site infection, and 10.62% from ventilator-associated events. Kaur *et al.,* (2015) observed that the distribution was as follows Obstetrics and gynaecology (33.33%), surgery (30.56%), medicine (19.44%), the intensive care unit (11.11%), and paediatrics (2.78%) and skin (2.78%) all had one case each of MRSA (resistant to commonly used antimicrobial drugs).(Kaur *et al.,* 2015) In current study, 58.2% isolates were found to be sensitive to both erythromycin and clindamycin, 10.9% cases were found to have Constitutive MLSB, 18.2% cases were found to have inducible clindamycin resistance and 12.7% cases had MS phenotype. Ciraj *et al.,* (2009) discovered that 32 (13.1%) of the 244 clinical isolates of staphylococci investigated had inducible clindamycin resistance and belonged to the iMLSB phenotype.(Ciraj *et al.,* 2009) In our study, Vancomycin was found to be Sensitive in 96.4% and intermediate in 3.6% MRSA isolates. Linezolid was found to be Sensitive in all (100.0%) isolates. Daptomycin was found in be Sensitive to all (100.0%) isolates. Al-Zoubi *et al.,* (2015) reported that Vancomycin was effective against all isolates (100%).

Bhavsar *et al.,* (2015) found that 96.93% MRSA isolates are susceptible to Vancomycin, 100% susceptible to Linezolid and 35.39% susceptible to Gentamicin and 12.31% susceptible to Azithromycin. Kamila *et al.,* (2017) found none of the MRSA isolates tested positive for resistance to linezolid. Regmi *et al.,* (2020) showed that all MRSA isolates were sensitive to vancomycin, teicoplanin and linezolid. It can be concluded that MRSA is a significant cause of both hospital and community acquired infections. As a result, it is necessary to monitor clinical and microbiological parameters in order to modify our existing infection control measures and treatment options in an appropriate manner.

Table.1 Interpretation of results of E Test. (CLSI, 2020)

Interpretive categories and MIC Breakpoints, µg/mL				
	S	**SDD**	**I**	**R**
Vancomycin	**≤2**	**-**	**4-8**	**≥16**
Linezolid	**≤4**	**-**	**-**	**≥8**
Daptomycin	**≤1**	**-**	**-**	**-**

MIC- Minimum Inhibitory Concentration, S- Susceptible, SDD- Susceptible dose dependent, I- Intermediate and R-Resistant.

Table.2 MRSA and MSSA by cefoxitin disc diffusion method

Sample	CDD		Total
	MRSA	MSSA	
Blood	19	27	46
	41.3%	58.7%	100.0%
Pus	91	63	154
	59.1%	40.9%	100.0%
Total	110	90	200
	55.0%	45.0%	100.0%
^{2} value = 4.527, p-value = 0.033*			

Table.3 Distribution of MRSA according to department

	Frequency	Percentage
Dental	1	0.9%
ENT	7	6.4%
ICU	2	1.8%
Medicine	1	0.9%
Obg	11	10.0%
Orthopaedics	24	21.8%
Paediatrics	12	10.9%
PICU	1	0.9%
Skin	27	24.5%
Sports medicine	3	2.7%
Surgery	21	19.1%

Table.4 Distribution of MRSA according to D-Zone Test

	Frequency	Percentage
Ery-S, CL-S	64	58.2%
Ery-R, CL-R (Constitutive MLS_B)	12	10.9%
Ery-R, CL-S (D-test positive, i MLS_B)	20	18.2%
Ery-R, CL-S (D-test negative, MS)	14	12.7%

Table.5 Distribution of MRSA according to MIC of vancomycin

VET (MIC)	Frequency	Percentage
0.25	1	0.9%
0.50	5	4.5%
0.75	6	5.5%
1.00	66	60.0%
1.50	28	25.5%
4.00	2	1.8%
6.00	2	1.8%

Table.6 Distribution of MRSA according to MIC of Linezolid

LET (MIC)	Frequency	Percentage
1.00	28	25.5%
1.50	71	64.5%
2.00	10	9.1%
3.00	1	0.9%
Total	110	100.0%

Table.7 Distribution of MRSA according to MIC of Daptomycin

DET (MIC)	Frequency	Percentage
0.25	5	4.5%
0.38	5	4.5%
0.50	68	61.8%
0.75	32	29.1%

References

Ajantha G, Kulkarni R, Shetty J, Shubhada C, Jain P. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates by using the lower limit of recommended inter-disk distance. Indian J Pathol Microbiol. 2008; 51:376-8. https://doi.org/10.4103/0377-4929.42515

Al-Zoubi M S, Al-Tayyar I A, Hussein E, Jabali A A, Khudairat S. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in Northern area of Jordan. Iran J Microbiol. 2015;7(5):265-72.

Bhavsar R, Garala N J, Garala R N, Patel P, Javadekar T B, Patel H, Tadvi J, Mehta K. Antimicrobial susceptibility pattern of methicillin resistant *staphylococcus aureus* isolated from various clinical samples at SSG hospital, Baroda. J Res Med Den Sci 2015;3(1):43-6. https://doi.org/10.5455/jrmds.20153110

Chaudhury N, Biswas T, Mondal R., Chatterjee A, Chattopadhyay S, Nag S. Antibiotic susceptibility and prevalence of Methicillin-resistant *Staphylococcus aureus* in different clinical isolates in a tertiary care hospital. Asian Journal of Medical Sciences. 2022;13:6. https://doi.org/10.3126/ajms.v13i6.43027

Ciraj A M, Vinod P, Sreejith G, Rajani K. Inducible clindamycin resistance among clinical isolates of staphylococci. Indian J Pathol Microbiol. 2009;52:49-51. https://doi.org/10.4103/0377-4929.44963

CLSI, Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement MI00. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.

Collee, J G, T J. Mackie, and J E. McCartney. Mackie & Mccartney Practical Medical Microbiology. New York: Churchill Livingstone, 1996.

Deleo F. R, Otto M, Kreiswirth B. N, Chambers H. F. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet.* 2010;375(9725):1557–68. https://doi.org/10.1016/S0140-6736(09)61999-1.

Gajdacs M, Spengler G, Urban E. Identification and antimicrobial susceptibility testing of anaerobic bacteria: Rubik's cube of clinical microbiology. Antibiotics (Basel). 2017;6(4):25. https://doi.org/10.3390/antibiotics6040025.

Barbara Kot, Kamila B. K., Małgorzata W, Grużewskab P. A. Antimicrobial Resistance

Patterns in Methicillin Resistant *Staphylococcus aureus* from Patients Hospitalized during 2015–2017 in Hospitals in Poland. Med Princ Pract 2020;29:61–68. https://doi.org/10.1159/000501788.

Kaur D C, Chate S S. Study of Antibiotic Resistance Pattern in Methicillin Resistant *Staphylococcus aureus* with Special Reference to Newer Antibiotic. J Glob Infect Dis. 2015 Apr-Jun;7(2):78-84. https://doi.org/10.4103/0974-777X.157245

Regmi, R. S, Khadka, S, Sapkota, S, Magar, S. T, Adhikari, S, Subedi, S, Shrestha, P, & Rana, J. C. (2020). Phenotypic Detection of Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* in Bharatpur Hospital. *Journal of College of Medical Sciences-Nepal*, *16*(3), 178–83. https://doi.org/10.3126/jcmsn.v16i3.28490

Sapkota J, Sharma M, Jha B, Bhatt C P. Prevalence of *Staphylococcus aureus* Isolated from Clinical Samples in a Tertiary Care Hospital: A Descriptive Cross-sectional Study. JNMA J Nepal Med Assoc. 2019 Nov-Dec;57(220):398-402. PMID: 32335648; PMCID: PMC7580409. https://doi.org/10.31729/jnma.4673.

Tiwari H K, Das A K, Sapkota D, Sivrajan K, Pahwa V K Methicillin resistant *Staphylococcus aureus*: Prevalence and antibiogram in a tertiary care hospital in western Nepal. The journal of infection in developing countries2009Oct22 3: 681-4. https://doi.org/10.3855/jidc.86.

WHO. Antimicrobial Resistance: Global Report on Surveillance; 2014. Available from: http://www.who.int/drugresistance/documents/surveillancereport/en/.

Zurenko G E, Ford C W, Hutchinson D K, Brickner S J, Barbachyn M R. Oxazolidinone antibacterial agents: development of the clinical candidates eperezolid and linezolid. Expert Opin Investig Drugs. 1997; 6(2):151-8.
https://doi.org/10.1517/13543784.6.2.151.

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