Volume 20 Issue 56
Originally issued as C4



Global Journal of Medical Research: C
Microbiology and Pathology
Volume 20 Issue 4 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Epidemiology of *Staphylococcus spp.* with Analysis of Various Available Methods for Detection of Methicillin Resistant Staphylococcus Aureus

By Gitali Bhagawati, Sania Paul, Rekha Saji Kumar, Mansi & Khushboo

Abstract- *Staphylococcus aureus* is one of the major resistant pathogens in clinical practice; Methicillin Resistant *Staphylococcus aureus* (MRSA) has come out as superbugs. Apart from this, with the increase in the number of hospitalized immunocompromised patients, Coagulase negative Staphylococcus (CONS) have become a major cause of nosocomial infections. Although molecular method like mecA gene detection is gold standard for MRSA, minimum inhibitory concentration (MIC) of cefoxitin or oxacillin can also be considered as standard where molecular methods are not available. Cefoxitin 30 µg disc or PBP 2a agglutination test can also be used as standard marker for MRSA identification. In this study, out of total 184 clinically significant, non-duplicate specimens, 150 (81.52%) isolates were *Staphylococcus aureus* and 34 (18.48%) were CONS. Among the CONS, the predominating isolate was *Staphylococcus haemolyticus* 15 (44.12%), followed by *Staphylococcus epidermidis* 10 (29.41%). In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%. PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. In both the methods, MIC of cefoxitin has been considered as gold standard.

GJMR-C Classification: *NLMC Code: WQ 4*



Strictly as per the compliance and regulations of:



© 2020. Gitali Bhagawati, Sania Paul, Rekha Saji Kumar, Mansi & Khushboo. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Epidemiology of *Staphylococcus spp.* with Analysis of Various Available Methods for Detection of Methicillin Resistant Staphylococcus Aureus

Gitali Bhagawati [α], Sania Paul [σ], Rekha Saji Kumar [ρ], Mansi [ω] & Khushboo [¥]

Abstract- ***Staphylococcus aureus*** **is one of the major resistant pathogens in clinical practice; Methicillin Resistant *Staphylococcus aureus* (MRSA) has come out as superbugs. Apart from this, with the increase in the number of hospitalized immunocompromised patients, Coagulase negative Staphylococcus (CONS) have become a major cause of nosocomial infections. Although molecular method like mecA gene detection is gold standard for MRSA, minimum inhibitory concentration (MIC) of cefoxitin or oxacillin can also be considered as standard where molecular methods are not available. Cefoxitin 30 µg disc or PBP 2a agglutination test can also be used as standard marker for MRSA identification. In this study, out of total 184 clinically significant, non-duplicate specimens, 150 (81.52%) isolates were *Staphylococcus aureus* and 34 (18.48%) were CONS. Among the CONS, the predominating isolate was *Staphylococcus haemolyticus* 15 (44.12%), followed by *Staphylococcus epidermidis* 10 (29.41%). In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%. PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. In both the methods, MIC of cefoxitin has been considered as gold standard.**

I. Introduction

Staphylococcus aureus is one of the major resistant pathogens in clinical practice. Methicillin Resistant *Staphylococcus aureus* (MRSA) is defined as a strain of *S. aureus* that is resistant to a large group of antibiotics called β-lactams, that includes penicillins and cephalosporins.[1] The first case of MRSA was reported in Britain in 1961 and is now "quite common" in hospitals.[1, 2]Methicillin resistance in *S. aureus* is primarily mediated by overproduction of PBP2a protein, an altered penicillin-binding protein with lower affinity for beta-lactam antibiotics than PBP2, the main physiological methicillin target. PBP2a is encoded by the *mecA* gene, a component of a larger DNA fragment designated the *mec* region.[1, 3, 4] Coagulase negative Staphylococcus (CONS) have been considered as non-pathogenic and were rarely reported to cause severe infections. However, with the increase in the number of hospitalized immunocompromised patients, CONS have become a major cause of nosocomial infection and they account for 9% of these infections.[5]

Author α σ ρ ω ¥: Department of Microbiology, Dharamshala Narayana Superspeciality Hospital, Delhi. e-mail: gitalibhagawati32@gmail.com

There are many traditional and commercial systems for detection of MRSA in clinical microbiology laboratories. Until 2006, Oxacillin disc and agar screening methods were used for detection of MRSA, however, in January 2006, Clinical Laboratory Standards Institute (CLSI) recommended use of Cefoxitin 30 µg disc as standard marker for MRSA identification.[6] The shift towards use of Cefoxitin disc is emphasized because of its property to induce production of PBP2a in-vitro, thus it has better predictive value for detection of hetero-resistance in MRSA isolates.[7] The gold standard method for antimicrobial susceptibility testing has been the minimum inhibitory concentration (MIC) test determined by dilution methods. In the recent years, MIC methods have been replaced by molecular methods which detect mecA gene as a gold standard for determining classical methicilin resistance in *S. aureus*. However, the use of molecular methods for detection of MRSA is largely restricted to reference laboratories and is not utilized in many microbiology laboratories as a routine test.[2]

The aims and objectives of the study were:

1. To detect the prevalence of *Staphylococcus aureus* and clinically significant CONS in various clinical specimens
2. Speciation of CONS
3. To isolate MRSA by easily available phenotypic methods: MIC level detection of Cefoxitin/Oxacillin, Disk diffution of Cefoxitin 30 µg disc and PBP 2a agglutination test.
4. To evaluate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of these methods for identification of these strains.

Since PCR was not available for routine tests in the laboratory, MIC level detection of Cefoxitin/Oxacillin was considered as a gold standard.

II. Materials and Method

This prospective study was done in the Department of Microbiology in a tertiary care hospital in Delhi over a period of one year. Isolates of *Staphylococcus aureus* and CONS were collected from various clinical samples that were grown in routine cultures. The clinical specimens comprised of pus, blood, urine, high vaginal swab (HVS), body fluid,

© 2020 Global Journals

endotracheal (ET) tube secretion, discharge from eye and ear, joint aspirate and Central venous catheter line (CVP) tip. A total of 184 consecutive, non-duplicate, clinically significant isolates were collected for this study.

a) Bacterial identification and antimicrobial susceptibility testing

The clinical specimens were inoculated on 5% sheep blood agar and MacConkey's agar (HiMedia, New Delhi, India), incubated at 37°C for 24-48 h, and examined for bacterial growth. The identification was done by manual as well as by Automated System (Vitek 2 Compact System, bioMérieux). Manual methods were based on colony morphology, Gram's stain, catalase test, mannitol fermentation, and coagulase test (slide and tube method). All the isolates were subjected to three methods of identification of methicillin resistance:

1. MIC breakpoints of oxacillin given by Vitek 2 Compact system or MIC level detection of Cefoxitin by E-test (Himedia). *Staphylococcus aureus* ATCC 29213 were used as control for MIC level detection.
2. Modified Kirby-Bauer disk diffusion method using Cefoxitin disks (30μg) on Mueller-Hinton agar (MHA). MHA plates were overlaid with clinical strain of the *S. aureus* with an inoculum of 0.5 McFarland turbidity standards. Cefoxitin 30 μg discs were used and incubated at 35°C for 24 hours. Cut off zone diameters for Cefoxitin was according to CLSI 2015. For quality control, ATCC controls strains for MRSA and MSSA were placed on the same plate.
3. PBP2' Latex agglutination test. (Oxoid, ThermoFisher Scientific, Basingstoke, England).

b) PBP2' Latex Agglutination test

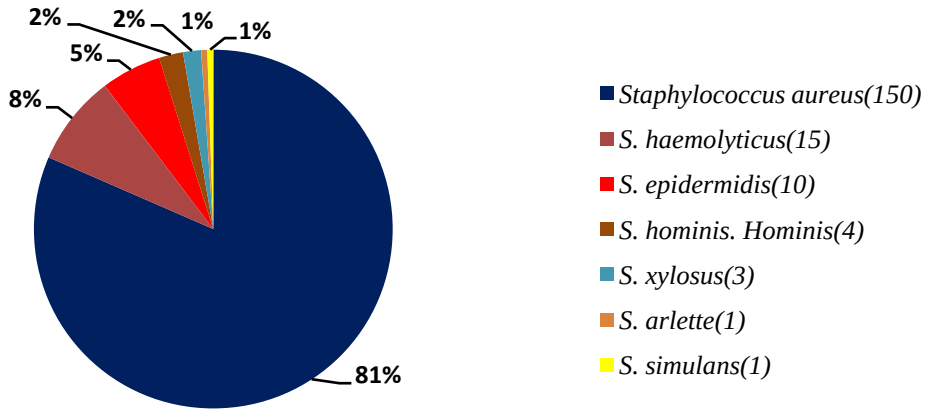
A loop-full of organisms was placed into a microcentrifuge tube with 4 drops of Extraction Reagent 1; the tubes were then placed in a heating block (>90°C), and after 5 minutes, the tubes were removed and allowed to cool to room temperature. A single drop of Extraction Reagent 2 was added to each tube, mixed well, and centrifuged at 1,500*g* for 5 minutes. The supernatant, 50 μL, was used for testing with 1 drop of the latex particles. The supernatant and latex particles were mixed together with a stick, and the test card was rocked for 3 minutes. Tests were read visually. Agglutination of the test but not the control latex was considered positive, while no agglutination was considered negative.

The data obtained was recorded on Microsoft excel (2007 version) and analyzed. The results are explained in frequency (number) and in percentage (%).

III. Results

Out of total 184 clinically significant, non-duplicate specimens, 150 (81.52%) isolates were *Staphylococcus aureus* and 34 (18.48%), were CONS. Among the CONS, the predominating isolate was *Staphylococcus haemolyticus*15 (44.12%), followed by *Staphylococcus epidermidis*10 (29.41%) *(Fig1)*

Fig1: Speciation of Staphylococcus in various samples



Out of total 184 Gram positive cocci, 104 (56.52%) were isolated from males and 80 (43.48%) from female patients. *(Table1)*

Staphylococcus aureus strains were isolated from106 (70.66%) indoor patients, followed by 29 (19.33%) intensive care unit (ICU) and neonatal ICU (NICU) patients. Among the clinically significant CONS, 23(67.65%) were isolated from indoor patients and the rest 11(32.36%) were from ICU and NICU patients. (*Table1)*

© 2020 Global Journals

Table1: Distribution of isolates according to sex and hospital admission

Isolates	Male	Female	ICU	NICU	Indoor	Outdoor
Staphylococcus aureus(150)	84	66	16	13	106	15
S. haemolyticus(15)	8	7	2	4	9	0
S. epidermidis(10)	6	4	2	0	8	0
S. hominis. hominis(4)	3	1	0	2	2	0
S. xylosus(3)	3	0	0	0	3	0
S. arlette(1)	0	1	0	1	0	0
S. simulans(1)	0	1	0	0	1	0
TOTAL(184)	104	80	20	20	129	15

Overall, the predominating specimen of isolating the Gram positive cocci was found to be pus 105 (57%), followed by blood 57(31%). Specimen wise distributions of *Staphylococcus aureus* and CONS have been shown in *Table2.*

Table 2: Specimen wise distribution of isolate

Specimen Isolates	Pus	Blood	Urine	High Vaginal Swab	Body Fluid	ET secretion	Eye Discharge	Ear Discharge	Joint Aspirate	CVP tip
Staphylococcus aureus (150)	86	45	9	4	1	2	2	0	1	0
S. haemolyticus (15)	9	4	0	0	0	0	0	0	1	1
S. epidermidis (10)	5	4	0	0	0	0	0	1	0	0
S. hominis. hominis (4)	2	2	0	0	0	0	0	0	0	0
S. xylosus (3)	2	1	0	0	0	0	0	0	0	0
S. arlette (1)	0	1	0	0	0	0	0	0	0	0
S. simulans (1)	1	0	0	0	0	0	0	0	0	0
Total	105	57	9	4	1	2	2	1	2	1

All the isolates of *Staphylococcus aureus* were subjected to three phenotypic methods of identifying methicillin resistance. Considering MIC level as gold standard, Cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.12% and negative predictive value (NPV) 100% while PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87%.

Table 3: Comparison between different phenotypic methods considering MIC level as Gold Standard in relation to MRSA

Phenotypic Methods	Result	MIC Level: Resistant	MIC Level: Susceptible	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
PBP2'a Latex Agglutination Test	Positive	102	1	99%	97.87%	99%	97.87%
	Indeterminate/ Negative	1	46				
Cefoxitin 30 µg Disk Diffusion	Resistant	99	0	100%	92.15%	96.12%	100%
	Susceptible	4	47				

© 2020 Global Journals

IV. Discussion

Among the Gram-positive pathogens, *S. aureus* continues to cause skin and soft tissue infections (SSTI) in the community as well as invasive infections in the hospitalized patients.[9] In our study, out of total 184 clinically significant, non-duplicate (except blood) specimens, 150 (81.52%) isolates were *S.aureus.* The most common clinical sample from which *S. aureus* have been isolated was pus or wound swabs 86 (57.33%). [Fig 1, Table 2] One similar finding corresponded *S.aureus* 165, out of which out of 131 (79.39%) were from pus samples.[1] In a Europian survey, the most common organisms in skin and soft tissue infections (SSTI) were *S. aureus* (71% cases) with 22% being MRSA.[10, 11]

In a study from Germany, out of 1037 bacteraemic episodes in children over 10 years, Gram-positive bacteria accounted for two third of all episodes in paediatric patients.[12] In another study from UK, out of 131 episodes of blood stream infection in a paediatric ICU over a period of 3 years, 63% was because of Gram-positive organisms.[13] In our set up, bacteraemia due to Gram positive cocci have been isolated in 57(31%) cases over a period of one year which is corresponding with above mentioned studies. However, our finding is in contrast to one Indian study [7 (4.24%)].[1]

Coagulase Negative Staphylococci (CONS) form a part of the normal commensal flora. To know the pathogenic potential, speciation of CONS is necessary. Out of total 184 clinically significant samples 34 (18.48%) were CONS. Among the CONS, the predominating isolate was *Staphylococcus haemolyticus* 15 (44.12%), followed by *Staphylococcus epidermidis* 10 (29.41%) [Fig1, Table 2]. This corresponds to other findings for *S. epidermidis,* 30.72%[14] and 44.8%[5]. Isolation rate of *Staphylococcus haemolyticus* 23.84%[14] and 19.7%[5] are not corresponding to our findings. Out of total 15 isolates of *Staphylococcus haemolyticus,* 9 (60%) isolates were from pus or wound swab, followed by blood 4(11.76%).[Table 2] This is almost similar to another study, *Staphylococcus haemolyticus,* 6 (13%) in blood and 7 (7.3%) in skin infection.[15]

Our study shows isolation rate of MRSA by cefoxitin disc diffusion was 99 (66%).[Table 3] This is similar to the study done by R. Kaur[4] in which out of 97 *S. aureus* strains, 53 (56.64%) were MRSA. The study done by INSAR[16] also shows similar pattern of resistance, 42 % in 2008 and 40% in 2009. The prevalence of MRSA varies between regions and between hospitals in the same region as seen in a study from Delhi, where the MRSA prevalence in nosocomial SSTI varied from 7.5 to 41.3 % between three tertiary care teaching hospitals.[16] The cause of varied prevalence rate of MRSA depends on multiple factors like proper sample collection, monitoring of infection control protocol implementations like hand hygiene protocol, barrier nursing or isolation policy, antibiotic policy of the hospital, prophylaxis policy protocol etc.

In our study, isolation rate of MRSA as per PBP2 a latex agglutination test was 102 (68%) [Table 3]; this is similar to findings of other studies, 42.4%[17] and 45.36%[4].

In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%.[Table 3] This is similar to study (sensitivity 100%, specificity 96.23% and NPV 100%)[4] but dissimilar to other studies (sensitivity 92% and specificity 98%)[3] and (sensitivity 90.9% and specificity 98.2%).[17]

Authors revealed in their study that low level Oxacillin resistance was detected better by Cefoxitin DD test.[18]

PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. [Table 3] This is in concordance with 97.6% sensitivity with this assay.[19] In one study, the authors have mentioned PBP2a latex agglutination 100% correlation with the oxacillin MIC which is almost similar with our finding.[20] Our finding is in contrast to another finding, sensitivity 100%, specificity 100% and NPV 100%[4].

Limitations of the Study

The limitation of the present study is that it *mec* A gene could not be detected among the isolates.

V. Conclusion

To know the prevalence of Gram positive cocci, *Staphylococcus aureus* along with MRSA in a hospital set up is an urgent need so that the spread of resistant strains can be controlled in that environment. Speciation of CONS, mainly in immunocompromised patients helps us to learn about diversity, epidemiological pattern and virulence. Correlation with patient's clinical status adds to the diagnosis. Proper quality control of the microbiological testing methods including Gram's staining to check the arrangements of Gram positive cocci, agglutination in coagulase testing, 0.5 Mac Farland Standard during Antimicrobial Susceptibility testing and measuring zone sizes according to CLSI guideline taking ATCC strains as control should not be subjective. Standardisation in each step can detect the resistant strains bythese fast and effective methods which are easily available and applicable without having the facility of detection of *mec*A gene.

Conflict of interest: None

References Références Referencias

1. Manju M Pillai, Ragunathan Latha, Gautam Sarkar. Detection of Methicillin Resistance in Staphylococcus Aureus by Polymerase Chain Reaction and Conventional Methods: A

© 2020 Global Journals

Comparative Study. J Lab Physicians. 2012 Jul-Dec; 4(2): 83–88.
2. Stephen T Odonkora, Kennedy K Addob. Evaluation of Three Methods for Detection of Methicillin Resistant Staphylococcus aureus (MRSA). Int J Biol Med Res. 2011; 2(4): 1031 – 1034.
3. Mimica M.J.,Berezin E.N., Carvalho R.L.B., Mimica I.M., Mimica L.M.J., Sáfadi M.A.P., Schneider E., Caiaffa-Filho H.H. Detection of methicillin resistance in *Staphylococcus aureus* isolated from pediatric patients: is the cefoxitin disk diffusion test accurate enough? Brazilian Journal of Infectious Diseases. 2007 August; 11(4).
4. Kaur R, Oberoi L, Aggarwal A. Comparative Evaluation of Latex agglutination method with the phenotypic methods for detection of Methicillin Resistant Staphylococcus aureus. IJMM 2012; 30:252-3.
5. Surekha.Y.Asangi, Mariraj.J, Sathyanarayan.M.S , Nagabhushan, Rashmi. Speciation of clinically significant Coagulase Negative Staphylococci and their antibiotic resistant patterns in a tertiary care hospital. International Journal of Biological & Medical Research. 2011; 2(3): 735-739.
6. National Committee for Clinical Laboratory Standard. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. M100-S16. Wayne, PA: NCCLS; 2006.
7. Mohammad Zeeshan, KausarJabeen, Erum Khan, Seema Irfan, Saira Ibrahim, ZebaParween and AfiaZafar. Comparison of Different Phenotypic Methods of Detection of Methicillin Resistance in *Staphylococcus aureus*with The Molecular Detection of *mec*-A Gene. JCPSP 2007, Vol. 17 (11): 666-670.
8. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute. M100-S25.2015, Vol 35 (3): 65-70.
9. Amilcar F. Cardona, Samuel E. Wilson. Skin and Soft-Tissue Infections: A Critical Review and the Role of Telavancin in Their Treatment. *Clinical Infectious Diseases*, Volume 61, Issue suppl_2, September 2015, Pages S69–S78.
10. Anant Parasher, Pushpender Khatana. Methicillin resistant Staphylococcus aureus: a review of the present Indian scenario and drug treatment. Int J Adv Med. 2020 May;7(5):860-868.
11. Sader HS, Farrell DJ, Jones RN. Antimicrobial susceptibility of Gram-positive cocci isolated from skin and skin-structure infections in European medical centres. Int J Antimicrob Agents. 2010; 36:28-32.
12. Berner R, Sauter S, Duffner U, Brandis M., Niemeyer CM. Bacteremic episodes in pediatric oncologic patients, especially caused by the Streptococcus viridans group. Clinical Padiatrics. 1998. 210; 256-260.
13. Gray *James, Gossain Savita,Morris P. Kevin* Three-year survey of bacteremia and fungemia in a pediatric intensive care unit. The Pediatric Infectious Disease Journal. 2001. 20(4):416-21.
14. Amita Jain, Astha Agarwal, Raj Kumar Verma, ShallyAwasthi* & K.P. Singh. Intravenous device associated blood stream staphylococcal infection in paediatric patients. Indian J Med Res, August 2011. 134, pp 193-199.
15. Robert H. K. Eng, Cynthia Wang, Audrey Person, Timothy E. Kiehn, Donald Armstrong. Species Identification of Coagulage-Negative Staphylococcal Isolates from Blood Cultures. Journal of Clinical Microbiology. March 1982.p 439-442.
16. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. Indian Journal of Medical Research. 2013. 137; 2; Page: 363-369.
17. Özel G[1], Aslan V, BaharErdem G, Çağatay M, Sencan I, Mert A. Comparison of oxacillin, cefoxitin, ceftizoxime, and moxalactam disk diffusion methods for detection of methicillin susceptibility in staphylococci. Mikrobiyol Bul. April 2011. 45(2); 258-65.
18. M Gupta, NP Singh, A Kumar, IR Kaur. Cefoxitin disk diffusion test - Better predictor of methicillin resistance in *Staphylococcus aureus*.IJMM. 2009; 27 (4): Page: 379-380.
19. Stephen T Odonkor, Kennedy K Addo. Evaluation of Three Methods For Detection of Methicillin Resistant Staphylococcus aureus (MRSA) 2011Int J Biol Med Res. 2011; 2(4): 1031 – 1034.
20. Elizabeth M. Marlowe, Andrea J. Linscott, Meganne Kanatani, and David A. Bruckner. Practical Therapeutic Application of the Oxoid PBP2' Latex Agglutination Test for the Rapid Identification of Methicillin-Resistant Staphylococcus aureus in Blood Cultures. American Society for Clinical Pathology Am J ClinPathol 2002; 118:287-291.

© 2020 Global Journals