ORIGINAL RESEARCH

Comparative Evaluation of Different Laboratory Methods for Detection of Methicillin Resistant Staphylococcus Aureus at Tertiary Care Centre

[1]Ashok Kumar Sharma, [2]Sweta Kumari, [3]Amber Prasad, [4]Kumari Seema

[1]Associate Professor, [2]Junior Resident, [3]Assistant Professor, [4]Research Scientist (VDRL), Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

Corresponding author
Dr. Sweta Kumari, Junior Resident, Department of Microbiology,
Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.
Email: sweta.rims04@gmail.com

ABSTRACT
Aim: An analysis and comparison of the many testing procedures used in the laboratory to identify methicillin-resistant staphylococcus aureus.
Materials and Methods: The research looked at a total of one hundred different Staphylococcus aureus strains. The strains were validated by slide and tube coagulase tests that were conducted with the conventional approach. The oxacillin screen agar was prepared using the direct colony suspension technique, and the turbidity was matched with 0.5 McFarland standards. On Mueller-Hinton agar that contained 5% NaCl and was supplemented with 6 g/ml of oxacillin, the suspension was inoculated. The plates were then kept in an incubator for 24 hours at 37 degrees Celsius. It was determined that any growth that occurred on the plate that contained oxacillin was resistant to methicillin.
Results: For the purpose of this investigation, one hundred different strains of Staphylococcus aureus were used. Oxacillin screen agar, cefoxitin disc diffusion, and oxacillin disc diffusion are the three standard procedures that were carried out. In addition to that, MIC determination was carried out. After that, the MIC determination was contrasted with the traditional testing techniques. It was possible to determine both their sensitivity and specificity. The MIC determination was used as the benchmark for quality control. It was possible to compute the total number of organisms that had MIC at each of the varied antibiotic concentrations. It was discovered that 6 species, or 6% of the total, had a MIC of 64 g/ml. However, the highest number of organisms that demonstrated a MIC of 32 g/ml was 37 (37%). The number of organisms that had MIC values of less than or equal to 0.5, 1,2,4,8, and 16 ranged from 9 (9%), to 2 (2%), to 15 (15%), to 9 (9%), to 10 (10%), and to 12 (12%), respectively. It was discovered that about 15 out of 26 MSSA strains, or 57.69 percent, displayed a higher range MIC of 2.
Conclusion: It was discovered that all three methods were similarly sensitive and specific. We do, however, suggest that any two of these procedures be used in conjunction with one another in order to get the desired level of precision.
Keywords: MRSA; MIC; Oxacillin; Cefoxitin.

INTRODUCTION

Staphylococcus aureus is a kind of gram-positive, non-motile cocci that may sometimes be seen clustered together in a manner resembling grapes. It has been a significant cause of human illness for centuries. Since its discovery by Alexander Fleming, penicillin has been considered nothing less than a wonder medication due to the fact that it has been shown to be extraordinarily successful in the treatment of staphylococcal infections. However, as time went on and the drug was used, the staphylococcus aureus bacteria naturally developed a resistance to the medication. This was primarily due to the adaptable nature of the bacteria, the rampant overuse of antibiotics in the early stages of their development, and the development of beta-lactamase resistance. Since the first report of methicillin-resistant Staphylococcus aureus (MRSA) as a prominent nosocomial pathogen in the 1960s, the number of infections that are caused by this bacterium has been steadily increasing.[1] MRSA refers to any strain of the bacterium Staphylococcus aureus that has become resistant to beta-lactamase antibiotics. These antibiotics include penicillins (such as methicillin, dicloxacillin, nafcillin, and oxacillin), as well as cephalosporins. MRSA is an extremely dangerous form of the infection. It is a significant reason for worry in medical settings, mostly because to the ease with which it may spread and its resistance to antibiotics that are often administered, which can result in delayed wound healing, sepsis, and a prolonged length of stay in the hospital. MRSA has traditionally been associated with patients residing in healthcare facilities such as hospitals and nursing homes; nevertheless, in recent years, outbreaks have been documented among previously healthy members of the population, which has further contributed to an increased awareness of MRSA.[2] It is believed that transiently colonised healthcare personnel are responsible for the majority of the transmission of MRSA from one patient to another, despite the fact that airborne dispersion and transmission via encounters with contaminated surfaces may also play a crucial role. Hand hygiene among healthcare personnel, limitation of antibiotic use, and the diagnosis and isolation of patients who are infected or colonised have been the primary focuses of efforts to reduce the spread of this infection.[3] The accurate diagnosis of MRSA is important not only for the treatment of the infection but also for limiting its spread across the healthcare facility where it was first found. There are a number of different tests, including the latex agglutination MRSA screen test, the quick ATB Staph test, and the automated Viteksystem. Other tests include the oxacillin disc diffusion test, the broth micro dilution test, and the oxacillin disc diffusion test. The sensitivity and specificity of each of these varies. It is clinically crucial to determine quickly whether S. aureus isolates are resistant to methicillin or not, as this is very important for both treatment and requires extensive hygienic precautions to limit the spread of such strains. This is very important for both treatment and requires extensive hygienic precautions.[4] The creation of an altered penicillin-binding protein, PBP2a, which is encoded by the mec gene complex is related with the development of methicillin resistance in S. aureus.[5,6] Genotypic assays that include the identification of the mecA gene by polymerase chain reaction (PCR) are the recommended methods[7,8], although many clinical labs do not find them to be practicable for usage on a regular basis. The high incidence of MRSA infections, on the other hand, highlights the need of developing a foolproof approach for the disease's diagnosis. This strategy need to not only be dependable, but also low-cost and time-efficient as well. Even in the most out of the way parts of the nation, one ought to be able to implement this strategy. Because of this, research was carried out to evaluate the sensitivity and specificity of three traditional techniques, namely oxacillin disc susceptibility, cefoxitin disc diffusion, and the oxacillin screen agar, in relation to the gold standard of MIC (minimum inhibitory concentration) determination.

MATERIALS AND METHODS

The cross-sectional study was conducted in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand (India) during October 2017 to March 2018. The research looked at a total of one hundred different Staphylococcus aureus strains. The strains were validated by slide and tube coagulase tests that were conducted with the conventional approach. The institutional ethics and research committee gave its approval before moving forward with the study. The guidelines provided by the Clinical Laboratory Standards Institute were followed for each and every test (CLSI).

Screening using oxacillin agar: The oxacillin screen agar was prepared using the direct colony suspension technique, and the turbidity was matched with 0.5 McFarland standards. On Mueller-Hinton agar that contained 5% NaCl and was supplemented with 6 g/ml of oxacillin, the suspension was inoculated. The plates were then kept in an incubator for 24 hours at 37 degrees Celsius. It was determined that any growth that occurred on the plate that contained oxacillin was resistant to methicillin.

Oxacillin disk diffusion

The susceptibility testing of the oxacillin disc was carried out in accordance with Clinical Laboratory Standards. In a nutshell, an inoculation was performed on Mueller-Hinton agar using a bacterial solution that had been adjusted to 0.5 McFarland. On top of the Mueller Hinton agar that had been infected, a filter paper disc that contained 1 g of oxacillin was inserted. The plate was kept in an incubator at 37 degrees Celsius for a whole day. A measurement was taken of the diameter of the zone of inhibition. The strain is resistant if the zone of inhibition is greater than 13 and less than 14. In a nutshell, a bacterial solution that had been modified to contain 0.5 McFarland was injected onto Mueller-Hinton agar. The cefoxitin disc diffusion test was carried out using a disc containing 30 micrograms, and the zone diameters were measured. The strain is resistant if the zone of inhibition is smaller than 20, which it always is.

Minimum inhibitory concentration determination

For the determination of the Minimum Inhibitory Concentration, the dilution with agar technique was performed (MIC). A quantitative approach for measuring the minimum inhibitory concentration of the antibiotics, this method has been developed. To prepare the necessary dilutions of the antibiotics, the following steps were taken: A stock solution of the antibiotic to be tested that contained 2000 g/ml of the antibiotic was produced. Using the accepted methods, the solutions were diluted to concentrations of 0.5 g/ml, 1 g/ml, 2 g/ml, 4 g/ml, 8 g/ml, 16 g/ml, 32 g/ml, and 64 g/ml respectively. After that, these antibiotic solutions were mixed with molten Mueller-Hinton agar and let to set for a while. As a result, plates of Mueller-Hinton agar with various doses of antibiotics were generated.

In addition, a control plate that was devoid of any antibiotic was created. After incubating the mixture for three to four hours, the turbidity was brought up to the 0.5 Macfarlands level after the organisms were added to the nutrient broth. They were incubated at 37 degrees Celsius for 18 to 20 hours after being spot injected onto the surface of the medium. The measurements were obtained at the end of the incubation period. The test plates were read after the control plates that did not contain any antibiotic were examined for signs of growth first.

The minimal inhibitory concentration (MIC) was determined to be the concentration at which growth was totally halted. The organisms that had a MIC of 4 or above were classified as MRSA, whereas those that had a MIC of 4 or below were classified as MSSA. On the basis of the MIC cut off, the organisms were then categorised as sensitive, intermediate, or resistant.

RESULTS

For the purpose of this investigation, one hundred different strains of Staphylococcus aureus were used. Oxacillin screen agar, cefoxitin disc diffusion, and oxacillin disc diffusion are the three standard procedures that were carried out. In addition to that, MIC determination was carried out. After that, the MIC determination was contrasted with the traditional testing techniques. It was possible to determine both their sensitivity and specificity. The MIC determination was used as the benchmark for quality control. It was possible to compute the total number of organisms that had MIC at each of the varied antibiotic concentrations (Table 1). It was discovered that 6 species, or 6% of the total, had a MIC of 64 g/ml. However, the highest number of organisms that demonstrated a MIC of 32 g/ml was 37 (37%). The number of organisms that had MIC values of less than or equal to 0.5, 1,2,4,8, and 16 ranged from 9 (9%), to 2 (2%), to 15 (15%), to 9 (9%), to 10 (10%), and to 12 (12%), respectively. It was discovered that about 15 out of 26 MSSA strains, or 57.69 percent, displayed a higher range MIC of 2.

Total MSSA=26(26%), Total MRSA=74 (74%). All of the approaches discovered 74 (74%) MRSA and 26 (26%) MSSA out of the 100 different strains of Staphylococcus aureus that were tested. This was in agreement with the findings that were obtained from the MIC determination. Therefore, it was discovered that all three techniques, oxacillin screen agar, cefoxitin disc diffusion, and oxacillin disc diffusion, exhibited sensitivity and specificity levels of one hundred percent respectively. Table 2.

Table 1: MIC values and susceptibility pattern of staphylococcus aureus strains

Minimum inhibitory concentration value (μg/ml)	Number and percentage of Staphylococcus aureus	Type of Staphylococcus aureus
0.5	9	MSSA
1	2	MSSA
2	15	MSSA
4	9	MRSA
8	10	MRSA
16	12	MRSA
32	37	MRSA
64	6	MRSA

Table 2: Sensitivity and specificity

Name of the test	Number of sensitive strains	Number of resistant strains	Sensitivity	Specificity
Cefoxitin disk screening	26	74	99	99
Oxacillin disk susceptibility	26	74	99	99
Oxacillin screen agar	26	74	99	99
MIC determination	26	74	99	99

DISCUSSION

The detection of mecA by PCR has emerged as the most reliable method for identifying MRSA in recent years. They examined a variety of additional approaches as potential replacements for PCR in this investigation.[9] In the current investigation, the minimum inhibitory concentration (MIC) was deemed to be the gold standard, and a comparison was made between cefoxitin disc diffusion, oxacillin disc diffusion, and oxacillin screen agar. In the process of identifying MRSA, it was discovered that each of the approaches had the same level of sensitivity as well as specificity. The sensitivity and specificity of the cefoxitin disc

diffusion technique was reported to be 97.3% and 100%, respectively, among 1,611 S. aureus isolates by Broekeme et al. The sensitivity and specificity of the cefoxitin disc diffusion test, on the other hand, were both 99% in the current investigation.[10] In this particular research endeavour, MIC demonstrated a sensitivity and specificity of around 99%, respectively. This was quite comparable to the findings that were achieved in a research that was carried out by Rahbar and colleagues, where the sensitivity and specificity of the MIC strip test were both 100%.[11] Cefoxitin disc diffusion can be a good alternative to molecular methods due to its low cost for clinical laboratories, as found in a study that was conducted by Mohammad Reza Pourmand and his colleagues.[12] The study found that cefoxitin disc diffusion had high sensitivity and specificity when compared with mecA PCR. This was quite comparable to the findings that we acquired from our research, which revealed that the cefoxitin disc diffusion technique had a sensitivity and specificity of one hundred percent; nevertheless, the gold standard that we employed was the MIC determination. According to the findings of a research conducted by Priya Datta and colleagues, another approach, ideally latex agglutination, should also be used consistently in hospitals to identify MRSA. This method should be used in addition to cefoxitin disc diffusion. For the most accurate results, we found that using any two of the following three procedures in combination produced the best outcomes: oxacillin disc diffusion, cefoxitin disc diffusion, and oxacillin screen agar.

CONCLUSION

In order to find an effective approach for the diagnosis of MRSA, this research was conducted on a total of one hundred different strains of Staphylococcus aureus. After carrying out the three traditional methods—namely, the cefoxitin disc susceptibility test, the oxacillin disc diffusion test, and the oxacillin screen agar test—a comparison was made with the lowest inhibitory concentration determination, which was regarded as the gold standard. The findings that were obtained by using cefoxitin disc susceptibility, oxacillin disc diffusion, and oxacillin screen agar corresponded well with those that were acquired by using MIC determination. It was discovered that all three methods were similarly sensitive and specific. We do, however, suggest that any two of these procedures be used in conjunction with one another in order to get the desired level of precision. This will improve the dependability of the final output.

REFERENCES

1. Gold, H. S., and R. C. Moellering, Jr.1996. Antimicrobial-drug resistance. N. Engl. J. Med. 335:1445-1453.
2. Joel W. Beam, Bernadette Buckley. Community Acquired Methicillin-Resistant Staphylococcus aureus: Prevalence and Risk Factors. Journal of Athletic Training. 2006; 41:337-340.
3. B S Cooper, S P Stone, C C Kibbler, B D Cookson, J A Roberts, G F Medley, S Ebrahim Isolation measures in the hospital management of methicillin resistant Staphylococcus aureus (MRSA): systematic review of the literature BMJ.2004:98
4. Araj GF, Talhouk RS, Simaan CJ, et al. Discrepancies between mecA PCR and conventional tests used for detection of methicillin resistant Staphylococcus aureus. Int J Antimicrob Agents,1999; 11: 47- 52.
5. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J Evaluation of MRSA screen, a simple anti-PBP- 2a slide latex agglutination kit, for rapid detection of methicillin resistance in Staphylococcus aureus. J Clin Microbiol.1999; 37:1591–1594.
6. Gerberding JL, Miick C, Liu HH, Chambers HF (Comparison of conventional susceptibility tests with direct detection of penicillin binding protein 2a in borderline

oxacillin-resistant strains of Staphylococcus aureus. Antimicrob Agents Chemother.1991; 35:2574–2579.

7. Thean YT. A comparison of PCR detection of mecA with two standard methods of oxacillin disk susceptibility testing for coagulase negative staphylococci. J Med Microbiol.2002; 51:83–85.

8. Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M. Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin resistant Staphylococcus aureus. Antimicrob Agents Chemother 1992; 36: 6–9

9. Bhutia KO, Singh TS, Biswas S, Adhikari L. Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in Staphylococcus aureus. Int J Appl Basic Med Res. 2012;2: 84–91.

10. Broekema NM, Van TT, Monson TA, Marshal SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in Staphylococcus aureus in a large-scale study. J Clin Microbiol. 2009;47:217–219.

11. Mohammad Reza Pourmand, Sepideh Hassanzadeh, Rahil Mashhadi, and Emran Askari. Comparison of four diagnostic methods for detection of methicillin resistant staphylococcus aureus Iran J Microbiol. 2014: 6(5):341-344

12. Priya Datta, Neelam Gulati, Nidhi Singla, Hena Rani Vasdeva, Kiran Bala, Jagdish Chander and Varsha Gupta. Evaluation of various methods for the detection of methicillin-resistant Staphylococcus aureus strains and susceptibility patterns. Journal of Medical Microbiology. 2011; 60:1613–1616