VOLUME-6 | ISSUE-2 | FEBRUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

RAPID SCREENING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM CONJUNCTIVAL REGION

Microbiology

KEYWORDS: MRSA, blood agar, CHROM agar, conjunctival region

Asokan S — Assistant professor, Department of Microbiology, Annai College of Arts and Sciences, Kumbakonam, Tamilnadu, India-612 503.

Jayanthi N — PhD scholar, Department of Microbiology, Marudupandiyar College of Arts and Sciences, Thanjavur, Tamilnadu, India-613 403.

ABSTRACT

This study investigated that the screening of methicillin resistant *staphylococcus aureus* from eye swabs were collected from eye care hospital, Trichy District, Tamilnadu, India. Two hundred of eye swabs were collected from the conjunctival region of infected patients using conjunctival swab. The swab samples were cultured and incubated at 37^{0}C for twenty four hours. *Staphyloccus aureus, Staphylococcus epidermis, Streptococcus pneumonia, Pseudomonas aeruginosa, Streptococcus pyogens, Haemophilus influenza, Bacillus subtilis, Proteus vulgaris, Escherichia coli, Klebsiella pneumonia* were identified in the eye swabs. The *staphylococcus aureus* was the most abundant species (55%) isolated from eye swab. Total Staphylococcus species were subjected into screening of MRSA used CHROM agar with oxacillin 4mg/l Mannitol Salt agar (MSA-4 OX) with oxacillin 4mg/l, Blood agar (BA) with oxacillin 2mg/l, Muller Hinton Agar (OX-MA agar) with 4% Nacl, Oxacillin 6 mg/l and Muller Hinton Agar with cefoxitin disk 30 µg. Our study revealed that 50% of the *S.aureus* isolates were MRSA. CHROM agar with oxacillin 4mg/l proved to be more sensitivity and specificity than other MRSA selective media. These provide an alternative for the detection of MRSA in clinical laboratories especially when PCR is unavailable.

INTRODUCTION

Staphylococcus aureus is among the most important and commonly isolated human ocular bacterial pathogens. *Staphylococcus aureus* resistant to methicillin usually resistant to other Beta lactum antimicrobial drugs, are termed as methicillin resistant *staphylococcus aureus* (MRSA). MRSA, first identified in the 1960s, but is now a dominant pathogen in community-associated infections. Methicillin Resistant *Staphylococcus aureus* (MRSA) has caused major problems in hospitals throughout the world (1). Detection of MRSA in clinical samples continues to be important, since infection due to MRSA has high morbidity and mortality rates (2). The isolation, recovery and identification methods used in any routine clinical laboratory are crucial for the detection of MRSA. The introduction of molecular methods (e.g., PCR) for the detection of MRSA has improved the reporting time of MRSA and utilization of infection control resources (3–5). However, not all laboratories can justify the added expense of molecular methods.

Numerous publications outline methods that include solid agar media, with or without prior broth enrichment, for the detection of MRSA from screening samples. Enrichment media are used to enhance the detection of MRSA by overnight incubation before plating on solid agar (6). Most media contain an indicator to distinguish *Staphylococcus aureus*, inhibitory substances to aid the selection of *Staphylococcus aureus* from other organisms and methicillin, oxacillin or, more recently, cefoxitin to select methicillin-resistant strains. Inhibitory substances have included NaCl, ciprofloxacin, polymyxin B, aztreonam, tellurite and desferrioxamine (7-11). An another alternative, cost-effective approach is the use of chromogenic media for screening of MRSA (12).

Detection of MRSA is based on phenotypic characterization of bacterial isolates. In most cases, phenotypic methods are faster and easier than genotypic methods, while genotypic methods may have better accuracy and precision (13). Phenotypic methods include broth microdilution, agar dilution, the agar screening method, disk diffusion, and latex agglutination methods (14). This study was performed to determine the relative importance of the agar screening methods.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF BACTERIA

The two hundred conjunctival samples were collected using swab from patients of eye care hospital,Trichy District, Tamilnadu, India from May 2015 to October 2015 by an ophthalmologist and technicians working in this hospital. Swab is directly inoculated onto previously prepared blood agar (aerobic incubation), Chocolate agar (5-10% CO_2) and Brucella Blood Agar (anaerobic). After incubation for 24 hours, some distinct colonies resembling the selected bacteria were picked and further subcultured three times on various specific media of MacConkey agar, Mannitol salt agar, Haemophilus selective agar medium and Robertson cooked meat medium so as to obtain pure colonies. After incubation the growth from culture plates were picked and subjected into Gram staining, colony morphology, motility and biochemical tests using standard procedure.

SCREENING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (CLSI,2012)

The following sensitivity media with appropriate antibiotic supplement was added and inoculated with the various strains of *Staphylococcus aureus* for the isolation of the MRSA.

*CHROM agar with oxacillin 4 mg/l
*Mannitol Salt agar (MSA-4 OX) with oxacillin 4mg/l.
* Blood agar (BA) with oxacillin 2mg/l
*Muller Hinton Agar (OX-MA agar) with 4% Nacl, oxacillin 6mg/l.
* Muller Hinton agar with cefoxitin disk 30 µg

The isolates of *Staphylococcus aureus* were inoculated on the above mentioned selective media plates. The plates were kept in incubation at 37^{0}C, in an inverted position for 24 to 48 hrs. After incubation, the plates were observed for growth.

RESULT

Two hundred of eye swabs were collected from the conjunctival region of infected patients using conjunctiva swab. The swab samples were cultured and incubated at 37^{0}C for twenty four hours. *Staphyloccus aureus, Staphylococcus epidermis, Streptococcus pneumonia, Pseudomonas aeruginosa, Streptococcus pyogens, Haemophilus influenza, Bacillus subtilis, Proteus vulgaris, Escherichia coli, Klebsiella pneumonia* were identified in the eye swab using various microbiological and biochemical methods. The *staphylococcus aureus* (55%) was the most abundant species isolated from eye swabs. Staphylococcus species were identified in 110 out of 200 eye swabs. Total 110 Staphylococcus species were subjected into a screening of MRSA is using CHROM agar with oxacillin 4mg/l, Mannitol Salt agar (MSA-4 OX) with oxacillin 6mg/l, Blood agar (BA) with oxacillin 2mg/l, Muller Hinton Agar (OX-MA agar) with 4% Nacl, Oxacillin 4mg/l and Muller Hinton Agar with 30 (µg) cefoxitin disk.

The MRSA was detected in 55 out of 110 *Staphylococcus aureus*

isolates. Thus, the frequency of detection of MRSA was 50% from total *Staphylococcus aureus*. CHROM agar proved to be more sensitivity and specificity than other MRSA selective media.

DISCUSSION

Staphylococcus aureus is one of the common causes of nosocomial and community acquired infections with high mortality and morbidity (15). Increase in methicillin resistance among Staphylococci has posed great difficulty in managing such infections (16). Hence, an accurate and rapid detection of methicillin resistance is essential not only to choose appropriate antibiotic but also to control the spread of MRSA (17). Many phenotypic methods to detect MRSA have been developed, but they are slow and vary in sensitivity and specificity (18). Currently, detection of mec-A gene by PCR is the gold standard for MRSA identification (19). However, use of molecular methods for routine practice is not affordable to many resource constraint laboratories. Therefore, it is essential to develop a rapid, accurate and sensitive phenotypic method for detection of MRSA (17). Our study revealed that 50% of the *Staphylococcus aureus* isolates were MRSA. The results were comparable to the studies carried out by others (20-22). CHROM agar proved to be more sensitivity and specificity than other MRSA selective media. Several workers have also reported higher sensitivity and specificity of CHROM agar than other MRSA selective media (23).

CONCLUSION

From this present study, we concluded that the CHROM agar medium is a reliable method for early detection of MRSA from *Staphylococcus aureus* species. CHROM agar proved to be more sensitivity and specificity than other MRSA selective media. These provide an alternative for the detection of MRSA in clinical laboratories, especially when PCR is unavailable.

REFERENCE

1. Waldvogel, F.A. (1995), Staphylococcus aureus (including toxic shock syndrome), p. 1754-1777. In G.L. Mandell, J.E.Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases, 4th ed,Churchill Livingstone, New York,
2. N.Y. Wertheim, H., Verbrugh, H, A., Pelt, C., Man, P., Belkum, A., & Margreet C. V. (2001), Improved detection of methicillin-resistant Staphylococcus aureus using phenyl mannitol broth containing Aztreonam and Ceftizoxime. J. Clin.Microbiol. 39(7): 2660-2662.
3. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966), Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.
4. Blanc, D. S., Wenger, A., & Bille, J. (2003), Evaluation of a novel medium for screening specimens from hospitalized patients to detect methicillin resistant Staphylococcus aureus. J. Clin. Microbiol. 41 (8): 3499-3502.
5. Brown, D. F. J., & Walpole, E. (2001), Evaluation of the Mastalex latex agglutination test for methicillin resistance in Staphylococcus aureus grown on different screening media. J. Antimicrob. Chemother. 47: 187-189.
6. Derek F. J. Brown (2005), Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA) Journal of Antimicrobial Chemotherapy (2005) 56, 1000–1018
7. Safdar N, Narans L, Gordon B et al., (2003), Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant Staphylococcus aureus: a prospective study comparing 32 methods. J Clin Microbiol 41: 3163–6.
8. Zadik PM, Davies S, Whittaker S et al.,(2001), Evaluation of a new selective medium for methicillin-resistant Staphyloccocus aureus. J Med Microbiol 50: 476–9.
9. Monsen T, Olofsson C, Granstrom et al.,(2003), A preliminary evaluation of a new selective agar supplemented with desferrioxamine for detection of methicillin-resistant Staphylococcus aureus. J Hosp Infect 54: 216–21.
10. Apfalter P, Assadian O, Kalczyk A et al.,(2002), Performance of a new chromogenic oxacillin resistance screen medium (Oxoid) in the detection and presumptive identification of methicillin-resistant Staphylococcus aureus. Diagn Microbiol Infect Dis 4: 209–11.
11. Davies S, Zadik PM, Mason CM et al.,(2000), Methicillin-resistant Staphylococcus aureus: evaluation of five selective media. Br J Biomed Sci 57: 269–72.
12. Blanc DS, Wenger A, Bille J (2003), Evaluation of a novel medium for screening specimens from hospitalized patients to detect methicillinresistant Staphylococcus aureus. J Clin Microbiol 41: 3499–502.
13. Perry JD, Davies A, Butterworth LA et al.,(2004), Development and evaluation of a chromogenic agar medium for methicillin-resistant Staphylococcus aureus. J Clin Microbiol 42: 4519–23.
14. Kluytmans J, van Griethuysen A, Willemse P et al.,(2002), Performance of CHROMagar selective medium and oxacillin resistance screening agar base for identifying Staphylococcus aureus and detecting methicillin resistance. J Clin Microbiol 40: 2480–2
15. Carrol KC (2008), Rapid diagnostics for methicillin- resistant Staphylococcus aureus. Mol Diag Ther. 12:15–24.
16. Brown DF, Edwards DI, Hawket PM, Morrison D, Ridgway GL, Tower KJ, et al.,(2005), Joint Working Party of the British Society for Antimicrobial Chemotherapy, Hospital Infection Society; Infection Control Nurses Association. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant Staphylococcus aureus. J Antimicrobial Chemother. 56:1000–18.
17. Mandell LA, Wunderink R (2012), Methicillin-Resistant Staphylococcus aureus and Community-Acquired Pneumonia: An Evolving Relationship. Clin Infect Dis. 54(8):1134-36
18. David MZ, Daum RS (2010), Community-Associated Methicillin-Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic Clin Microbiol Rev. 23(3):616–87.
19. Pillai MM, Latha R, Sarkar G (2012), Detection of Methicillin Resistance in Staphylococcus aureus by Polymerase Chain Reaction and Conventional Methods: A Comparative Study. J Lab Physicians. 4(2):83–88
20. Bhutia KO, Shantikumar Singh T, Biswas S, Adhikari L (2012), Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in Staphylococcus aureus. Int J Appl Basic Med Res. 2(2):84–91.
21. Ahmad MK, Asrar A (2014), Prevalence of Methicillin Resistant Staphylococcus aureus in pyogenic community and hospital acquired skin and soft tissues infections. J Pak Med Assoc.64(8):892-95.
22. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP (2013), MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. PLOS ONE. 8(7):1-12. | www. plosone.org
23. KV Vaerenbergh, R. Cartuyvels ,G Coppens, J Frans, A Abeele, H Beenhouwer (2010) BILULU Group Performance of a New Chromogenic Medium, BBL CHROMagar MRSA II (BD), for Detection of Methicillin- Resistant Staphylococcus aureus in Screening Samples. J Clin Microbiol . 2010; 48(4) : 1450 1451