ORIGINAL ARTICLE

DETECTION OF MRSA NASAL CARRIAGE IN HOSPITAL POPULATION USING CHROMAGAR

FATIMA S.,[1] SALEEM S.,[2] ABBASI M. H.[3] AND HAMAN A.[4]
[1,2]*Department of Microbiology, University of Health Sciences*
[3]*Cell and Molecular Biology Lab, Department of Zoology, University, of Punjab, Lahore – Pakistan*

Abstract

Background and Objectives: Methicillin-resistant Staphylococcus *aureus (MRSA) is an important human pathogen and normally colonize in body parts including skin, nose, inguinal folds, perineum and throat and accounts for nearly two-third of all* S.aureus *infections in hospitals.* MRSA *is resistant not only to all β-lactam group but also other antibiotics including aminoglycosides, tetracycline and macrolides. The study was undertaken for the rapid detection of MRSA utilizing CHROMagar MRSA (CMRSA).*

Methods: Two hundred nasal samples were taken with sterile wooden swab, inoculated on CMRSA and Mueller Hinton agar (MHA) with cefoxitin disc (30µg). All the studied samples were taken from 3 days old hospitalized patients.

Results: At 37°C of incubation after 24h, 10.5% of the total studied samples were positive for MRSA while another 12% samples showed positive results with an extended period of incubation upto 48 h on CMRSA plates. Thus a total of 22.5% were positive for MRSA. 6.5% of the total samples showed methicillin resistance confirmed with novobicin disc (30µg) and were identified as Staphyloccocus *epidermidis (MRSE) on MHA with cefoxitin disc after 24h of incubation.*

Conclusion: It is concluded that CMRSA is equal in activity as compared to Mueller Hinton agar with cefoxitin disc which requires at least 3 days of sample processing, so CMRSA can be used for the rapid detection of MRSA without utilization of additional sources.

Keywords: Staphyloccocus aureus; beta-lactam; *Methicillin-resistant* Staphylococcus *aureus (MRSA);* Staphyloccocus *epidermidis; CMRSA; Mueller Hinton agar; Cefoxitin (FOX).*

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a human pathogen that is capable of causing infections of skin and soft tissue, surgical site, respiratory and urinary tract that may results into severe morbidity and mortality.[1,2] It is resistant to all β lactam and other antibiotics like aminoglycosides, tetracycline, macrolides etc.[3] The most important reservoir for its spread are symptomatic/asymptomatic patients and healthcare workers that carry MRSA in their anterior nares.[4,5] Asymptomatic colonization with it leads to high risk of its subsequent infection not only for carrier but to other patients as well.[6] Worldwide, nasal carriage is recommended for screening of MRSA for its appropriate antibiotic selection and timely treatment of asymptomatic carriers.[7]

Screening for MRSA is very important for therapeutic and epidemiological purposes employing (Diederen, 2005) various methods including enriched media, differential solid media and broth culture enhancement media (Bukhari, 2007). Several studies have utilized phenotypic and genotypic methods for rapid identification of MRSA, including direct identification, susceptibility testing, DNA probes, real-time PCR, and immunologic approaches (Flayhart, 2005, Chapin, 2003, Eigner, 2005, Marlowe, 2002 & Paule, 2005). Each method has its own advantages and disadvantages over other conventional techniques like diffusion method are still available and routinely used.[8] *CHROMagar* (CMRSA) is a selective and differential medium used for early detection of MRSA within 24 hrs from clinical specimens including nasal, peri-anal swabs and sputum.[9] MRSA strains grow in the presence of antibiotic (cefoxitin) and after hydrolysis of chromogenic substrate produces blue colored colonies. Growth of other bacteria can be suppressed by the addition of selective agents.[10]

The aim of the current study was to determine the frequency of nasal carriage of MRSA amongst hospital population and comparison of CMRSA with conventional methods for its detection.

Corresponding Author: Dr. Sana Fatima
Department of Microbiology, University of Health Sciences, Lahore
E-mail: sf.koolsun21@gmail.com

METHODS

Materials

Sterile wooden swab (Copan, Italy), CMRSA (Lab M, UK), Blood agar (Oxoid, UK), cefoxitin disc (Oxoid, UK) Mueller Hinton agar (MHA) (Oxoid, UK), Microbank beads (Pro-Lab Diagnostics, UK).

Standard Control Strain

Staphylococcus aureus ATCC 25923 and MRSA ATCC 33591 (obtained from UHS Lahore).

Exclusion Criteria

Patients having treatment with intranasal anti-MRSA ointments and other antibiotics in the last 14 days were excluded from the study.

Ethical Issues

Patients and controls were informed about the study and were explained that their samples were used for the research purpose. A written informed consent was taken from all of the participants before the collection of samples.

Settings

The study was carried out in Department of Microbiology, University of Health Sciences, Lahore, Pakistan from August 2010- January 2011.

Sample Collection

A total of 200 nasal samples for bacterial isolation were taken from the hospital staff as well as patients exceeding 3 days of the hospitalization with sterile wooden swab.

Media Preparation

CMRSA and blood agar plates were prepared according to manufacturers, instructions.(ref.)

Processing of Samples

All samples were separately and evenly spreaded over the plated mediums, CMRSA and blood agar. The inoculated plates were then incubated at 37°C and growth was checked after 24h. CMRSA plates that were negative for any type of growth were given subsequent 24h incubation.

Working stock culture of the pure cultures thus obtained was maintained on nutrient agar slants and the pure strains were incubated with Microbank beads and stored at minus 70°C.

Characterization of the Bacterial Isolates

For determination of morphological and biochemical characteristics, the bacteria were processed as described by Mackie and McCartney, 1996. Based upon their susceptibility to cefoxitin disc (30 µg) in Mueller-Hinton agar plates with 4% NaCl, the bacterial isolates were identified as MRSA after measuring the zone diameter. Zone size ≤ 21mm was considered to be resistant and zone size ≥ 22 mm was considered to be sensitive according to the CLSI 2010 recommendations.

Statistical Analysis

The data was analyzed using SPSS (18.0). Frequencies, percentages and graphs were given for qualitative variables. Diagnostic statistics like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were calculated.

RESULTS

A total of 200 nasal samples were taken from the indoor patients exceeding 3 days of hospitalization irrespective of the age and sex. Samples collected from different wards of sheikh zayed Hospital is given in Table 1. Out of total samples, 142 (71%) were susceptible to

Table 1: *Percentage of MRSA from different wards.*

Wards	*MRSA*	*Staphylococci other than MRSA*	*Total number of samples collected*	*Percentage of MRSA (out of 200 samples)*
Cardiothoracic	2	9	11	1
ICU	3	13	16	1.5
Medical Unit	6	16	22	3
CCU	2	7	9	1
Orthopaedic	2	24	26	1
Urology	5	16	23	2.5
Paediatric	3	7	10	1.5
ENT	2	3	5	1
Surgical	2	6	8	1

Gynaecology	2	10	12	1
Gastroentrology	6	16	22	3
Pulmonology	4	4	8	2
Nephrology	5	2	7	2.5
Hospital staff	1	20	21	0.5
Total	45	155	200	22.5

cefoxitin (30 µg), and were methicillin sensitive *S.aureus* (MSSA).

The samples were inoculated on CMRSA and blood agar. 45; 21 (10.5%) and 24 (22.5%) samples showed positive results for MRSA after 24 and subsequent 48h of incubation at 37°C with blue colored colonies on CMRSA agar plates (Fig. 1) while after 24h of incubation at 37°C on blood agar utilizing cefoxitin disc diffusion method, 45 samples were isolated and identified as MRSA. 7 samples were taken from Nephrology ward out of which 5 samples (71.4%) were positive for MRSA (1% out of total 200 samples). Of total MRSA isolated, one was obtained from hospital staff and the rest 44 were isolated from the patients.

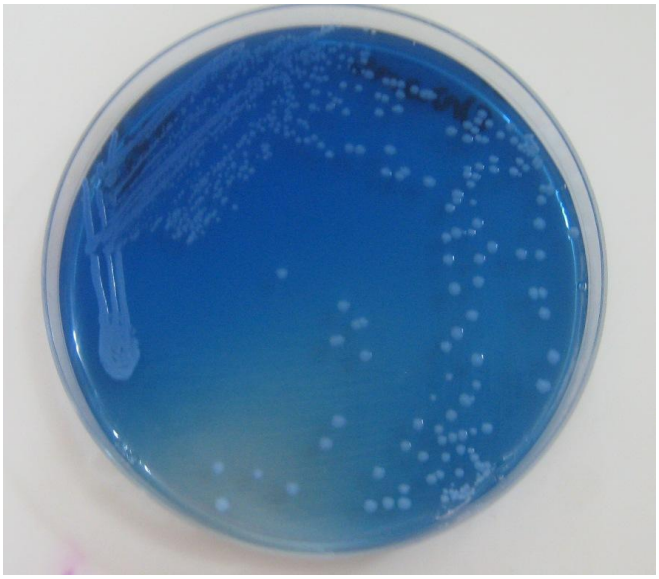

Fig. 1: *Plate of CHROMagar MRSA showing blue colored colonies of MRSA.*

46.7% sensitivity was noted for CMRSA after 24h of incubation while further 24h incubation causes rise in its sensitivity up to 100% (Table 2). 6 cultures were appeared with blue colonies on CMRSA and identified as coagulase negative *Staphylococci* (CoNs). After 48h, 7 more cultures (total 13 i.e. 6.5%) demonstrated blue colonies on same agar plates which were confirmed by Kirby Bauer disc diffusion method according to CLSI 2010 guidelines. The organisms were identified as *S.epidermidis* and were susceptible to novobicin disc (30 µg). Overall sensitivity of CMRSA was noted 100% when excluding blue colonies that were coagulase negative or had a gram stain not consistent with MRSA after 48 h of incubation.

Table 2: *Specificity and sensitivity of MRSA isolation media.*

MRSA Isolation Media	*CHROMagar MRSA*		*MHA with cefoxitin*
	24 hours	*48 Hours*	
Specificity in %age	100	100	100
Sensitivity in %age	46.7	100	100

DISCUSSION

Detection of MRSA from clinical samples is of crucial importance for choosing appropriate antimicrobial therapy and to control MRSA endemicity.[13] The present study was aimed to find out the rapid, reliable, cost-effective and easily applicable method for MRSA detection in routine microbiology laboratory.

CMRSA was found to have improved sensitivity and specificity for the recovery of MRSA within 24-48 hrs. It was also observed that negative cultures (at 24 hrs) when reincubated for another 24 hrs, grew 24 more MRSA (Figure 1). According to *Taguchi et al* in 2003, CMRSA gave 100% specificity and sensitivity to detect MRSA when compared to that of conventional media.[14] *Stoakes et al* in 2006, found that only 3% of colored colonies at 24 hrs gave false-positive results on CMRSA but they were not MRSA whereas in the present study, 6.5% of colored colonies were found to be coagulase negative Staphylococci i.e. Methicllin resistant 5764we456w7 *Staphylococcus epidermidis* (MRSE).[10] *Vaerenbergh et al* in 2010 found CMRSA to be 98.9% sensitive and 89.4% specific at 48 hrs.[15] The results of the above mentioned studies are in accordance with the present study. On the basis of the results, it is suggested that the blue colored colonies on CMRSA must be carefully examined by gram staining, catalase, coagulase and other biochemical tests.

The cost of one plate of CMRSA is more than that of Mueller-Hinton agar plates with cefoxitin disc. However, identification of other colonies that grew on

blood agar plates, need further subculturing and additional biochemical tests. So culturing the specimen on CMRSA from clinical specimens remains economical.

In Pakistan, several independent studies regarding the frequency of MRSA have been reported that show a remarkable difference. In the present study, 22.5% were MRSA, 71% were MSSA and the rest 6.5% were MRSE. Bukhari et al in 2007 reported 27.9% of their isolates as MRSA of all *S. aureus* isolates in a study carried out in King Edward Medical University, Lahore.[16] Khatoon et al reported 38.5% prevalence of MRSA infections in a study carried out at Mayo Hospital Lahore in 2000.[17]

According to another study at Military Hospital Rawalpindi (2006), 42.01% of all nasal staphylococci were MRSA. The samples were taken after 72 hrs of admission. None of the patient had MRSA infection at the admission time.[18] This observation clearly indicates that there is a need to screen all the indoor patients if they stay more than 48 hrs in the hospital.

There is a difference in MRSA isolation frequency in developed parts of world. According to one study conducted in Chicago (2005), 7.9% of all staphylococci were MRSA with high isolation rate from nasal site sample (84% of total isolates).[19] This low frequency is due to their implementation of infection control awareness programmes such as hand washing, gloves, gowns, masks, isolation of MRSA patients and use of suitable disinfectants in clinical practice. It is important that antimicrobial policy should promote that rational use of antibiotics.

It is ***concluded*** that this study showed a high percentage (22.5%) of MRSA nasal carriage in a tertiary care hospital. CMRSA is a chromogenic culture medium with improved sensitivity and specificity for isolation of MRSA for surveillance and screening specimens. CMRSA is useful in the rapid identification of MRSA in 24-48 hrs in a single step directly from clinical specimens thereby reducing detection time without additional susceptibility testing, enhanced recovery of MRSA, suppression of MSSA and other non-MRSA species that might be present in the nose. It is relatively inexpensive as compared to molecular methods. The reduced time of detection of MRSA could facilitate rapid implementation of recommended infection control barrier precautions.

RECOMMENDATIONS

The high prevalence of MRSA should never be ignored and its occurrence demands serious concern. There is an urgent need to adopt basic principles of asepsis and sterilization.

Proper precautionary measures should be followed to prevent MRSA infection.

Implementation of infection control awareness programmes such as hand washing, gloves, gowns, masks, isolation of MRSA patients and use of suitable disinfectants in clinical use will help to reduce the risk factors that associated with the emergence of multidrug resistance.

There should be proper isolation precautions in hospitals.

Blue colored colonies from direct specimen inoculation on CMRSA should be carefully examined using colony characteristics; gram stain and coagulase test (slide and tube both) because other species of Staphylococci, that are resistant to cefoxitin, also grow on it.

Yearly data should be collected from different hospitals to observe the prevalence of MRSA.

Authors' Contribution

SF: Research work, literature review, main write-up.
SS: MHA: Helped in write-up and data analysis.

ACKNOWLEDGMENTS

We express our gratitude to University of Health Sciences, Lahore for fully supporting this research project. We are extremely thankful to Sheikh Zayed Hospital, Lahore for providing clinical samples. We also thank Mr. Muhammad Yasir, Mr. Usman, Mr. Abdul Quddus Tariq, and Mr. Wasim Akhtar for their valuable technical assistance and help.

REFERENCES

1. Foster TJ. The Staphylococcus aureus "superbug." J Clin Invest. 2004; 114: 1693-6.
2. De San N, Denis O, Gasasira MF, De Mendonça R, Nonhoff C, Struelens MJ. Controlled evaluation of the IDI-MRSA assay for detection of colonization by methicillin-resistant *Staphylococcus aureus* in diverse mucocutaneous specimens. J Clin Microbiol. 2007; 45 (4): 1098-01.
3. Bukhari MH, Iqbal A, Khatoon N, Iqbal N, Naeem S, Qureshi GR, Naveed IA. A laboratory study of susceptibility of Methicillin resistant *Staphylococcus aureus* (MRSA). Pak J Med Sci. 2004; 20 (3): 229-33.
4. Skiest DJ, Brown K, Cooper TW, Hoffman RH. Prospective comparison of methicillin-susceptible and methicillin-resistant community-associated *Staphylococcus aureus* infections in hospitalized patients. J Infect. 2007; 54 (5): 427-34.
5. Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. Int J Antimicrob Agents, 2003; 22: 406-19.
6. Flayhart D, Hindler J, Bruckner DA, Hall G. Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *S. aureus* from surveillance cultures of the anterior nares. J Clin Microbiol. 2005; 43: 5536–40.
7. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J et al. Comparison of Community- and Health Care–Associated Methicillin-Resistant Staphylococcus aureus Infection. JAMA. 2003; 290: 2976-84.

8. Eigner U, Weizenegger M, Fahr M, Witte W. Evaluation of a rapid direct assay for identification of bacteria and the mecA and van genes from positive-testing blood cultures. J Clin Microbiol 2005; 43: 5256–5262.
9. Pape J, Wadlin J and Nachamkin I. Use of BBL CHROMagar MRSA medium for Identification of MRSA directly from blood cultures. J Clin Microbiol. 2006; 44: 2575–76.
10. Stoakes L, Reyes R, Daniel J, Lennox G, John M, Lannigan R, Hussain Z. Prospective comparison of a new chromogenic medium, MRSA Select, to CHROMagar MRSA and mannitol-salt medium supplemented with oxacillin or cefoxitin for detection of MRSA. J Clin Microbiol. 2006; 44: 637–39.
11. Duguid JP, Staining Methods. In: Collee JG, Fraser AG, Marmion BP, Simmons A, (editors) Mackie and McCartney Practical Medical Microbiology, New Dehli, Reed India Elsevier Private Limited, 14th edition; 1996: 793-812.
12. Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC et al. Charts. In: Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Ed. Lippincott Williams and Wilkins, 2006; 1442-1535.
13. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E: Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet, 2006; 368: 874-85.
14. Taguchi H, Kaneko T, Onozaki M, Kubo R, Kamiya S. CHROMagar MRSA–Evaluation of a New Chromogenic Medium for Isolation of MRSA. J Jap Assoc Infect Dis. 2004: 54-8.
15. Vaerenbergh KV, Cartuyvels R, Coppens G, Frans J and Beenhouwer HD. 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: 1450-51.
16. Bukhari SZ and Ahmed S. Prevalence of methicillin resistance among *Staphylococcus aureus* isolates in Pakistan and its clinical outcome. J Hosp Infect. 2007; 67: 101-2.
17. Khatoon N, Bukhari MH, Raja RJ. Prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) infection: Laboratory study at Mayo Hospital Lahore. Biomedica, 2002; 18: 49-52.
18. Ali AM, Abbasi SA, Arif S, Mirza IA. Nosocomial infections due to Methicillin Resistant *Staphylococcus aureus* (MRSA) in hospitalized patients. Pak J Med Sci. 2007; 23: 593-6.
19. Lucet JC, Grenet K, Lefevre LA, Harnal M, Bouvet E, Regnier B, Andremont A. High prevalence of MRSA at hospital admission in elderly patients: Implication for infection control strategies. Infection Control and Hospital Epidemiology, 2005; 26: 120-6.