

Detection of MRSA nasal carriage in hospital population using chromagar

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human pathogen and normally colonized in body parts including skin, nose, inguinal folds, perineum and throat. It accounts for nearly two-third of all *S. aureus* infections in hospitals. MRSA is resistant not only to all β -lactam groups but also other antibiotics including aminoglycosides, tetracycline and macrolides. The study was undertaken for the rapid detection of MRSA utilizing CHROMagar MRSA (CMRSA) at Department of Microbiology, University of Health Sciences, Lahore for the period of six months.

Methods: Two hundred nasal samples were taken from 3 days old hospitalized subjects with sterile wooden swab, inoculated on CMRSA and Mueller Hinton agar (MHA) with cefoxitin disc (30 μ g). After 24hr, the colonies were identified on both the agar plates which were then further reincubated for the next 48hr.

Results: At 37°C of incubation after 24hr, 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 48hr on CMRSA plates. Thus a total of 22.5% were positive for MRSA. 6.5% of the total samples showed methicillin resistance confirmed with novobycin disc (30 μ g) and were identified as *Staphylococcus 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 also be used for the rapid detection of MRSA without utilization of additional sources.

Keywords: *staphylococcus aureus*, beta-lactam, methicillin-resistant, *staphylococcus aureus* (MRSA), *staphylococcus epidermidis*, CHROMagar MRSA, 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 In Pakistan, a study was done in 2015 in Abbottabad in which MRSA was found to be 70.8% in the total samples.⁸ Screening for MRSA is done employing various methods including enriched media, differential solid media and broth culture enhancement media.

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. Each method has its own advantages and disadvantages over the others.⁹ CHROMagar (CMRSA) is a selective and differential medium used for early detection of MRSA within 24hrs from clinical specimens including nasal, peri-anal swabs and a sputum.¹⁰ MRSA

strain grow in the presence of antibiotic (cefoxitin) and after hydrolysis of chromogenic substrate produces blue colored colonies. Growth of other microbes can be suppressed by the addition of selective agents.¹¹ 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.

Materials and methods

Materials

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

Standard control strain

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

Exclusion criteria

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

Inclusion criteria

Patients with more than 3 days of hospital stay, doctors and healthcare workers were included in 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 samples were taken from the different wards of Sheikh Zayed Hospital, Lahore and the study period of six months was carried out at the Department of Microbiology, University of Health Sciences, Lahore Pakistan

Sample collection and media preparation

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

Processing of samples

All samples were separately and evenly spreaded over the plated media, CMRSA and blood agar. The inoculated plates were then incubated at 37°C and growth checked after 24hr. CMRSA plates that were negative for any type of growth were given subsequent incubation of 24hr. 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 (30µg) 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>22mm was considered to be sensitive according to the CLSI 2010 recommendations.

Statistical analysis

The data was analyzed using SPSS (19.0). 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 healthcare unit (Figure 1). Out of total samples, 142(71%) were susceptible to cefoxitin (30µg), and were methicillin sensitive Saureus (MSSA). The 45 samples were inoculated on CMRSA and blood agar simultaneously. Of the total MRSA isolated, one was obtained from hospital staff and the rest 44 were isolated from the patients. 7 samples were taken from Nephrology ward out of which 5 samples (71.4%) were positive for MRSA. Among 45 samples, a total of 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 (Figure 2) while after 24hr of incubation at 37°C on blood agar utilizing cefoxitin disc diffusion method the same shown resistance

and identified as MRSA. An increase in sensitivity was noted for CMRSA from 46.7% upto 100% upon twice incubation one after the other (Figures 2) (Figure 3).¹² Blue colonies on CMRSA were identified as coagulase negative Staphylococci (CoNs). The organisms were identified as *S. epidermidis* and were susceptible to novobycin disc (30µg). 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 hr of incubation.

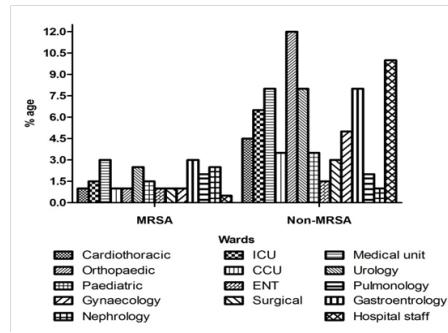


Figure 1 Comparison of Percentages of Methicillin-resistant Staphylococcus aureus (MRSA) with Staphylococci other than MRSA (Non-MRSA) from different wards of hospital.

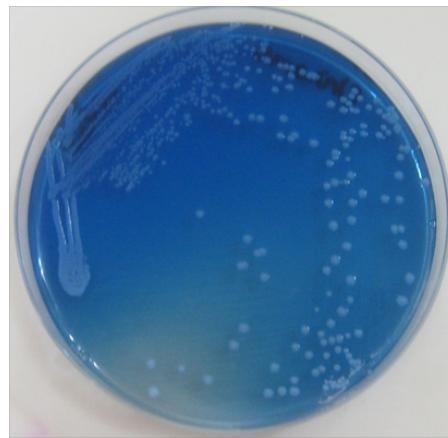


Figure 2 Plate of CHROMagar MRSA showing blue colored colonies of MRSA.

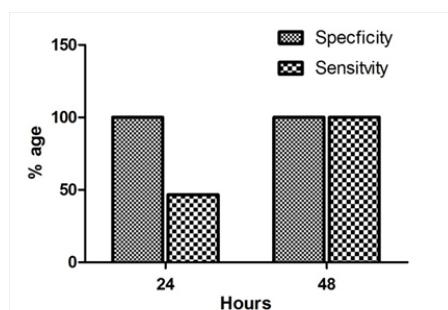


Figure 3 Comparison of specificity and sensitivity of MRSA in CHROM-agar MRSA (CMRSA) after 24 and 48h of incubation.

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 24hrs) when reincubated for another 24hrs, grew 24 more MRSA (Figure 1). CMRSA gave 100% specificity and sensitivity to detect MRSA when compared to that of conventional media.¹⁴ Stoakes et al.¹¹ found that only 3% of colored colonies at 24hrs 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. Methicillin resistant Staphylococcus epidermidis (MRSE).¹¹ Vaerenbergh et al.¹⁴ found CMRSA to be 98.9% sensitive and 89.4% specific at 48hrs.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.¹⁴ In Pakistan, several independent studies regarding the frequency of MRSA have been reported that show a remarkable difference. In our present study, 22.5% were MRSA, 71% were MSSA and the rest 6.5% were MRSE. Bukhari et al.¹⁶ 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 hr 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).^{19,20} 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.

Conclusion

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.

Recommendations

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

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Conflict of interest

The author declares no conflict of interest.

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