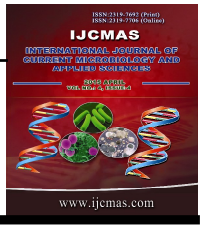
*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 4 Number 4 (2015) pp. 939-947**
**http://www.ijcmas.com**




**Original Research Article**

# Rapid identification of Meticillin Resistant *Staphylococcus aureus* (MRSA) using chromogenic media (BBL™ CHROM agar™ MRSA) compared with conventional methods

**AA Poojary* and LD Bhandarkar**

Department of Pathology and Microbiology, Breach Candy Hospital Trust, Mumbai, India
**Corresponding author*

**Keywords**

MRSA, Chrom agar, rapid identification

**A B S T R A C T**

*Meticillin Resistant Staphylococcus aureus* (MRSA) has become a major infection control challenge within the hospital set up. Adequate infection control measures require rapid turnaround time (TAT) and accurate results in order to optimize hospital resources and reduce the spread of MRSA. This study was undertaken to compare the Time around time (TAT) for the detection of MRSA using chromogenic medium (BBL™ CHROM agar™ MRSA) with conventional methods for different clinical specimens. 246 specimens comprising of nasal swabs, pus samples and blood cultures were analyzed during the study period from September to December 2013. All samples were processed by conventional methods as well as with the use of chromogenic media, BBL™ CHROM agar™ MRSA. MRSA was isolated from 40 (16.26 %) out of 246 specimens. 36 isolates (90%) of the MRSA isolates were identified at 24 hours using the BBL CHROM agar . The remaining 4 isolates (10% ) were identified at 48hours. The overall sensitivity and specificity of the BBL™ CHROM agar™ MRSA when compared with the conventional techniques is 100% and 98.4% respectively. A time study analysis showed time savings of 59.37 hours per month of the technologist's time with the use of BBL™ CHROM agar™ MRSA. BBL™ CHROM agar™ MRSA. medium is a reliable method for rapid identification of MRSA from various clinical specimens and is especially helpful in isolating infected patients and initiate decolonization measures in carriers.

## Introduction

*Meticillin Resistant Staphylococcus aureus* (MRSA) infections pose a major infection control challenge in hospitals. [1] Preventive strategies include strengthening hand hygiene practices, thorough environmental cleaning and disinfection, timely identification of MRSA-colonized or infected patients and their contact isolation. [2] While isolation of infected patients reduces the direct and airborne transmission, screening measures help identify asymptomatic individuals who serve as reservoirs. Screening therefore is a useful technique to identify the reservoir, initiate contact precautions and eradication measures. [3,4] Adequate infection control

measures require rapid turnaround time (TAT) and accurate results in order to optimize hospital resources and reduce the spread. This study was undertaken to determine the utility of a chromogenic agar containing cefoxitin in rapid diagnosis of MRSA carriers and infections.

## Materials and Methods

The study was conducted from September to December 2013. A total of 246 specimens comprising of 109 nasal swab specimens from patients and healthcare workers , 125 pus specimens (swabs and frank pus samples) and 12 positive blood cultures (showing gram positive cocci on smear) were processed using conventional methods and on chromogenic agar (BBL ™ CHROM agar™ MRSA BD Diagnostics, Sparks, MD), hence forth called as BBL CHROM agar. This medium contains 6 mg/l of cefoxitin and inhibitory substances and MRSA are visualized as mauve colored colonies by the presence of a specific chromogenic substrate (proprietary formulation). The medium also contains additional selective agents for the inhibition of Gram-negative organisms, yeasts and other Gram positive cocci.

The conventional methods included inoculation onto Mannitol Salt agar (MSA) (Beckton Dickinson Pvt.Ltd.) for nasal swab specimens. Pus specimens and blood cultures were inoculated onto 5% sheep blood agar (SBA) in Columbia agar base (Beckton Dickinson Pvt.Ltd.) and Chocolate agar and MacConkeys agar (Beckton Dickinson Pvt.Ltd ). All conventional media were prepared in house and were subjected to strict internal quality control using Oxacillin resistant *S.aureus* (ATCC 43300) and Oxacillin sensitive *S.aureus* (ATCC 29213). The control strains were also inoculated onto the BBL CHROM agar prior to its use. For blood cultures only smears which showed gram positive cocci were inoculated on the BBL CHROM agar. Sheep blood agar and Chocolate agar cultures were incubated for 18 to 24 hours in 5% $CO_2$ at 35°C, and MacConkeys agar and BBL CHROM agar cultures were incubated for 18 to 24 hours in ambient air at 35°C. The negative results of the BBL CHROM agar were incubated further for 24 hours as per the manufacturer's instructions and the final report was interpreted at 48 hours. All interpretations done at 48 hours were confirmed with a slide coagulase test as per the manufacturer's instructions. *Staphylococcus aureus* strains isolated were further processed for susceptibility testing using cefoxitin (30µg) disc test as per Clinical Laboratory Standards Institute (CLSI) guidelines.

For nasal specimens, *Staphylococcus aureus* appeared as yellow colored colonies due to fermentation of mannitol on MSA after 24 to 48 hours of incubation. These colonies were then confirmed by Gram stain. A subculture was done onto blood agar to perform additional testing like catalase and coagulase tests. At 48 hours, the isolate was tested for catalase, coagulase then screened for resistance using the Kirby Bauer disc diffusion method with cefoxitin disc (30 µg) as per the Clinical Laboratory Standards Institute (CLSI) guidelines. The results of the cefoxitin disc screening were available after another 24 hours. The BBL CHROM agar was read first after 24 hours of incubation at 35 °C. MRSA were identified on the BBL CHROM agar as mauve colored colonies. (Fig 1) If there were no mauve colored colonies, the plate was further incubated for 24 hours before final interpretation of the result.

From pus specimens, S.aureus was identified by Gram stain, catalase and

coagulase tests. The resistance screen was performed by the Cefoxitin disc method and the Vitek 2 compact. Similarly, positive blood cultures were stained with Gram stain. Those showing gram positive cocci were inoculated onto the conventional media along with the BBL CHROM agar.

**Statistical analysis**

Data analysis was done using standard statistical formulas for sensitivity, specificity, positive predictive value, negative predictive value & Kappa value.

**Results and Discussion**

MRSA was isolated from 40 (16.26 %) out of 246 specimens. 12 (11%) out of 109 nasal swabs, 25 (20%) out of 125 pus specimens and 3 (25%) out of 12 blood culture specimens were MRSA positive . (Fig 2)

The earliest turnaround time (TAT) for MRSA identification with the conventional methods was 48 hours. 12 (30%) isolates were reported at 48 hours, 15 (37.5%) isolates were reported at 72 hours, 8 (20%) isolates after 96 hours and 5 (12.5%) isolates were reported after 120 hours. For nasal specimens and some pus specimens which were mainly polymicrobial in origin, additional subcultures and biochemical testing caused delays in reporting beyond 48 hours and sometimes upto 120 hours. (Fig 3) 36 isolates (90%) of the MRSA isolates were identified at 24 hours using the BBL CHROM agar. The remaining 4 isolates (10% ) were identified at 48hours. (Fig 3)

A time study analysis to compare the supplemental workload for MRSA identification by the two methods was undertaken. (Fig 4)

Technologist's time required for the isolation and identification of the MRSA by both methods was assessed for one week. The processes taken into consideration were Grams stain (140 secs), subculture onto SBA (45 secs), catalase test (10 secs), coagulase test (60secs) and cefoxitin disc (30μg) susceptibility testing (90 secs). This showed that the time taken for the above supplemental tests by the conventional method was 5.75 minutes versus 60 seconds (slide coagulase to be performed as per manufacturer's recommendation) with the BBL CHROM agar method per culture.

The overall sensitivity and specificity of the BBL CHROM agar when compared with the conventional techniques is 100% and 98.4% respectively. (Table 1)The strength of agreement between the two methods as assessed by the kappa value 'κ' was 0.957 indicating very good agreement of the chromogenic medium with the conventional methods.

The emergence and rapid spread of MRSA has posed a formidable infection control challenge over the last four decades. Their ability to colonize human subjects within the community and the healthcare setting serves as an important reservoir in the chain of transmission. The anterior nares are the primary site for colonization, followed by other body sites, such as the perineum, axillae, scalp, throat and rectum. [1,5,6]

A careful assessment of the prevalence of MRSA in the local hospital environment is important to lay down infection prevention strategies as part of the hospital's infection control programme. [7] In our hospital, the annual prevalence of MRSA infections is about 5% to 6% and the Healthcare worker (HCW) carriage rates range from 10% to 12%. Therefore, we have adopted the policy of conducting annual nasal surveillance for MRSA carriers among our staff working in the operation theaters, maternity wards, pediatric wards and the intensive care units.

The knowledge of baseline trends of MRSA carriage allows targeting and strengthening infection control measures in target areas. Active surveillance is also conducted during outbreaks. On the other hand, passive surveillance from clinical specimens serves as a guide towards complete screening and eradication of carriers among infected patients. Decolonization measures include the use of 1% nasal mupirocin ointment along with 4% Chlorhexidine baths for five days followed by repeat nasal specimens for three consecutive days to declare the carrier negative.

In the present study, the earliest TAT for BBL CHROM agar was 24 hours as compared to a minimum of 48 hours with the conventional methods. 36 (90%) MRSA isolates were recovered at 24 hours of incubation. Additionally, two isolates which did not show the typical mauve color were also recovered at 24 hours. These were subjected to gram stain and coagulase test and identified as *Coagulase negative Staphylococcus species (CoNS)* and *bacillus* species. The short TAT of BBL CHROM agar medium was very useful for surveillance specimens as it allowed early institution of contact isolation precautions and treatment of carriers. A study conducted to determine the effectiveness of different infection control measures using rapid MRSA identification techniques found that rapid diagnosis could reduce the need for isolation precautions by > 90% in low endemic settings and by 20% in high endemic settings. [8]

The early identification of MRSA from blood cultures was also useful as it could be interpreted in less than 48 hours after arrival in the laboratory allowing the clinician to make important therapeutic decisions. Other studies have also demonstrated the utility of BBL CHROM agar in being a simple, rapid , highly sensitive and specific assay for detecting MRSA from blood cultures. [9,10] Complication rates in sepsis due to *S.aureus* increase with the duration of bacteremia and delay in appropriate therapy causing increased morbidity and mortality. [11]

Another important finding, with regards to blood cultures observed in this study was the early identification of *Meticillin resistant S. hemolyticus (*MRSH). MRSH is a common nosocomial pathogen in the neonatal intensive care unit (NICU). [12] Two blood cultures from the NICU grew *MRSH* which appeared as pale pink colonies at 24 hours and became dark pink colored colonies at 48 hours. (Fig 5) Both these isolates were reported as *Meticillin resistant Coagulase negative Staphylococcus sp (MRCoNS)* at 24 hours after performing a gram stain and slide coagulase test . In both the cases the therapy was modified to cover a methicillin resistant *Staphylococcus sp* and patients responded favorably. The above observation suggests that BBL CHROM agar may have an important role in optimizing therapy towards other *Meticillin resistant Coagulase negative Staphylococcus sp (MRCoNS)* in certain clinical situations. Therefore the routine use of BBL CHROM agar for blood cultures showing gram positive cocci on microscopy can be used to optimize patient outcomes.

In this study, the overall sensitivity and specificity of the BBL CHROM agar medium was 96% and 99% respectively, at 48 hours. When BBL CHROM agar was compared with Oxacillin MIC (broth microdilution), Oxacillin screen agar , PBP2a latex agglutination, cefoxitin disk diffusion and *mecA* PCR for direct identification of MRSA from nasal specimens , its overall specificity was found to be 99.7%. [13] When compared with the cefoxitin disc diffusion method alone, its the

sensitivity & specificity was found to be 95.0% and 98.1%. [13]

For wound specimens, BBL CHROM agar showed a sensitivity and specificity of 92.1% and 94.6% . [14] In the present study, the sensitivity and specificity of CRHOM agar for pus specimens was 100% and 99% respectively. Though the numbers of blood cultures were small in this study, sensitivity and specificity matched up to two other studies that evaluated the use of BBL CHROM agar for inoculating positive blood cultures. [8, 9] The growth of Methicillin Sensitive S.aureus (MSSA) on chromogenic MRSA ID agar (bioMe´rieux, Marcy l'Etoile, France ) with direct blood culture inoculation has also been observed in one study. [15] This was probably as a result of breakthrough due to the large inoculum present in blood cultures. This phenomenon was not observed in studies that used BBL CHROM agar for direct inoculation of blood cultures. [8, 9] Our study also did not experience any breakthrough growth of MSSA when BBL CHROM agar was used for direct inoculation of positive blood cultures.

False positives due to other organisms like *S. epidermidis, S. hominis, S.caprae and Corynebacterium sp.* have been reported with the use of BBL CHROM agar . [8,16]This study also had three false positive mauve colored colonies; *Bacillus species and S.epidermidis* grew at 48 hours from nasal specimens, but were easily identified by performing the gram stain and coagulase test.

The third false positive isolate from the pus swab was also identified by gram stain and further confirmed by VITEK 2 Compact (Marcy l' Etiole, bioMeriuex, France ) as Acinetobacter sp. Acinetobacter has not been isolated from other studies performed with BBL CHROM agar. [ 8,16]

The increase in infection control awareness due to MRSA has led to an increase in workload of microbiology laboratories in the form of increased staff, space and material requirements. Therefore, MRSA screening methods must be studied with respect to reduction in workload and the reporting time. A significant reduction in the overall workload in the microbiology laboratory was demonstrated with the use of chromogenic media. [17]

The utility of BBL CHROM agar in reducing our workload was also assessed in this study. The processes taken into consideration to signify 'extra workload' were gram stain, subculture onto SBA, catalase test, coagulase test and the cefoxitin screen test. Primary inoculation of the specimens and the time taken for reporting were not taken into consideration. By the conventional methods, supplemental tests needed to confirm MRSA could be accomplished in 5.75 minutes. With BBL CHROM agar, supplemental tests needed one minute taking into consideration the coagulase test as per the manufacturer's recommendation. Thus direct specimen inoculation onto BBL CHROM agar saved 4.75 minutes of the technologist's time. Extrapolated to the 750 specimens processed by my laboratory this amounts to savings of 59.37 hours per month of the technologist's time.

The effectiveness of any screening assay in reducing the cost depends on its sensitivity, specificity and turnaround time (TAT) required for obtaining the results. [17] The cost of BBL CHROM agar was calculated to be Rs.118/- per plate as against the conventional screening methods which came to Rs.75/- (cost of Mannitol salt agar plate, Blood agar plate for subculture, gram stain, catalase test , coagulase test and screening test using cefoxitin ) per nasal specimen.

**Table.1** Comparison of results between conventional media and BBL CHROM agar for all specimens

| Method | Conventional (BAP, Mannitol Salt Agar/Cefoxitin screen | | | |
|---|---|---|---|---|
| BBL CHROM agar | | Positive | negative | Total |
| | Positive | 40 | 3 | 43 |
| | Negative | 0 | 203 | 203 |
| | Total | 40 | 206 | 246 |

**Fig.1** BBL CHROM agar showing mauve colored colonies of MRSA

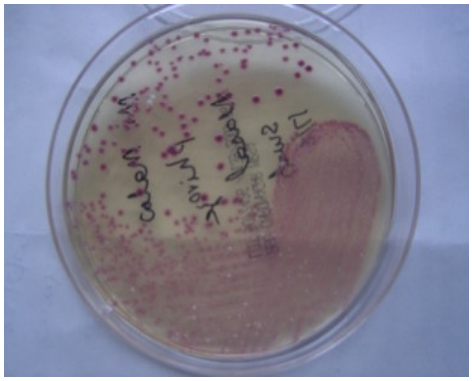


**Fig.2** Distribution of samples in the study

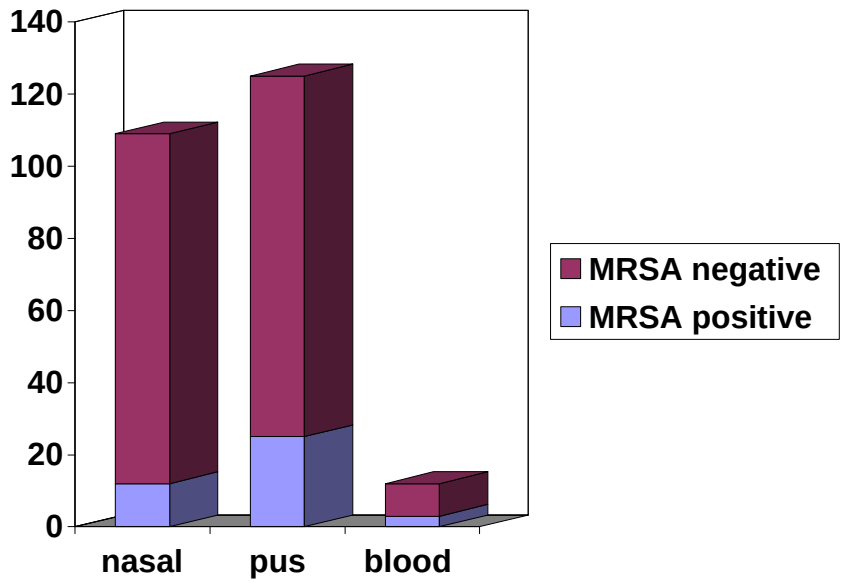


**Figure.3** Comparison of turn around time (TAT) for conventional methods and BBL CHROM agar

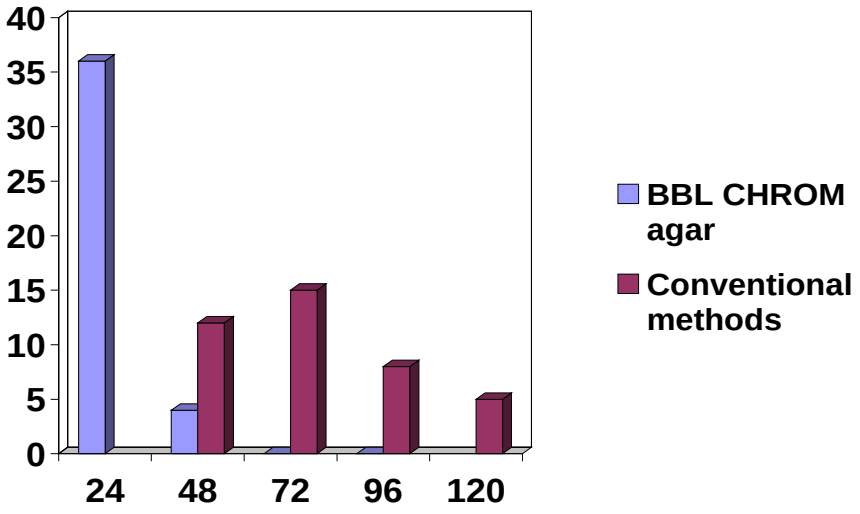
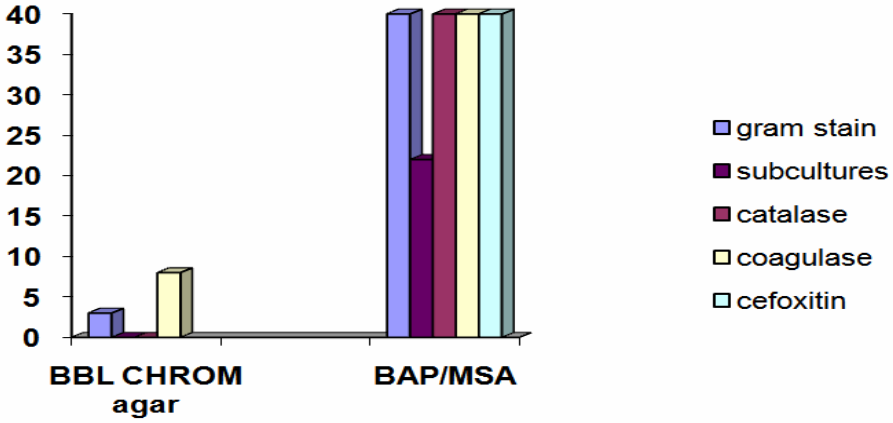



**Figure.4** Comparison of supplemental workload for MRSA identification by the two methods




Despite the increase in the cost per specimen, the time savings of 48 hours with the use of the chromogenic medium goes a long way as a cost saving measure in preventing the control and spread of MRSA outbreaks in the hospital.

The present study has a few shortcomings. Firstly, the sample size of the study was small a larger sample size would be more useful in assessing the utility of BBL CHROM agar. This study compared a selective medium which directly identifies

MRSA with a conventional non selective medium which has no advantage in terms of turnaround time for reporting. Thus there is an obvious selection bias. Though broth enrichment techniques have been recommended [18] for surveillance specimens, the same was not done in this study as this would increase the turnaround time by almost 18 to 24 hours for both the methods. Such prolonged incubation not only leads to delays in reporting but may also reduce the specificity of the media used. [19] No confirmatory method like a latex agglutination assay or a molecular assay was used in this study. The conventional method was considered as the gold standard as the other techniques were either not easily available or unsuitable in the present infrastructure of the laboratory settings.

To conclude, BBL Chrom agar MRSA medium is a reliable method for early detection of MRSA from various clinical specimens. The reduced turnaround time (TAT) for reporting MRSA is especially useful from the infection control point of view to isolate the patient and initiate decolonization measures.

## References


1) H Boucher, L G. Miller, R R Razonable. Serious Infections Caused by Methicillin-Resistant *Staphylococcus aureus. Clin Infect Dis.2010; 51(S2):S183–S197*
2) DJ Diekema, M Climo. Preventing MRSA infections: finding it is not enough. *JAMA 2008; 299:1190–1192.*
3) J. C Lucet , B Regnier. Screening and Decolonization: Does Methicillin-Susceptible *Staphylococcus aureus* Hold Lessons for Methicillin-Resistant *S. aureus*? *Clin. Infect. Dis. 2010; 51(5):585–590*
4) CA Muto, JA Jernigan , BE Ostrowsky et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus. Infect Control Hosp Epidemiol 2003; 24:362–386*
5) HF Wertheim, DC Melles, MC Vos,W van Leeuwen , A van Belkum , HA Verbrugh , JL Nouwen . The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis. 2005; 5(12): 751–762*
6) P Nilsson , T Ripa . *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. *J Clin Microbiol. 2006; 44(9):3334–3339*
7) E. Coia, G.J. Duckworth, D.I. Edwards, M. Farrington, C. Fry, H. Humphreys, C. Mallaghan, D.R. Tucker, for the Joint Working Party of the British Society of Antimicrobial Chemotherapy, the Hospital Infection Society, and the Infection Control Nurses Association. Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus (MRSA)* in healthcare facilities. *J Hosp Infect 2006;63:S1–S44*
8) M. C. J. Bootsma , O. Diekmann, and M. J. M. Bonten. Controlling methicillin-resistant *Staphylococcus aureus:* Quantifying the effects of interventions and rapid diagnostic testing. *Proc Natl Acad Sci U S A. 2006; 103(14): 5620–5625*
9) C. Wendt, N. L. Havill, K. C. Chapin, J. M. Boyce, R. Dickenson, U. Eigner,S. Schu¨tt, A. M. Fahr. Evaluation of a New Selective Medium, BD BBL CHROMagar MRSA II, for Detection of Methicillin-Resistant *Staphylococcus aureus* in Different Specimens. *J Clin Microbiol. 2010; 48(6) : 2223–2227*

10) J Pape, J Wadlin, I Nachamkin. Use of BBL CHROMagar MRSA Medium for Identification of Methicillin-Resistant *Staphylococcus aureus* Directly from Blood Cultures. *J Clin Microbiol. 2006 ; 44(7): 2575–2576*

11) R Khatib, L. B. Johnson, M. G. Fakih, K. Riederer, A. Khosrovaneh, M. S.Tabriz, M. Sharma, S. Saeed. Persistence in *Staphylococcus aureus* bacteremia: incidence, characteristics of patients and outcome. *Scand. J. Infect. Dis. 2006; 38:7–14*

12) O Raimundo , H Heussler , JB Bruhn , S Suntrarachun , N Kelly , MA Deighton , SM Garland . Molecular epidemiology of coagulase-negative staphylococcal bacteremia in a newborn intensive care unit. *J Hosp Infect. 2000; 51(1):33-42*

13) D. Flayhart, J.F. Hindler, D.A. Bruckner, G Hall, R.K. Shrestha, SA. Vogel, S S. Richter, W.H.RWalther, K C. Carroll. Multicenter Evaluation of BBL CHROMagar MRSA Medium for Direct Detection of Methicillin-Resistant *Staphylococcus aureus* from Surveillance Cultures of the Anterior Nares. *J Clin. Microbiol 2005; 43(11): 5536–5540*

14) A T. Harrington, S D. Mahlen, J E. Clarridge Significantly Larger Numbers of Methicillin-Resistant *Staphylococcus aureus* Bacteria Are Recovered from Polymicrobial Respiratory and Wound Sites by Use of Chromogenic Primary Media than by Use of Conventional Culture. *J Clin Microbiol. 2010 ; 48(4) 1350–1353*

15) S Colakoglu, S Aliskan, S. S. Senger, T. Turunc, Y. Z. Demiroglu, H. Arslan Performance of MRSA ID chromogenic medium for detection of methicillin-resistant *Staphylococcus aureus* directly from blood cultures and clinical specimens. *Diagn. Microbiol. Infect. Dis. 2007; 59:319–323*

16) Z Han, E Lautenbach, N Fishman, I Nachamkin Evaluation of mannitol salt agar, CHROMagar Staph aureus and CHROMagar MRSA for detection of meticillin-resistant *Staphylococcus aureus* from nasal swab specimens. *J Med Microbiol. 2007; 56: 43–46*

17) P R. S. Lagace´-Wiens, M J. Alfa, KManickam and G. K. M. Harding Reductions in Workload and Reporting Time by Use of Methicillin-Resistant *Staphylococcus aureus* Screening with MRSASelect Medium Compared to Mannitol-Salt Medium Supplemented with Oxacillin. *J Clin Microbiol. 2008; 46(4) 1174–1177*

18) M Gardam , J Brunton , B Willey ,A McGeer ,D Low , J Conly . A blinded comparison of three laboratory protocols for the identification of patients colonized with methicillin-resistant *Staphylococcus aureus. Infect Control Hosp Epidemiol. 2001 ; 22(3):152-6.*

19) K V Vaerenbergh, R. Cartuyvels ,G Coppens, J Frans, A Abeele, H Beenhouwer 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*