Methicillin Resistant Staphylococcus Aureus Screening in Blood Culture, Simple Anti-PBP2a Slide Agglutination Kit, MRSA Chromagar and Conventional Disc Diffusion Method

Thesis for fulfillment for master degree in Clinical and Chemical Pathology

by

May Mohamed Sherif Soliman

MBBCH, Faculty of Medicine, Cairo University.

Supervisors

Prof. Dr. Afaf Mohamed Sami EL-Banna

Professor of Clinical and Chemical Pathology, Cairo University

Prof. Dr. Iman Kamal Behery

Assistant Professor of Clinical and Chemical Pathology, Cairo University

Prof. Dr. Mervat Gaber Abdel-Wahab

Assistant Professor of Clinical and Chemical Pathology, Cairo University

Cairo University

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"Glory be to You, we have no knowledge except what You have taught us. Verily, it is You, the All-Knower, the All-Wise." *(Al-Bakarah – 32)*

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ABSTRACT

In critically ill patients MRSA bacteraemia was found to have a higher attributable mortality in comparison to MSSA bacteremia. The optimal phenotypic method for detecting methicillin resistance in *S. aureus* remains controversial. This work aimed at evaluating the utility of chromagar directly from positive blood culture bottles for rapid diagnosis of MRSA, and comparing it with MRSA latex test, and disc diffusion method.All positive blood culture samples showing Gram positive cocci in clusters, were included in the study (n=84) and examined further for Staphylococcus aureus content by Slidex, Slide coagulase, Mannitol Salt and DNAse, adopting Slidex test as a gold standard, and for MRSA by Cefoxitin Disc diffusion susceptibility testing, MRSA latex test and MRSA chromagar, with Disc diffusion adopted as the reference method. Concerning S.aureus identification, Coagulase test was the most sensitive (100%), specific (97.4) and accurate (98.8), followed by Mannitol salt test (100%, 94.9%, and 97.6% respectively) then DNAse test (97.7%, 82.1% and 80.4% respectively). As regards MRSA detection, MRSA latex was the most sensitive, specific and accurate relative to our gold standard. MRSA chromagar provided most rapid result delivery time and was the least labour intensive at reasonable cost, despite the slight difference from the gold standard, where it demonstrated 98.1% specificity, 98.8% accuracy. MRSA CHROMagar appears to be dependable for detection of MRSA directly from blood culture bottles, while still being speedy and cost effective. As regards identification of staphylococcus aureus, Slidex remains the gold standard in this domain.

Keywords:

MRSA
Chromagar
PBP2a
Slidex

Contents

List of Abbreviations

- CAMRSA : community acquired methicillin resistant staphylococcus aureus
- CATs : chloramphenicol acetyltransferases.
- $CFUml^{-1}$: colonies forming unit per ml
- CLSI : Clinical and Laboratory Standard Institute
- CoNS : coagulase negative
- CSA : Chromagar staph aureus
- CMRSA : chromagar MRSA
- CVL : central venous line
- G+C : guanine-plus-cytosine
- HA-MRSA: Healthcare-associated MRSA
- ICU : Intensive care unit
- MHA : Mueller-Hinton agar
- MLST :Multilocus sequence typing.
- MRCNS : Methicillin resistant coagulase negative staph
- MRSA : Methicillin-resistant S.aureus
- MSA :Mannitol Salt agar
- MSCNS : Methicillin sensitive coagulase negative staph
- MSSA : Methicillin sensitive S. aureus
- NPV : negative predictive value
- PBP : penicillin binding protein

- PCR : polymerase chain reaction
- PFGE :pulsed-field gel electrophoresis
- PPV : positive predictive value
- PRSA : Penicillin-resistant S aureus
- SCC mec : S.aureus cassette chromosome mec
- TNase : thermonuclease
- TSS : Toxic shock syndrome
- UTI : urinary tract infection
- VISA :vancomycin-intermediate S. aureus

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INTRODUCTION

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AIM OF WORK

In the early 1960s, methicillin resistance appeared among clinical isolates of Staphylococcus aureus. Since then, methicillin-resistant Staphylococcus aureus (MRSA) has become one of the most virulent pathogens in hospitals especially in intensive care units. Identification of MRSA from blood cultures usually takes 1 to 2 days following detection in semiautomated blood culture systems. Since bloodstream infections with MRSA are associated with significant morbidity and mortality, therefore early detection of MRSA in blood cultures plays an important role in patient management ***(Pape et al., 2006).***

A selective and differential medium; Chromagar MRSA medium (C-MRSA) has been evaluated for identifying MRSA accurately from subcultures of positive blood cultures ***(Pape et al., 2006).***

Nearly all MRSA isolates produce an additional penicillin-binding protein; PBP2a. The latter binds β-lactams with a lower affinity than PBP2, the major physiologic methicillin target. PBP2a can be detected by a latex agglutination test; MRSA latex test ***(Felten et al., 2002).***

This work aims at evaluating the utility of chromagar directly from positive blood culture bottles for rapid diagnosis of MRSA, and comparing it with MRSA latex test, and disc diffusion method.

Review Of Literature

HISTORICAL BACKGROUND

Staphylococcus aureus is one of the major health hazards with global presence and impact. In the early turn of the twentieth century, staphylococcal septicemia was associated with high mortality rate until the discovery and propagation of penicillin in the late 1940s and 1950s with the subsequent decline in fatality. However, penicillin-resistant strains were discovered by several investigators thereafter ***(Gardam, 2000).***

It is clear that beta-lactamase-producing strains of S aureus existed before the discovery of penicillin. The majority of isolates responsible for outbreaks of staphylococcal food poisoning before 1940 were, in retrospect, found to be penicillin resistant. The ultimate origin of beta lactamase is yet unsettled ***(Gardam, 2000).*** Despite the fact that its production is commonly induced by the presence of beta-lactam antibiotics, nevertheless, spontaneous production of beta-lactamase has been reported in some strains ***(Rosdahl and Rosendal, 1983)***. The plasmid-based encoding gene is easily transferred between staphylococcal species, both horizontally (between members of the same species that are not in a parent-child relationship) and vertically (from generation to generation within the same species, from parent to child) (***Gardam, 2000).***

Penicillin-resistant S. aureus (PRSA) rose to prominence in the hospital setting in the 1950s and 1960s. PRSA strains were discovered in the community shortly after they were found in hospitals, making hospital control of PRSA essentially meaningless within two decades of the strains' appearance**.** Within

the last decades, over 90% of North American community and hospital isolates of S. aureus have been found to be penicillin resistant ***(Gardam, 2000).***

This widespread can be explained. Bacteria harbouring the resistance plasmid were promoted over sensitive strains during the 1950s and 1960s because of the widespread and indiscriminant use of penicillin. By the 1960s, plasmid-carrying strains essentially replaced penicillin-sensitive strains as 'normal' human flora ***(Gardam, 2000).*** That, in addition to the easy propagation of the plasmid-based resistance mechanism, has contributed to the booming flare of PRSA.

The 1960s were a turning point in infection control, with the evolution of beta-lactamase-resistant penicillins such as methicillin and oxacillin. Beta-lactamase-resistant penicillins had a special impact on control of staphylococcal infections. Unfortunately, within merely a year of their release, resistant S.aureus strains were reported ***(Maranan et al., 1997).*** The origin and the mode of propagation of the DNA-based resistance mechanism are controversial. Some investigators are in favor of vertical transmission and trace back resistance to a single parent strain, while others propose horizontal transmission with the subsequent modification in structure and function.

Kreiswirth et al., (1993) constructed an evolutionary tree for the encoding gene in 450 MRSA strains isolated over 30 years from around the world. These researchers showed that all of the studied isolates could be linked to a single parent strain, indicating that essentially all of the studied MRSA strains arose from a single clone. They concluded that the horizontal transfer of

the mecA gene between staphylococcal species is likely an extremely rare event. On the other hand, ***Mussuer and Kapur (1992)*** constructed a similar evolutionary hierarchy of MRSA isolates but found quite different results. While they concluded that European and northern African isolates were likely derived from a single clone, isolates from North America exhibited considerable diversity, more in keeping with ongoing horizontal acquisition of the encoding gene by S.aureus.

A third group of investigators analyzed 105 MRSA isolates obtained worldwide over a 30-year span and found that MRSA isolates dating from the 1960s had identical sequences, suggesting that they may have arisen from the same clone. Those isolates from the 1970s onwards, however, contained additional DNA sequences and were more heterogeneous, suggesting that they arose independent of the 1960s clone and that horizontal transfer of the encoding gene was likely occurring ***(Archer et al., 1994)***.

Finally, a study by ***(Hiramatsu et al., 1996)*** has again found somewhat different results. These investigators were able to group MRSA isolates into three distinct categories: those isolates prevalent exclusively in Britain; those prevalent in Japan and the United States; and finally, those prevalent in Britain, Europe, and former British colonies in the Middle East and South East Asia. It was concluded that these different categories of MRSA strains appear to have developed independently, thus arguing against a single clonal origin for MRSA. Furthermore, considerable genetic diversity was found within each category supporting the opinion that genetic material is being shared between organisms.