



EFFECTIVENESS OF MRSA DETECTION METHODS IN THE LABORATORY PRACTICE – A BRIEF REVIEW

Neli M. Ermenlieva¹, Tatina T. Todorova², Gabriela S. Tsankova², Tsvetelina K. Popova¹, Emilia P. Georgieva¹

1) Medical College, Medical University Varna, Bulgaria

2) Department of Preclinical and Clinical Sciences, Faculty of Pharmacy, Medical University Varna, Bulgaria

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) are bacteria, responsible for severe and hard-to-manage infections in human. They are resistant to beta-lactam antibiotics - penicillins (methicillin, dicloxacillin, nafcillin, and oxacillin), cephalosporins and carbapenems, but can also be resistant to the new-generation MRSA-active cephalosporins (such as ceftaroline) or other groups of antibiotics, including aminoglycosides, macrolides, clindamycin, amphenicols, quinolones and tetracyclines. MRSA bacteria are pandemic and are often isolated in medical practice and nosocomial infections.

The MRSA detection is a challenge to any clinical microbiology laboratory and demands implementation of strict protocols for active screening. While more expensive molecular techniques have the potential of offering highly sensitive and rapid results, the cultural methods require longer time but can achieve a comparable sensitivity for lower price.

Keywords: Methicillin-resistant *Staphylococcus aureus*, drug resistance, MRSA detection methods

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus*– MRSA (also called multi-drug resistant staphylococci or oxacillin resistant *Staphylococcus aureus* – MRSA) are bacteria, responsible for severe and hard-to-manage infections in human. They show resistance to beta-lactam antibiotics – penicillins (methicillin, dicloxacillin, nafcillin, and oxacillin), cephalosporins and carbapenems, but can also be resistant to the new-generation MRSA-active cephalosporins (such as ceftaroline) or other groups of antibiotics, including aminoglycosides, macrolides, clindamycin, ampheni-

cols, quinolones and tetracyclines. MRSA bacteria are global pandemic and are often isolated in medical practice and nosocomial infections [1]: 19000 lethal cases are registered annually in USA– a number higher than HIV-related deaths [2].

The major risk factor for appearance of resistant *Staphylococcus aureus* is the uncontrolled and wide use of beta-lactam antibiotics (penicillins, cephalosporins and carbapenems). Methicillin resistant *S. aureus* should be considered as resistant to all penicillins, cepheems, carbapenems and other beta-lactams, such as amoxicillin/clavulanate, ampicillin/sulbactam, ticarcillin/clavulanate, piperacillin/tazobactam and imipenem, regardless the antimicrobial testing results, as most of the isolates react weakly to beta-lactam therapy [3].

Actually glycopeptides are the most adequate choice for treatment of staphylococcal infections and vancomycin is the leading option in severe cases [4, 5]. However in 1996 the first strains with decreased sensitivity as well as with complete resistance towards vancomycin were detected [6]. In these cases, vancomycin can be successfully replaced with linezolid [7].

MRSA detection methods

The fast proper detection and specific therapeutic approaches to MRSA are important questions for the public health policy of each country but to date no consensus exists for the most appropriate MRSA screening method [8]. In general, two big groups of methods are used – classical cultural techniques and molecular methods. While the expensive molecular techniques offer highly sensitive and rapid results, the cultural methods require longer time but can achieve a comparable sensitivity for lower price (Table 1).

Table 1. MRSA screening methods, according [9, 10]

Method	Sensitivity	Specificity (%)	Time to result (h)	Cost	Level of staff competence
Cultural	Low ^a / High ^b	100 ^a / 86-99 ^b	18–48 / 24-48	Low	Moderate
Molecular	High	< 100	< 24	High	Moderate to high

^a In standard agar media and enriched broths

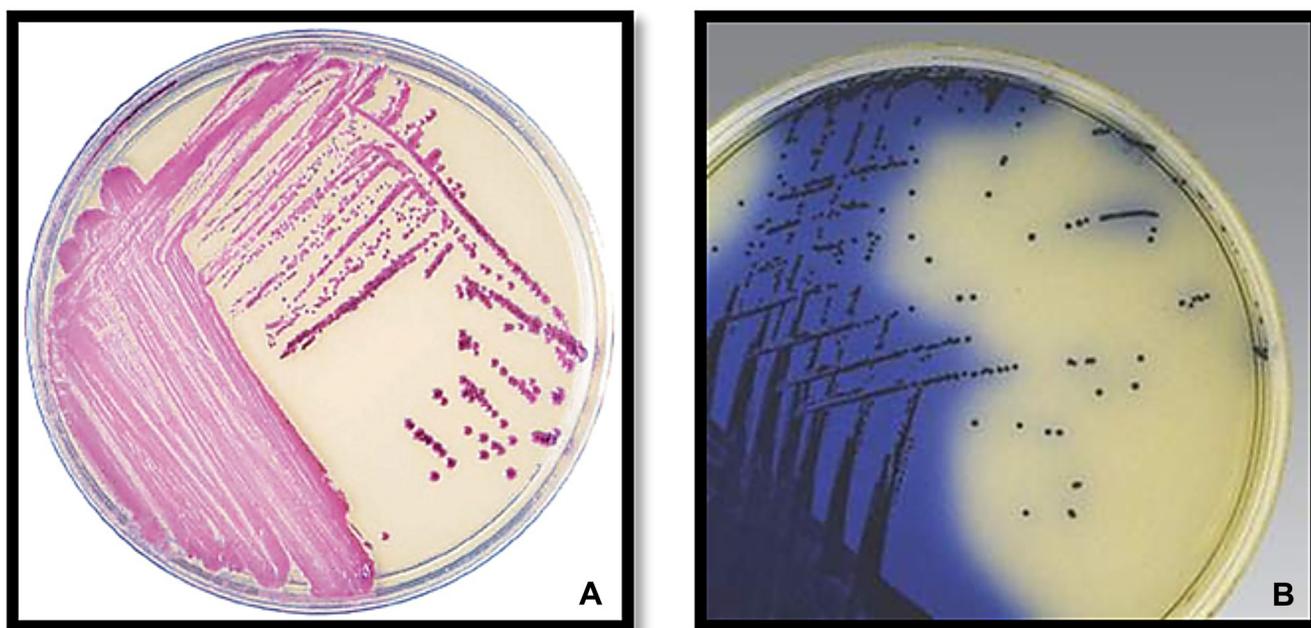
^b In MRSA selective agar media

Culture is the gold standard for MRSA detection [11]. Other commonly used classical methods include disc-diffusion method with cefoxitin and latex agglutination test for PBP2a (penicillin-binding protein 2a) detection. In addition, selective chromogenic agar media (ChromID MRSA, BBL CHROMagar MRSA – Fig. 1A) were also approved (by FDA (Food and Drug Administration – USA) [6]. They possess very high and improved when compared with standard selective media sensitivity (from 93 to 99%) [11, 12].

A widely used medium is Oxacillin Resistance Screening Agar Base (ORSAB). It can be used for MRSA screening directly from routine swab samples and is based on man-

nitol-salt agar but has a reduced salt concentration (7,5% - 5,5%). However, this salt content inhibits most of the other bacteria. The medium contains oxacillin to inhibit the growth of Methicillin-sensitive *Staphylococcus aureus* (MSSA) and polymyxin B to inhibit the growth of salt-resistant gram-negative bacteria (*Proteus spp.*) [13]. The cultivation is under aerobic conditions at 37°C for 24 hours. The observed blue colonies (Fig. 1B) are therefore confirmed with coagulase test. A negative result demands additional 24 hours of reincubation. The typical MRSA positive colonies are intensively blue on colorless background and this gives the possibility for easier identification in mixed culture.

Fig. 1. MRSA, cultured on ORSAB (A) [13] and on BBL CHROMagar (B) [14]



Because of the lower reliability of disc-diffusion method with oxacillin, cefoxitin is preferred for MRSA detection with breakpoints of ≤ 27 mm. In this method, the whole surface of Muller-Hinton agar is covered with inoculum, and then is air dried for 15 min; the cefoxitin disk is placed and finally the dish is incubated for 18 hours at 37°C. The technique is highly effective and is accepted as the most reliable for MRSA detection with 100% sensitivity [6, 15].

The automate VITEK 2 system (bioMérieux) or MRSA-screen latex agglutination test (Denka) are other popular choices for MRSA-detection. The VITEK 2 system can identify bacterial isolates in less than 8 hours and the ag-

glutination test – in less than 1 hour. They have respectively 94% and 97.6% sensitivity and 100% specificity to detect MRSA [15]. Other studies also favors latex agglutination test with sensitivity of 100% and specificity of 99.1% [16].

PCR (polymerase chain reaction) can also be used for rapid MRSA detection (2-4 hours). In this way the presence of *mecA* gene, widely associated with the staphylococcal resistance towards oxacillin can be detected. However, other limitations exist – PCR cannot find new resistance mechanisms, such as *mecC* gene or borderline oxacillin resistance [6]. The sensitivity of the PCR method varies between 98,6 – 98,7 % (Table 2).

Table 2. Sensitivity and time consumption of different MRSA detection methods

MRSA detection method	Sensitivity (%)	Time to results (h)
MRSA-selective media (BBL CHROMagar, ORSAB)	93-99	24-48
Disc-diffusion with cefoxitim	100	18-24
MRSA-screening latex agglutination	97,6-100	1
Vitek 2 system	94	5-8
PCR	91,6-98,7	2-4

CONCLUSION

The MRSA detection is a challenge to any clinical microbiology laboratory and demands implementation of strict protocols for active screening. Different methods for successful and rapid screening exist and their proper use can improve significantly the detection of both community-acquired and nosocomial resistant *Staphylococcus aureus*.

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Address for correspondence:

Neli Mitkova Ermenlieva
Medical College, Medical University - Varna, Bulgaria,
55, Marin Drinov Str., 9002 Varna, Bulgaria
e-mail: Neli.Ermenlieva@mu-varna.bg