



Performance of CHROM Agar and Oxacillin Resistant Screening Agar Base Media for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Chronic Wound

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Abstract

CHROM agar *Staphylococcus aureus* and Oxacillin Resistant Screening Agar Base (ORSAB) media with oxacillin were evaluated for the screening of Methicillin Resistant *Staphylococcus aureus* (MRSA). Among 190 samples, totally 126 confirmed *Staphylococcus aureus* strains were used for screening of MRSA used CHROM agar and ORSAB media were compared with the other MRSA screening media like Baird Park agar (BPA) with ciprofloxacin, Mannitol salt agar (MSA) with oxacillin, Blood agar (BA) with oxacillin and Muller Hinton agar (MHA) with oxacillin. Totally 54 MRSA strains were confirmed using PCR, among that 83% and 92% of the MRSA stains were isolated as mauve colonies on CHROM agar and blue colonies on ORSAB medium in 24 hrs incubation, compare with 64%, 61%, 50%, and 42% of the strains that were isolated on BPA, MSA, BA and MHA respectively. After 48 hrs of incubation 100%, 98%, 77%, 77%, 72% and 68% of the MRSA strains were isolated on CHROM agar, ORSAB, BPA, MSA, BA and MHA respectively. CHROM agar *Staphylococcus aureus* and ORSAB agar proved to be more sensitivity and specificity than other MRSA selective media. These provide an alternative for the detection of MRSA in clinical laboratories, especially when PCR is unavailable.

Keywords: MRSA, Specificity, Sensitivity, PCR, *mecA* gene

1. Introduction

In 1961 the first case of Methicillin Resistant *Staphylococcus aureus* (MRSA) was documented (Barber., 1961). Over the last three decades Methicillin Resistant *Staphylococcus aureus* (MRSA) has caused major problems in hospitals throughout the world (Waldvogel., 1995). Detection of MRSA in clinical samples continues to be important, since infection due to MRSA have high morbidity and mortality rates (Wertheim *et al.*, 2001). Approximately 75% of hospital strains are resistant to methicillin (York *et al.*, 1996). Mechanisms of methicillin resistant in *S. aureus* is based on the production of an additional low affinity penicillin binding protein (PBP; PBP2a), which is encoded by the *mecA* gene (Chambers., 1997). Accurate and quick identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) in clinical specimen is essential for timely decision on isolation procedures and effective antimicrobial chemotherapy (Jonas *et al.*, 1999).

The isolation, recovery and identification methods used in any routine clinical laboratory are crucial for the detection of MRSA. PCR based methods have recently been developed for the direct detection of MRSA in clinical specimen. However the use of these assays is largely restricted to reference centers and they are not currently utilized by most routine diagnostic laboratories. In this study, we evaluated the performance of CHROM agar and ORSAB as compared to that other conventional media with proper supplement for recovery of MRSA strains in clinical specimens, also studied specificity and sensitivity of the selected media compared with PCR analysis detection of *mecA* gene.

2. Experiments

2.1 Isolation of Clinical Samples

Totally 190 samples were obtained for the infected wound included accident, bite, septic and burn wounds. No duplicate isolates from a single patient were included in this study. Isolates were plated onto Mannitol Salt Agar (MSA) and incubate at 37°C, after 48 hrs of incubation results were observed.

2.2 Identification and sensitivity test

Method of identification included Gram staining, morphology, catalase, mannitol salt fermentation, slide and tube coagulase. The antibiotic sensitivity profile of the 126 *S. aureus* isolates were determined according to the method of Bauer-Kirby (Bauer *et al.*, 1966) using discs of antibiotics places on the surface of MHA medium seeded with the test organism. Inhibition zones were measured after 48 hrs of incubation at 37°C. Interpretation of resistance was based on the National Committee for Clinical Laboratory Standards (NCCLS) criteria. The antibiotics used were ciprofloxacin, methicillin, oxacillin and vancomycin.

2.3 Screening of MRSA

All confirmed *Staphylococcus aureus* isolates were inoculated onto a CHROM agar *Staphylococcus aureus* media with Oxacillin 4mg/l, Oxacillin Resistant Screening Agar Base (ORSAB) supplement with 50 000 IU of polymyxin B and 2.0 mg/l of oxacillin and 5.5% sodium chloride, Baird Park Agar (BPA) with 8mg/l ciprofloxacin and 7% sodium chloride, Mannitol Salt Agar (MSA) with 2mg/l oxacillin, Blood agar with 2mg/l oxacillin, Muller Hinton Agar (MHA) with 6mg/l oxacillin. Confirmed *Staphylococcus aureus* strains were streaked on the plates and incubated at 37°C. All the culture plates were interpreted after 24 hrs and 48 hrs of incubation. If any growth with expected color was detected, the isolates were considered as MRSA.

2.4 Detection of *mecA* gene

The PCR procedure was based on a modification by Unal *et al.*, (1992) and this were used as the gold standard for all isolates. Oligonucleotide used were *mecA* F primer 1282 (5'-AAA-ATC-GAT-GGT-AAA-GGT-TGG-C-3') and *mecA* R primer 1793 (5'-AGT-TCT-GCA-GTA-CCG-GAT-TTG-C-3'), which gives a PCR products of 533bp. PCR was performed on cooled thermocycler 5333, Eppendorf version 2.30.33-09, using a reaction mixture of 20 µl consisting of *Taq* polymerase buffer 2µl, 1 µl of each primer, DNA sample 1 µl, *Taq* polymerase enzyme 0.2 µl and distilled water 12.8 µl. 20µl of PCR product was then analyzed by 1.2% agarose gel electrophoresis.

3. Results and Discussion

3.1 Isolation and Sensitivity test

Totally 190 clinical specimens were directly tested on MSA. Out of these specimens 126 *S. aureus* were isolated after 48 hrs of incubation *S. aureus* colonies appeared yellow on MSA plate and later confirmed by tube and slide coagulase test. Four antibiotics discs were used for antimicrobial susceptibility test against *S. aureus*. It showed 49%, 44%, 39% and 50% of isolates resistant to ciprofloxacin, methicillin, oxacillin and vancomycin respectively. All *S. aureus* strains were resistant to any one of the above antibiotics.

3.2 Screening of MRSA

All *S. aureus* isolates were cultured on CHROM agar *S. aureus* with Ox, ORSAB with Ox, Baird Parker agar with ciprofloxacin, Mannitol Salt agar with 4-Ox, Blood agar with Ox, and Muller Hinton agar media with Ox and incubated for 48 hrs at 37°C. The plates were examined at 24 and 48 hrs, which were mauve color on CHROM agar, blue on ORSAB, yellow on MSA-4 Ox, black on Baird Park agar, white on Blood agar and Ox-MH agar. 83.30% of the MRSA strains were recovered by the use of CHROM agar after 24 hrs of incubation and this rate increased to 100% after the plate had been incubated for 48 hrs. Of 126 *S. aureus* isolates tested on 6 different MRSA selective media, 45 and 54 isolates were grown on CHROM agar after 24 and 48 hrs respectively. Similarly ORSAB, BPA, MSA Ox, BA, Ox-MHA were successfully in identifying MRSA in 24 and 48 hrs (Table 1).

3.3 Sensitivity and Specificity of MRSA

Multiple antibiotic resistant *S. aureus* strains constitute a major health care problem, therefore the availability of sensitive and specific method for the accurate detection of antibiotic resistance in these bacteria has become an important tool in clinical diagnosis. CHROM agar and ORSAB media showed a substantially better performance than any of the other media tested, and its sensitivity after 24 hrs of incubation were superior to that of other BPA, MSA-4Ox, BA and Ox-MHA. ORSAB was slightly less sensitive than CHROM agar after both 24 and 48 hrs of incubation. BPA, MSA-Ox, BA, Ox-MHA media were less sensitive than ORSAB and CHROM agar in 24 hrs 64.80%, 61.10%, 50% and 42.60%, in 48 hrs 77.80%, 77.80%, 72.20% and 68.50% respectively (Table I). Six media were showed high specificity (>90%) at 24 hrs incubation, but it was reduced after 48 hrs incubation except CHROM agar. Simor *et al.*, (2001) reported that the incubation time of 24 hrs should be extended to 48 hrs for the detection of MRSA.

CHROM agar MRSA achieved 100% in both specificity and sensitivity. MSA and ORSAB both achieved 91.50% and 91.50% respectively (Taguchi *et al.*, 2004). Apfalter *et al.*, (2002) also examined the performance of ORSAB at 24 and 48 hrs and found that the sensitivity of ORSAB increased from 50.80% to 68.20%. Blanc *et al.*, (2003) found that 38% of blue MRSA colonies on ORSAB were visible only after 48 hrs of incubation. Out of 114 *S. aureus* isolates tested on CHROM agar *S. aureus*, all the isolates were grown and were identified chromogenically as *S. aureus* by a pink to mauve color change after 18 to 24 hrs of incubation and also DNase and MSA were successful in identifying *S. aureus* in 112 of 114 isolates (98%) (Merlino *et al.*, 2002).

The similar results were observed by Lally *et al.*, (1985) that the use of MSA-4Ox resulted in a small number of false positive and false negative results. Brown and Walpole (2001) reported that a positive results were markedly lower for test on colonies from Blood agar and Baird Parker agar than from those on MSA and also reported blood agar plates with and without oxacillin were incubated at 30°C and 37°C respectively and the lower temperature of incubation of the plates with oxacillin may have contributed to better expression at PBP2a. Baird Parker agar contains ciprofloxacin was a useful medium for isolation of MRSA in situations where ciprofloxacin resistant strains were endemic (Davies and Zadik, 1997).

MDR-MRSA isolates were grown on CHROM agar *S. aureus* and ORSAB when supplemented with oxacillin 4mg/l, 2mg/l respectively in 48 hrs as well as on the isolation of NMDR-MRSA made difficult phenotypic interpretation. These isolates were usually resistant to β -lactam antibiotic but often susceptible to agents such as gentamicin, tetracycline, trimethoprim and variably erythromycin and ciprofloxacin (Maguire *et al.*, 1998).

3.4 Correlation between antibiotics groups and PCR

The correlation between major three antibiotics with Methicillin resistant (M, Ox), (M, Cf), (M, V) and the presence and absence of the *mecA* gene. In MRSA, there were 34 strains of Ox^r /*mecA*⁺, 40 of Cf /*mecA*⁺ and 39 of V^r /*mecA*⁺ and Ox, Cf, V sensitive and the presence of *mecA* gene were 14, 8 and 9 isolates respectively. In MSSA, these were none of the strain of Ox^r /*mecA*⁻, 2 of Cf /*mecA*⁻ and 5 of V^r /*mecA*⁻ and Ox, Cf, V sensitive and the absence of *mecA* gene were 8, 6 and 3 isolates respectively. In MRSA, the correlation between three antibiotics with Methicillin sensitive and the presence of *mecA* gene isolates were observed very low percentage (<3.2%). In MSSA class methicillin sensitive and absence of *mecA* gene were 12 strains of Ox^r, 16 of Cf and 17 of V^r and sensitivity of all three antibiotic 52, 48 and 47 isolates respectively (Table II).

3.5 Detection of *mecA* gene by PCR

Currently, multiple antibiotic resistant *S. aureus* strains constitute a major healthcare problem, since they are the etiologic agent of several nosocomial and skin infection. For that reason, accurate detection of resistant isolates constitutes a critical goal of clinical microbiology. The phenotypic expression of pre-confirmed 126 clinical wounds *S. aureus* isolates were examined in this study. These isolates were tested for the phenotypic confirmation for MRSA and hence were all genetically confirmed to be MRSA using PCR. Among 126 *S. aureus*, 54 isolates were positive for the *mecA* gene by PCR, remaining 74 *S. aureus* were negative (Table I).

Schmitz *et al.*, (1997) and Tokue *et al.*, (1992) previously reported that the utility of PCR for the accurate detection of the *mecA* gene and the possibility of simultaneous identification of *S. aureus* and detection of *mecA* gene. During the last decade, many studies have demonstrated the extremely high capacity of PCR for specifically detecting bacteria and genes of interest (Salisbury *et al.*, 1996). That ability has revealed PCR as a powerful tool in clinical microbiology studies (Cockerill, 1999). Several authors have already shown the feasibility of the PCR methodology for the identification of *S. aureus* strains and for the detection of antibiotic resistance genes (Cockerill, 1999; Jonas *et al.*, 1999). We concluded that the procedure still require a minimum of 2 days before final results can be obtained, but these media proved to be more sensitive and specific than other media. It may be an alternative for the detection of MRSA in clinical laboratories, especially when the PCR is unavailable.

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Table 1. COMPARISON OF THE LEVELS AND ACCURACY OF REACTIONS OF MRSA AND MSSA ISOLATES ON DIFFERENT MEDIA AFTER 24 AND 48 HRS

S. No	Media	24 hrs				48hrs							
		Positive Test Results		Accuracy of media		Positive Test Results		Accuracy of media					
		MRSA (mec+) n=54 Isolates	MSSA (mec-) n=72 Isolates	Sensitivity (%)	Specificity (%)	MRSA (mec+) n=54 Isolates	MSSA (mec-) n=72 Isolates	Sensitivity (%)	Specificity (%)				
1.	CHROM Agar ^a	45	83.3	0	0	83.30	100.00	54	100.0	0	0	100.00	100.00
2.	ORSAB ^b	42	77.8	1	1.4	77.80	98.60	53	98.1	5	6.9	98.10	93.10
3.	BPAgar ^c	35	64.8	0	0	64.80	100.00	42	77.8	3	4.2	77.80	95.80
4.	MSA-4 Ox ^d	33	61.1	2	2.7	61.10	97.30	42	77.8	8	11.1	77.80	88.90
5.	BAgar ^e	27	50.0	4	5.5	50.00	94.50	39	72.2	13	18.1	72.20	81.90
6.	Ox- MH Agar ^f	23	42.6	5	7	42.60	93.00	37	68.5	16	22.2	68.50	77.80

n - Number of strains

^a - CHROM agar *Staphylococcus aureus* with oxacillin 4mg/l.

^b - Oxacillin Resistant Screening Agar Base (ORSAB) with Oxacillin 2mg/l.

^c - Baird Parker Agar (BPA) with Ciprofloxacin 8mg/l.

^d - Mannitol Salt Agar (MSA-4 Ox) with Oxacillin 4mg/l.

^e - Blood Agar (BA) with Oxacillin 2mg/l.

^f - Mueller Hinton Agar (Ox- MH Agar) with 4% NaCl, 6mg/l Oxacillin.

Table 2. CORRELATION BETWEEN ANTIBIOTIC GROUPS AND PCR RESULTS

S. No	Bacterial Class	Antibiotics		No. of Isolates	<i>mecA</i> gene %		Antibiotics		No. of Isolates	<i>mecA</i> gene %		Antibiotics		No. of Isolates	<i>mecA</i> gene %	
		M	Ox		M	Cf	M	Va		M	Va					
1	MRSA	R	R	34	+	27.00	R	R	40	+	31.70	R	R	39	+	31.00
2	MRSA	R	S	14	+	11.00	R	S	8	+	6.35	R	S	9	+	7.10
3	MRSA	S	R	4	+	3.20	S	R	4	+	3.20	S	R	2	+	1.60
4	MRSA	S	S	2	+	1.60	S	S	2	+	1.60	S	S	4	+	3.20
5	MSSA	R	R	0	-	0.00	R	R	2	-	1.60	R	R	5	-	4.00
6	MSSA	R	S	8	-	6.30	R	S	6	-	4.80	R	S	3	-	2.40
7	MSSA	S	R	12	-	9.50	S	R	16	-	12.70	S	R	17	-	13.50
8	MSSA	S	S	52	-	41.30	S	S	48	-	38.10	S	S	47	-	37.30

MRSA : Methicillin Resistant *Staphylococcus aureus*

MSSA : Methicillin Sensitive *Staphylococcus aureus*

+: Positive, - : Negative,

R: Resistant, S: Sensitive, %: Percentage

M: Methicillin, Ox: oxacillin, Va: Vancomycin, Cf: Ciprofloxacin