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ORIGINAL ARTICLE

Detection of Methicillin-Resistant *Staphylococcus aureus* from Clinical Samples by CHROMagar™ and the *In-Vitro* Effect of Topical Antiseptics

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Key words:

Antiseptics, Chromogenic media, Methicillin-resistant Staphylococcus aureus, mannitol salt agar, Wound management

ABSTRACT

Background: *Rapid identification of MRSA isolates is mandatory to control its spread and management as well, especially with the continuous emergence of antimicrobial resistance.* ***Objectives:*** *Our study aimed to compare the sensitivity and specificity of chromagar™ in the detection of MRSA isolates in comparison to conventional bacteriological diagnostic methods. Testing the effect of some local antiseptics (Acetic acid, povidone iodine, hydrogen peroxide and diluted sodium hypochlorite) on identified MRSA isolates was also performed to examine for increased resistance in comparison to currently used concentrations.* ***Methodology:*** *Ninety-five samples were collected. MRSA was identified using conventional bacteriological methods along with chromogenic agar. Bactericidal concentrations of commonly used antiseptics were determined for isolated MRSA from patients.* ***Results:*** *Our study revealed 100% sensitivity, specificity, PPV and NPV of cefoxitin disc diffusion method versus 93.8% sensitivity, 100% specificity, 100% PPV and 97% NPV of chromogenic agar in the identification of MRSA. All MRSA isolates were killed at 5%, 10%, 5% and 0.25% concentrations of acetic acid, povidone iodine, hydrogen peroxide and Dakin's solution respectively.* ***Conclusion:*** *CHROMagar™ can be used as a rapid method for MRSA screening. The use of Hydrogen peroxide 2.5% and betadine 10% in wound management are recommended for use than Dakin's solution and acetic acid.*

INTRODUCTION

Diagnosis and treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is an important challenge to microbiologist and clinicians. The rapid identification of MRSA isolates is mandatory to control its spread, improve the therapeutic management and hence decreasing hospital stay, care cost and morbidity and mortality incidences [1,2]. More than one type of chromogenic media, with different constituents, is readily available for rapid and precise identification of MRSA isolates. Chromogenic media possess the advantage over conventional culture media; as it allows direct identification of pathogen based on a color change without the need for subculture and/or further confirmatory tests [3]. Previous studies based their research mainly on evaluating different types of chromogenic media for screening of MRSA from pure isolates or clinical samples (nasal, throat, groin,…..*etc.*) to screen for carriers[4-6], compared to different conventional methods as culture on blood agar and mannitol salt agar (MSA) supplemented with oxacillin [6].

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Multiple risk factors exist for MRSA colonization such as long term stay in hospitals and the use of medical devices as catheters. The presence of a traumatic wound, deep burn, skin diseases and immune deficiencies are also contributing factors [7,8]. Wound colonized by pathogenic bacteria is associated with delayed healing mandating for the use of topical antibiotics and antiseptics as well; to which unfortunately resistance has risen as the extensive use of antibiotics and biocides in treatment of patients and cleaning of surfaces and medical equipment has led to the emergence of resistant microorganisms [9-11].

In the present study, we aimed to evaluate CHROMagar™ for detection of MRSA isolates directly from a clinical wound and burn discharge samples as well as from nasal swabs of health care workers in comparison to conventional culture on MSA and identification of MRSA through testing its susceptibility to cefoxitin[12]. Testing the effect of some local antiseptics (Acetic acid, povidone iodine, hydrogen peroxide and sodium hypochlorite) on identified MRSA isolates to examine for increased resistance in comparison to currently used concentrations.

METHODOLOGY

1. Samples:

The present study was conducted at Intensive Care Units of Surgery Department & Burn Units of Ain Shams University Hospitals; from April 2015 to January 2016 and it was approved by the Institutional Review Board of Faculty of Medicine, Ain Shams University.
Ninety-five samples were collected; 70 of them were exudates or pus from patients admitted for chronic wound and burn infections. The rest were twenty-five nasal swabs collected from health care personnel of these units.

Culture and Identification:

Clinical specimens were collected by cotton sterile ordinary swabs and inoculated on both mannitol salt agar (MSA) **(HIMEDIA, India)** and CHROMagar™ **(CHROMagar ™, France).** After 24hrs incubation of the culture plates at 37°C, golden-yellow colonies (fig. 1) on MSA were identified as *Staphylococcus aureus* after confirmation by Gram stain, catalase and coagulase tests [13]. Rose to mauve colored colonies (fig.2) grown on the chromogenic media was considered positive for MRSA according to the manufacturer's instructions (table 1).

Colonies of *Staphylococcus aureus* from MSA were sub-cultured on nutrient agar (Lab M Limited, United Kingdom) and incubated at 37°C for 24 hrs. A bacterial suspension equivalent in density to 0.5 McFarland was prepared from separate colonies grown on nutrient agar, followed by its inoculation on Muller Hinton agar (Oxoid, UK). A commercially prepared cefoxitin disk (30µg, 6mm in diameter) (Bioanalyse, Turkey) was then added to test the *Staphylococcus aureus* susceptibility (Kirby- Bauer method) and detect MRSA. Inhibition zone diameters ≤21 mm was considered to be cefoxitin resistant (MRSA) [12].

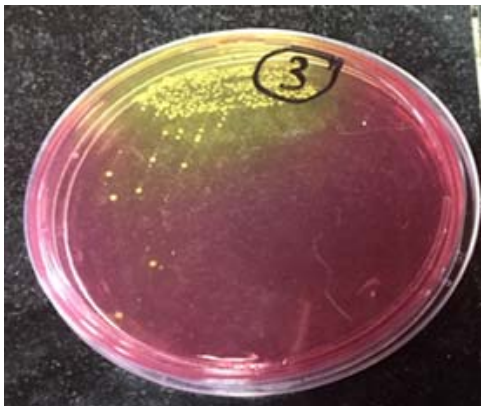

Fig. 1: Golden yellow colonies of *Staphylococcus aureus* on MSA

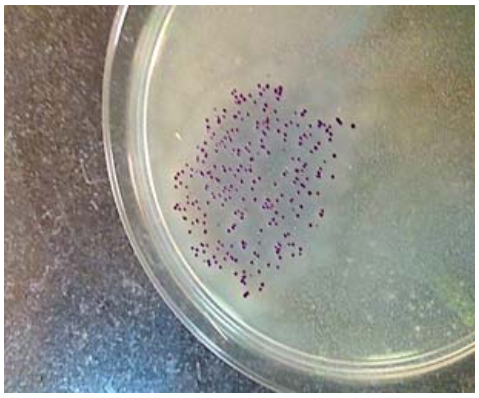

Fig. 2: Rose to mauve colonies of suspected MRSA on chromogenic screening agar

Table 1: Typical Appearance of microorganisms on chromogenic MRSA screening agar media

Methicillin Resistant Staphylococcus aureus *(MRSA)*	rose to mauve
Methicillin Susceptible Staphylococcus aureus (MSSA)	Inhibited
Other bacteria	blue, colorless or inhibited

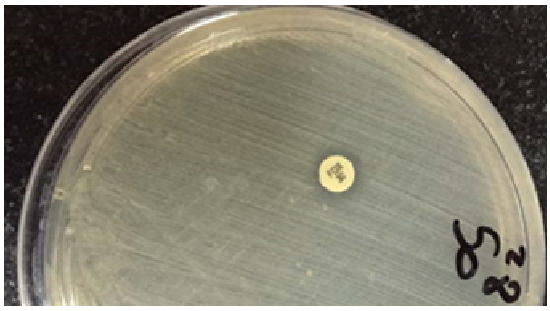

Fig. 3: Cefoxitin disc diffusion (Inhibition zone diameters ≤ 21 mm)

3. Effects of some local antiseptics against *MRSA* strains:

Some local antiseptics agents including Acetic acid (5%) (ElGomhouria, Egypt), Hydrogen peroxide (10%) (ElGomhouria, Egypt), Betadine (povidone iodine) (10%) (ElNile Company, Egypt), and Dakin's solution (diluted sodium hypochlorite, 4%) (ElNile Company, Egypt) were tested against MRSA isolates to observe their *in-vitro* effect.

Serial 2 fold dilution was performed from 100% concentration of the antiseptic solution, thus preparing 7 different concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.12% and 1.56%).

Separate colonies of MRSA, previously cultured on nutrient agar, were inoculated into sterile tryptone soya broth (Lab M Limited, United Kingdom) and the

suspension was adjusted to match a turbidity of 0.5 McFarland standards to get a final volume of 15 ml of the mixture of tryptone soya broth & 0.15 ml of the standardized bacterial suspension. One ml of the standardized bacterial suspension was added to the serially diluted tubes. After overnight incubation aerobically at 36-37°C, the tubes were examined macroscopically for visible evidence of bacterial growth in the form of turbidity, by comparing with the control tubes. Two control tubes were employed; one was a positive control tube containing the broth and each *MRSA* strain while negative controls contained the antiseptic agent only.

The last four clear tubes were sub-cultured on a nutrient agar plate, incubated for 24hrs at 37˚C. Growth was observed to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of these agents for each MRSA strain.

4. Data analysis:

All results are presented as mean and SD values or as median and interquartile range according to the distribution of data. Categorical results are presented as numbers of cases and percentages. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

This study was conducted at surgical intensive care units and burn unit of Ain Shams University Hospitals in the period from April 2015 to January 2016. Ninety-five samples were collected from 70 patients admitted for wound and burn infections as well as 25 nasal swabs from health care workers. They were 56 male and 39 female (58.6 % and 41.4% respectively). Their mean age was 39.9 years (table 2). From conventional culture and susceptibility results; 22 culture negative samples, 30 isolates of *Staphylococcus aureus* (27 MRSA; 3 methicillin- sensitive *Staphylococcus aureus* "MSSA") and 18 *Coagulase negative Staphylococci* (CoNS) isolates were identified from a wound and burn discharge samples. Eight culture negative samples, 5 MRSA, and 12 CoNS isolates were identified from 25 health care personnel working at ICU of surgery hospital.

Twenty-two (68.75%) out of 32 isolated MRSA; were identified from wound discharge samples (fig. 4)

MRSA identified by cefoxitin disc diffusion from isolated *Staphylococcus aureus* on MSA were; 5, 5 and 22 strains from nasal swabs, burn and wound discharge respectively versus 3, 5 and 22 strains identified by chromogenic agar (CHROMagar^{TM}) directly from clinical specimens (table 3).

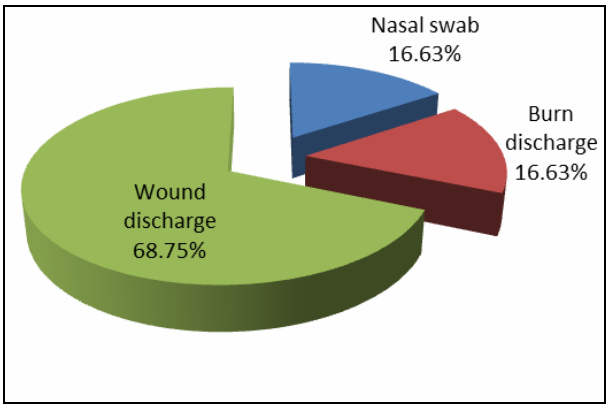

Figure (4): Percentage of identified MRSA (32) from different samples

Table 2: Demographic data of subjects and types of sample

Demographic data and type of sample	*N=95 (%)*
Age (mean ± SD)	39.2±2.1
Sex	
Male	56 (58.6%)
Female	39 (41.4%)
Type of sample	
Nasal swabs	25 (26.32%)
Burn discharge	25 (26.32%)
Wound discharge	45 (47.37%)

Table 3: MRSA isolates identified by cefoxitin disc diffusion versus chromogenic agar

Type of sample	*No. (%) of MRSA isolates identified by cefoxitin disc diffusion*	*No. (%) of MRSA isolates identified by Chromogenic screening agar*
Nasal swabs (HCW)	5 (15.6%)	3 (9.4%)
Burn discharge	5 (15.6%)	5 (21.9%)
Wound discharge	22 (68.8%)	22 (68.8%)

Our study revealed 100% sensitivity, specificity, PPV and NPV of cefoxitin disc diffusion method versus 93.8% sensitivity, 100% specificity, 100% PPV and 97% NPV of chromogenic agar in the identification of *MRSA* strains from nasal swabs, burn and wound discharge samples collectively (table 4).

Table 4: Predictive values, sensitivity and specificity of chromogenic agar compared to cefoxitin disc diffusion in identification of MRSA from different samples

	Total = 95		*Burn discharge = 25*		*Wound discharge = 45*		*Nasal swabs =25*	
	Cefoxitin disc diffusion	**Chromogenic screening agar**	**Cefoxitin disc diffusion**	**Chromogenic screening agar**	**Cefoxitin disc diffusion**	**Chromogenic screening agar**	**Cefoxitin disc diffusion**	**Chromogenic screening agar**
Sensitivity	100%	93.8%	100%	100%	100%	100%	100%	60%
Specificity	100%	100%	100%	100%	100%	100%	100%	100%
PPV	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	97%	100%	100%	100%	100%	100%	91%

From nasal swabs specimens, chromogenic agar revealed 60% sensitivity and 91% NPV with 100% specificity and PPV as well. From wound and burn discharge, 100% sensitivity, specificity, and predictive values were documented by chromogenic agar.

Testing the effect of local antiseptics/disinfectant on MRSA isolated from a wound and burn discharge samples revealed that; 100% of strains were inhibited at 0.63%, 0.63% and 0.063% concentrations of acetic acid, hydrogen peroxide, and Dakin's solution respectively, reflecting the minimal inhibitory concentration (MIC). While all strains were killed at 5%, 10%, 2.5% and 0.25% concentrations of acetic acid, betadine, hydrogen peroxide and Dakin's solution respectively reflecting the minimal bactericidal concentration (MBC) (table 5). The least effective concentration of betadine (1.25%) could not inhibit all isolates (92.6%).

Table 5: Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of some antiseptics on MRSA

Antiseptic agent	***10%***		***5%***		***2.5%***		***1.25%***		***0.63%***	
	+	-	+	-	+	-	+	-	+	-
Acetic acid	Concentration not tested		0	27 (100%) (MBC)	22	5	12	15	27 (100%) (MIC)	0
Povidone iodine	0	27 (100%) (MBC)	15	12	21	4	25	2	27 (100%) Resistant	
Hydrogen peroxide	Concentration not tested		0	27	0	27 (100%) (MBC)	20	7	27 (100%) (MIC)	0
Dakin's solution	**0.5%**		**0.25%**		**0.125%**		**0.063%**		-	
	0	27	0	27 (100%) (MBC)	17	10	27 (100%) (MIC)	0	Concentration not tested	

+ Growth after overnight culture on nutrient agar
- No growth after overnight culture on nutrient agar

DISCUSSION

In the present study, we identified MRSA from different samples (nose of health care workers, burn and wound discharge) using 2 cultural microbiological methods.

Concerning isolation of MRSA from nasal specimens, we reported 60% sensitivity and 100% specificity, 91% NPV and 100% PPV of chromogenic agar in detecting *MRSA* strains. Our sensitivity and NPV results are little lower than those reported by Loulergue et al. [14] (95.8% sensitivity 100% specificity, 100% positive predictive value and 95.6% negative predictive value, of chromogenic agar in identifying MRSA directly from clinical specimen). They concluded that chromogenic agar for the screening of MRSA nasal colonization provided accurate results in 24 h without requiring any complementary tests in comparison to conventional culture on Trypticase soy agar supplemented with 5% horse blood and antimicrobial susceptibility testing (cefoxitin disc diffusion). The difference may be related to the lower

number of nasal swab samples and identified MRSA in our study.

Our sensitivity and specificity results concerning identified MRSA from wound and burn discharge samples (100%), agree with those reported by Rahbar et al.[15] who examined previously collected 97 isolates of *Staphylococcus aureus* (58 MRSA and 39 MSSA) and examined them by chromogenic agar and reported 100% sensitivity and specificity. Our results are better ensuring the efficacy of CHROMagar™, as it could identify MRSA from clinical samples that might contain other organisms and flora.

Our results are supported by Malhotra-Kumar et al.[16] who documented that CHROMagar™ has the highest specificity and positive predictive value in detecting MRSA from clinical samples (Nasal and groin screening swabs) compared to another three types of chromogenic media. They also stated that CHROMagar gave the best overall results for the detection of MRSA, irrespective of the sample concentration, investigator, or incubation period.

Dakin's solution is used in the management of chronic wounds such as pressure ulcers, especially those with necrotic tissue and clinical infection in the concentration of 0.0125% to 0.5%, (diluted version of household bleach, which is a 5% solution of sodium hypochlorite) [17].

Our isolated MRSA were killed at concentrations of 0.5% and 0.25% of Dakin's solution which is higher from the concentration reported by Heggers et al. [17]. This can be explained by the increase in bacterial resistance. They found that a 0.025% concentration of Dakin's solution was effective (bactericidal) against MRSA, *Streptococcus mitis, Staphylococcus epidermidis* and some gram- negative bacilli. They confirmed that tissue toxicity, both *in-vitro* and *in-vivo*, was observed at concentrations of 0.25% but not at a concentration of 0.025%.

In contrast, Mama et al**.** [18] isolated *Staphylococcus aureus*, CoNS and other gram-negative bacilli from wound infection samples which were resistant to concentrations of 0.025%, 0.25% of Dakin's solution after incubation for 24 hrs. The minimum inhibitory and bactericidal concentration of the solution (Dakin's solution) was 0.5% and 1% which is higher from inhibitory and bactericidal concentrations (0.063% and 0.25% respectively) observed in our study. The difference may be attributed to their testing the effect of Dakin's solution, not only on MRSA but also on other gram positive and gram negative organism which may have acquired other resistant mechanisms.

A 3% hydrogen peroxide solution has been used as a wound cleansing agent for years; with a remarkable bactericidal activity [19].

The MIC and MBC for hydrogen peroxide in our study were; 0.63% and 2.5% respectively, while both Lineaweaver et al. [20] and Mama et al. [18] studied various concentrations of hydrogen peroxide for effectiveness against *Staphylococcus aureus*. They found that; 3% concentration is bactericidal.

Isolated MRSA in our study were killed at 5% concentration of acetic acid which is much higher than the bactericidal concentration (0.5%) reported by Mama et al.[18] indicating the high resistance of MRSA to an acetic acid solution. Lineaweaver et al. [20] tested 0.25% acetic acid for its ability to kill *Staphylococcus aureus*; 78% of the bacteria survived 24 hours of exposure to acetic acid. McKenna et al. [21] tested a 0.0025% solution (a concentration which is non-cytotoxic to fibroblasts) against *Staphylococcus aureus* and other gram- positive and negative pathogens. The acetic acid showed slight inhibition of staphylococcal growth and moderate inhibition of *Pseudomonas* but was not found to be bactericidal to either of these organisms. The other organisms tested were unaffected by the 0.0025% concentration of acetic acid. Cooper *et al*.,[22] found that a 0.125% solution of acetic acid to be cytotoxic to both fibroblasts and keratinocytes, but a 0.0125% solution caused no damage. Hence, the MBC (5%) detected in our study for MRSA is not recommended for use as a wound antiseptic to avoid tissue cytotoxicity.

Betadine (povidone-iodine) solution is manufactured to contain a 10% of polyvinylpyrrolidone iodine, providing a gradual liberation of free iodine (the bactericidal component of the solution). The actual concentration of free iodine is usually 1 ppm. This low concentration of free iodine was proved to kill most bacteria in 60 seconds [23,24] which is equal to the MBC documented in our study for betadine solution.

CONCLUSION

Chromogenic screening agar (CHROMagar™) for MRSA Identification was proved to be simple, with sufficient sensitivity and specificity comparable to conventional media. Both time and laboratory workload were saved as identification of MRSA was achieved in a single step and so we recommend its use for rapid MRSA screening. Hydrogen peroxide 2.5% and betadine 10% are recommended for use in wound management than Dakin's solution and acetic acid. Searching for a new antiseptic substitute should be carried on as bacterial resistance is currently rising.

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