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ORIGINAL ARTICLE

Evaluation of a Chromogenic Methicillin Resistant *Staphylococcus Aureus* Selective Medium

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Abstract

Background: **One of the main sources of acquired bacterial infections in the community and hospitals is *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA) strains.**

Aim: **To compare between the CHROMagar MRSA selective medium (as a rapid method) and the other laboratory conventional methods. Also, to evaluate of the presence of *mecA* gene within MRSA isolates using PCR.**

Materials and methods: **The samples were screened for antibiotic susceptibility testing and growth on a particular chromogenic medium for the detection of MRSA and their resistance rates to other routinely used antibiotics. Additionally, gel-based PCR technique was used for the detection of *mecA* gene.**

Results: **Of the patients under investigation, 200 (26.7%) had *Staphylococcus aureus* isolates.**

MRSA was detected in 86 (43%) cases. MRSA was mostly isolated from newly admitted patients (75.6%) and medical personnel (24.4%). The clinical samples with the greatest MRSA prevalence (43.3%) were pus swabs. In 97.6% of cases, chromogenic agar was successfully identified MRSA isolates. All isolates that were resistant to oxacillin disc had positive PCR results.

Conclusion: **Using of chromogenic media as a rapid screening method and accurate diagnosis of MRSA strains is depended upon its high sensitivity and specificity compared to PCR method.**

Keywords: MRSA, CHROMagar TM MRSA medium, PCR

1. Introduction

The skin and mucous membranes of humans frequently harbor *Staphylococcus aureus*.[1] But among the top three principal bacteria responsible for community and hospital acquired infections is *S. aureus*.[2]

Higher morbidity and mortality are being caused by the emergence of antimicrobial resistance, specifically to methicillin, in a number of healthcare settings. This is particularly true for systemic methicillin-resistant *S. aureus* (MRSA) infections,[3] which can be further divided into healthcare-associated and community-acquired types.[4] The penicillin-binding protein 2a or 2′ (PBP2a or PBP2′ having poor affinity for beta-lactam antibiotics) is encoded by the methicillin-resistant gene A (*mecA*), which was integrated into the chromosomal element (SCC mec) of methicillin-susceptible *S. aureus*.[5]

Since the *mecA* gene is widely dispersed among staphylococcal species, its amplification is regarded as a crucial way to diagnose MRSA in nosocomial and community settings.[6] Consequently, the use of PCR to detect the *mecA* gene is thought to be the most accurate method for identifying MRSA.[7]

The use of chromogenic medium has proven to be a beneficial tool for the fast detection of MRSA in clinical samples. Chromogenic medium permit the initial culture's immediate colony identification of the resistant bacterium, in contrast to conventional culture medium.[8] The purpose of this study was to compare CHROMagar MRSA selective medium with other widely used laboratory techniques.

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Additionally, PCR was used to assess if MRSA isolates had the *mecA* gene.

2. Patients and methods

This study was carried out between April 2021 and May 2022 in Al-Azhar University Hospital (Assiut branch). Seven hundred forty-two clinical samples were taken from patients who had been admitted in various wards and healthcare professionals. Samples were processed in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Al-Azhar University (Assiut branch). Two hundred clinical isolates of *S. aureus* were detected from the 742 clinical samples which were used in this investigation. Demographic information was gathered from every patient. Clinical traits such as the length of the hospital stay, the existence of underlying disorders, risk factors (such as the use of invasive devices), prior investigations, and antibiotic treatment were recorded.

Samples, such as blood samples, midstream urine, nasal samples, pus, morning sputum, suctions from endotracheal tube, and urinary catheter were taken. Clinical samples were cultured on mannitol salt Agar, MacConkey's agar, blood agar, and cysteine lactose and electrolyte deficient agar and incubated aerobically at 37 °C for 24–48 h. Gram stain, colony morphology, catalase test, and coagulase test 'tube method' were used to identify *S. aureus* isolates. As recommended by CLSI,[9] disc diffusion sensitivity tests against oxacillin were used to determine antibiotic susceptibility. The inhibitory zones' sizes were measured in millimetres. MRSA was identified as having a zone diameter of less than 11 mm, while methicillin-sensitive *S. aureus* was identified as having a zone diameter of less than 12 mm. The MRSA isolates were subjected to a disc diffusion test to determine their sensitivity to several antibiotics, including vancomycin (30 g), clindamycin (2 g), gentamycin (10 g), erythromycin (15 g), trimethoprim/sulfamethoxazole (1.25 + 23.75 g), and chloramphenicol (30 g). Purified colonies were obtained and kept at −20 °C. MRSA CHROMagar (the inventor and pioneer in chromogenic culture media technology, Paris, France) was used. The presence of rose to mauve colonies was checked by looking at the color of the colonies on CHROMagar MRSA agar.

DNA from the genome was retrieved by boiling. TransGen Biotech Co. (Beijing, China) provided the primers utilized in this work (amplifier size is 147 bp; primer forward: GTGAAGATATACCAAGTGATT; primer reverse: ATGCGTATAGATTGAAAGGAT). A 50 l volume containing 1 l of extracted DNA, 22 l of 2 × Easy Taq PCR SuperMix, 1 l of primer mix 1 forward, 1 l of primer mix 2 reverse, and 25 l nuclease-free water were used for the amplification reaction. The following conditions were used to carry out the PCR amplification procedure in a DNA Thermal Cycler (Biometra): denaturation at 94 °C for 4 min, then 35 cycles of annealing at 50 °C for 30 s, extension at 72 °C for 1 min, and finally extension was carried out at 72 °C for 7 min. The amplified PCR products were seen by electrophoresis on 2% agarose gels stained with ethidium bromide, analysed by UV *trans*-illumination, and captured on camera.[10,11]

2.1. Ethical consideration

This study was conducted after approval by Al-Azhar Faculty of Medicine Research Ethical Committee. Written consent was obtained from each patient or patient's family.

2.2. Statistical analysis

The statistical tool (SPSS, North Castle Drive, Armonk, New York, United States of America), version 15 has been employed. Data were presented by percentages for categorical variables. The χ^2 test was used to test for statistical significance of categorical variables and was set at a P value less than or equal to 0.05.

3. Results

Of the total of 742 clinical samples, 200 (26.9%) clinical isolates of *S. aureus* were detected followed by *Klebsiella pneumoniae* (25.2%) then *Pseudomonas aeruginosa* (17.9%). Males were more than females (136, 68% vs. 64, 32%, respectively) with highly significant difference ($P = 0.0005$). Patients involved in the study were of different age groups ranging from 17 to 80 years, with mean ± SD age, 54.18 ± 16.5 years. Rate of isolation of *S. aureus* was higher in 31–40 years ($n = 84/200$) (42%) with no statistical difference ($P = 0.07$) (Table 1).

S. aureus was detected among healthcare workers (HCWs) in 44.4% ($n = 51/200$). *S. aureus* was isolated in among nurses (47.05%) followed by doctors (41.17%) then laboratory technician (11.78%). There was no statistical significant difference ($P = 0.85$).

S. aureus infection between in-patients was (149/200) 47.5%. The highest prevalence was among medical ward (40.2%) followed by surgical ward (37.6%) then ICU (22.2%). There was no statistical significant differences between clinical departments ($P = 0.67$). Demographic and clinical data are shown in Table 1.

Table 1. Demographic and clinical data of S. aureus isolates.

Items	*n* (%)	*P* value
Sex		
Male	136 (68)	0.0005
Female	64 (32)	
Age (years)		
17–30	46 (23)	0.78
31–40	84 (42)	
41–60	48 (24)	
61–80	22 (11)	
HCWs		0.85
Nurses	24 (47.05)	
Doctors	21 (41.17)	
Laboratory technicians	6 (11.76)	
Hospital department		0.76
Medical ward	60 (40.2)	
Surgical ward	56 (37.6)	
ICU	33 (22.2)	

The isolation of *S. aureus* in clinical samples was more observed in nasal swabs (49.5%) followed by pus (25%) then sputum (16.5%), urinary catheter (3%), endotracheal tube (2.5%), urine samples (2%), and the least was in bronchial lavage (1.5%) (Fig. 1).

MRSA was detected in 86 (43%) out of 200 patients. MRSA had high resistance rates for the used antibiotics. The most common resistance rates were for trimethoprim/sulfamethozole (51.1%) followed by ciprofloxacin (46.5%) then erythromycin (45.3%), chloramphenicol (43%), and gentamycin (41.8%) and the least resistance was toward vancomycin (13.9%) (Fig. 2).

Males were more affected than female patient (63.9 vs. 36.0%). The most affected age group was of 31–40 years (48.8%) from the total MRSA population. The isolation rate of MRSA in relation to sex was not significantly associated ($P = 0.36$) as well as any of age groups ($P = 0.34$).

MRSA was also detected among HCWs in 24.4% ($n = 21/86$). Nurses (57.14%) followed by doctors (33.33%) then laboratory technician (9.53%). There was no statistical significant difference ($P = 0.64$).

Prevalence of MRSA between inpatients were ($n = 65/86$) 75.58%. The highest prevalence was among surgical ward (53.85%) followed by medical ward (29.23%) then ICU (16.92%). There was no statistical significant difference ($P = 0.39$). Demographic and clinical data are shown in Table 2.

Among all clinical samples the highest rate of MRSA isolates was from pus swab (43.3%) followed by nasal swab (31.3%), sputum (22%), urinary catheter (3.4%) and the least urine and endotracheal tube (2.2%; each) (Fig. 3).

The sensitivities of the CHROMagar MRSA were 93% ($n = 80/86$) after 24 h of incubation and 97.7% ($n = 84/86$) after 48 h. Also, the specificities were 88.3% after 24 h; and 92.1% after 48 h.

PCR targeting the *mecA* gene in *MRSA* was positive in all isolates with resistance to oxacillin disc ($n = 86/86$).

4. Discussion

S. aureus was the most common pathogen isolated overall (26.9%) from the diverse clinical specimens in this study, followed by *K. pneumoniae* (25.2%) and *P. aeruginosa* (17.9%). The most common bacteria

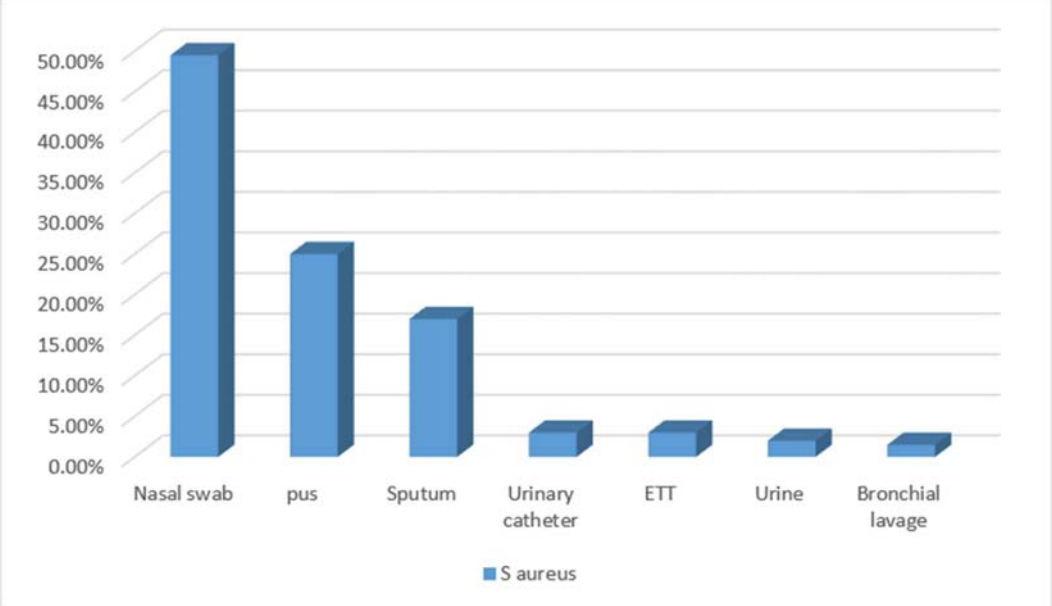

Fig. 1. Distribution of S. aureus in clinical samples.

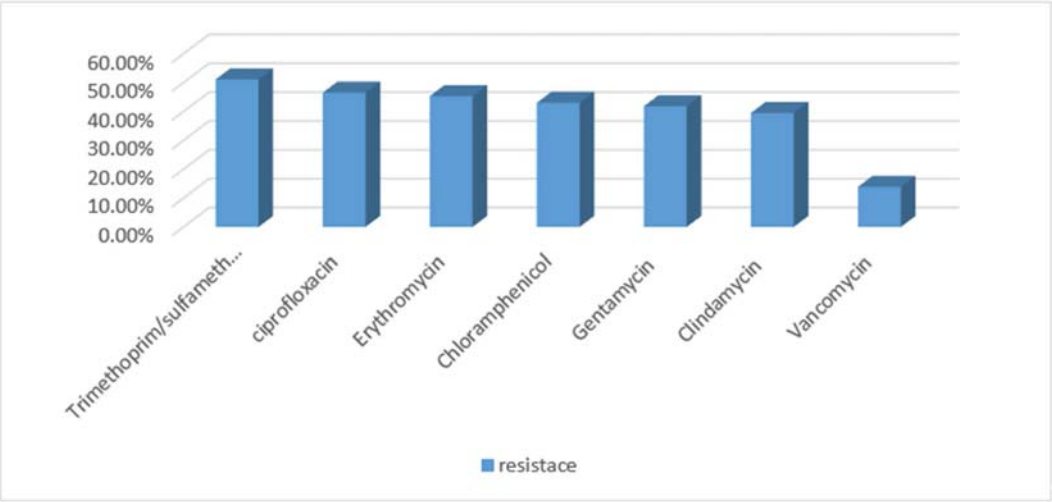

Fig. 2. MRSA antibiotic susceptibility test.

Table 2. Demographic and clinical data of methicillin-resistant S. aureus isolates.

Items	*n* (%)	*P* value
Sex		
Male	55 (63.9)	0.36
Female	31 (36.0 %)	
Age (years)		
17–30	14 (16.27)	0.34
31–40	42 (48.88)	
41–60	23 (26.72)	
61–80	7 (8.13)	
HCWs		0.64
Nurses	12 (57.14)	
Doctors	7 (33.33)	
Laboratory technicians	2 (9.53)	
Hospital department		0.39
Surgical ward	35 (53.85)	
Medical ward	19 (29.23)	
ICU	11 (16.92)	

found, according to Dhungel et al.[12] findings was *S. aureus* (27.1%).

Males outnumbered females in this study of 200 *S. aureus* isolates by a margin of 136 to 64 (68–32%). These findings corroborated those of Dilnessa and Bitew,[13] who identified *S. aureus* from clinical samples. Regarding the relationship between age and *S. aureus* infection, this study showed that *S. aureus* infection rates varied according to the patient age, with greater rates found in those between the ages of 31 and 40 (42%), 41–60 (24%), and 17–30 (18%). These findings were consistent with those of Hussain et al.[14] who found that the higher rate was observed in the age group of 31–45 years with 42% followed by the age group of 46–60 years with 25% while the least rate was in the age group of 16–30 years with 17%.

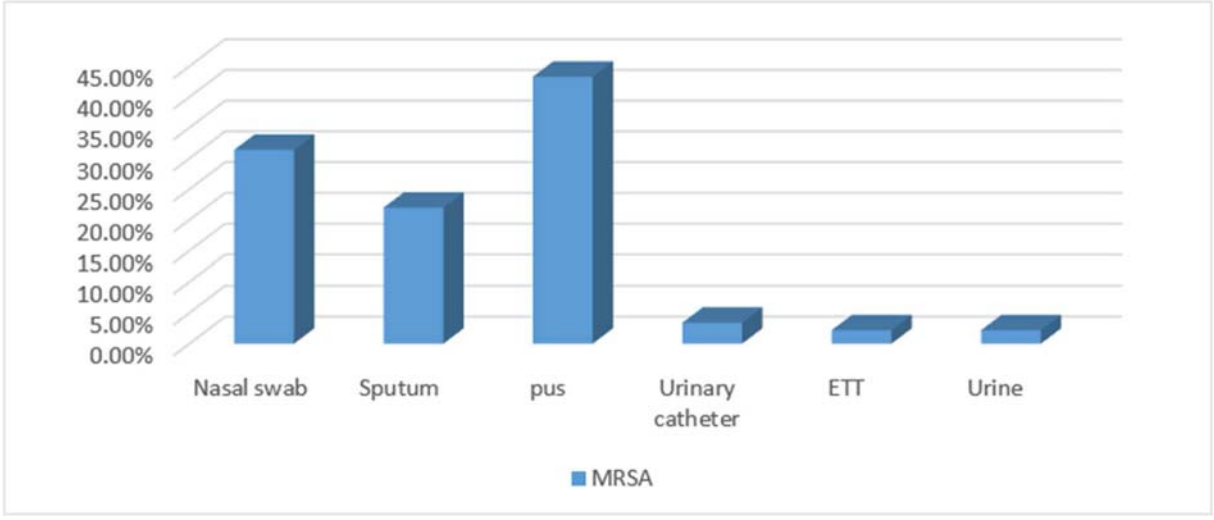

Fig. 3. Distribution of MRSA in clinical samples.

Our research found that 44.4% (51/200) of HCWs had *S. aureus* colonization. The study's results were similar to those from Tanzania (41.4%) 45 and India (40.8%),[15] however they were greater than those from Oman (20.5%)[16] and Northeast Ethiopia (28.8%).[17] Laboratory technicians had the lowest prevalence of *S. aureus* carriage among HCWs (11.78%) and nurses had the highest incidence (47.05%), which is consistent with the findings of Joachim et al.[18] This was in contrast to a research done by Radhakrishna et al.,[19] who found that nurses had the lowest *S. aureus* carriage (13.6%) while doctors had the highest (32.5%) one.

In the present study, the isolated *S. aureus* between admitted patients was the highest among medical ward (40.2%) followed by surgical ward (37.6%) then ICU (22.2%). This study was similar to study carried out by Ginawi et al.[20] who reported that the frequency of *S. aureus* infection in admitted patients was 39.1% among medical ward followed by 30.9% in surgical ward.

In this study, nasal swabs had a higher rate of *S. aureus* (49.5%), followed by pus (25%) and bronchial lavage (1.5%). Finding that is in line with the findings of Chigbu and Ezeronye.[21] The highest incidence rate was found in wound specimens (29.4%) by Nsofor et al.,[22] pus (55.6%) by Kumar et al.,[23] and blood samples (43.6%) by Dhungel et al.[12] Our findings, however, were in contrast to other results.

In the current study, there were 86 (43%) isolated MRSA cases among *S. aureus*. This outcome is consistent with observations made by Dalela et al.[24] Other studies, however, reported rates of (35.8%) in South Africa[25] and (30.7%) in India.[26] MRSA prevalence varied from 16 to 44% in different European nations, and there was also an increase in other antimicrobial group resistance http://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2018. The existence of active MRSA surveillance programmes, the popularity of infection control, antibiotic policies, the different geographical region, specimens, and testing techniques should all be aspects responsible for the price of editions evaluated with distinctive research.[27]

According to the antimicrobial susceptibility pattern, trimethoprim/sulfamethozole had the highest resistance (51.1%) while the lowest resistance was detected toward vancomycin (13.9%) indicating that vancomycin can be employed as a treatment option for MRSA infections in our settings. The current results are remarkably similar to those published by Lohan et al.[26] who found that 12.3% of MRSA isolates were resistant to vancomycin.

Male patients were more affected than female patients when comparing the sex distribution of MRSA strain infections (63.7 vs. 36.3%), and Lohan et al.[26] also noted this pattern. In this study, the afflicted age group was between the ages of 31 and 40 (48.8%), whereas Dhungel et al.[12] stated that the affected age group with the highest frequency was between the ages of 60 and above (29.8%).

Significant MRSA strains were recovered in the current study, mostly from HCWs (24.4%). MRSA strain isolation rates from HCWs have been reported at various rates, ranging from 0 to 59%.[28] Nurses had the highest rate (57.14%). This observation might be explained by the fact that nurses spend more time interacting with patients who were possibly ill or colonized, making them at a greater risk for the spread and acquisition of microorganisms than other fitness professionals.[29]

In this study, the surgical ward had the greatest percentage of isolated MRSA from patients who had been hospitalized, followed by the medical ward (29.2%) and the ICU (16.9%). Similar findings were reported by Lohan et al.,[26] who speculated that the reason surgery produced the highest number of MRSA isolates was because the bacteria had colonized the skin and had increased the likelihood of invasion during invasive procedures and due to indwelling devices in ICUs.

In the current investigation, pus swabs had the highest rate of MRSA isolates (43.3%) among all clinical samples. Tiwari et al.[30] observed very similar results, with pus (42%) being the most common clinical sample found. This predominance of MRSA in pus may result from wound exposure to MRSA in the environment and on the skin as well as from improper use of antibiotic therapy.[26]

In the present study, sensitivities of the *CHROMagar* MRSA were 93% ($n = 80$) after 24 h of incubation and 97.7% ($n = 84$) after 48 h. The specificities were 88.3% after 24 h and 92.1% after 48 h. This finding is consistent with that report by Yang et al.[31] who detected that the sensitivity and specificity of *CHROMagar* MRSA was 91.9 and 99.5% after 24 h then 99.8 and 99% after 48 h, respectively.

In the current study, PCR assay was performed and all 86 MRSA isolates were *mecA* positive isolates. This had been established by previous studies reported by Karmakar et al.[32]

4.1. Conclusion

The excellent specificity of MRSA detection made possible by the use of chromogenic substrates in chromogenic media as a screening fast approach is due in part to the presence of the

optimal combinations of selective agents, which avoid interference from nontarget microorganisms. Chromogenic medium has a sensitivity and specificity of 97.6 and 92.1%, respectively.

Conflicts of interest

There are no conflicts of interest.

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