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Detection of Staphylococcus Aureus And Methicillin-Resistant Staphylococcus Aureus (MRSA) From Human Clinical Specimens Using Conventional Biochemical Tests And Chromogenic Media

KEYWORDS: S.aureus, MRSA, biochemical test, chromoagar

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ABSTRACT *Objective: To identify of Staphylococcus aureus and methicillin – resistant staphylococcus aureus (MRSA) by two methods; conventional biochemical test and chromogenic media from various clinical specimens in Basrah, Iraq.*

Methods: A total of 82 isolates from clinical specimens were collected (wounds , skin infections, urine samples and stool samples) . All these isolates were tested for comparative detection S. aureus by conventional biochemical test and by CHROMagar Staph. aureus , all positive samples were detected MRSA by CHROMagar MRSA.

Results: 26 (31.7%) isolates of 82 clinical samples were detected as S. aureus by conventional biochemical test, while 23 (28%) were positive by CHROMagar Staph. aureus. 17 (65%) from 26 isolates as MRSA detected by CHROMagar MRSA.

Conclusion: A screening procedure using chromogenic media give better results, faster and more sensitive in identifying S. aureus and MRSA than conventional methods.

1. INTRODUCTION

Staphylococcus aureus is one of the most frequent bacterial pathogens in humans [1]. It's a common cause of hospital and community-acquired infections, and causes skin diseases , osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals [2].

Over the last several decades, the resistance ability of *S. aureus* increased due to the widespread abuse of common antibiotics.

Resistance to methicillin has reached global proportions [3]. The integration of staphylococcus cassette chromosome mec (SCCmec) element into *S. aureus* changed it into methicillin-resistant *Staphyloccocus aureus* (MRSA) [4].

MRSA has occurred in many countries since its discovery in 1961[5]. It is a significant cause of morbidity, mortality, and healthcare costs[6] .

The global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections [7].

There are many laboratory methods for detection of MRSA[8]. One of these methods use detective and selective media like Chromogenic media, which is new media for identification of many bacterial species, It's a rapid test with high sensitivity, specificity and it's rapid.

The study aimed to identify *S. aureus* and MRSA by routine biochemical tests and chromogenic media .

2. MATERIALS AND METHODS

2.1 Specimen Collection

Eighty two swab samples were collected over four months (February to May 2013). from many clinical samples(wounds , skin infections, urine samples and stool samples). The samples inoculated on mannitol salt broth media were incubated at 37°C for 24 h and then inoculated onto mannitol salt agar media and incubated at 37°C for 24-48 h.

2.2 Biochemical test

All positive isolates on mannitol salt agar medium were identified as *S. aureus* by conventional biochemical tests (gram stain, catalase positive , mannitol fermenting test, coagulase test, oxidation- fermentation test, detection of haemolysin, DNase test).

2.3 Chromogenic screening medium for *S. aureus*

Samples identified as a *S. aureus* by biochemical tests have been inoculated on CHROMagar *Staph. aureus* (CHROMagar™, Paris, France).

2.4 Chromogenic Culture for MRSA

All positive sample (*S. aureus*) have been inoculated on CHROMagar MRSA (CHROMagar™, Paris, France).

3. RESULTS

Twenty six samples out of the eighty two isolates (31.7%) were Gram-positive, cluster forming cocci , non-motile, non-spore forming, catalase positive and coagulase positive. They fermented mannitol and formed β-hemolysis on blood agar. These isolates were identified as *S. aureus* . While twenty three samples (28 %) identified as *S. aureus* on chromo agar medium(Fig.1). The results are summarized in Table I.

Table1: *S. aureus* identified by biochemical and chromoagar medium

No. of positive isolate(26)	*S. aureus* (Biochemical) (26)	*S.aureus* (Chromagar) (23)	Colonies appearance In Chromoagar plates
2	+	+	Typical mauve colonies (1-3mm)
4	+	+	Small mauve colonies (1mm)
7	+	+	Small mauve colonies (1mm)
8	+	+	Typical mauve colonies (1-2mm)
9	+	-	Colourless colonies
10	+	+	Small mauve colonies (1mm)
12	+	+	Pink colonies(1-2mm)
13	+	+	Pink colonies(1-2mm)
17	+	+	Small mauve colonies (1mm)
19	+	+	Pink colonies(1-3mm)
20	+	+	Small mauve colonies (1mm)
28	+	+	Typical mauve colonies (1-2mm)
31	+	+	Typical mauve colonies (1-3mm)
32	+	+	Small mauve colonies (1mm)
37	+	+	Small mauve colonies (1mm)

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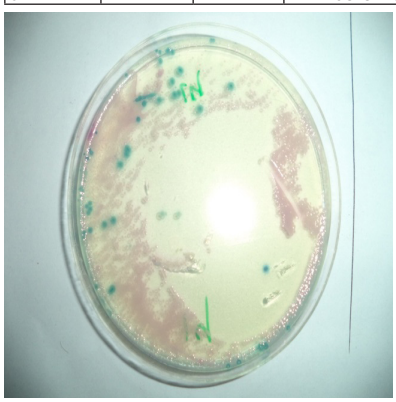
40	+	-	Colourless colonies
49	+	+	Pink colonies(1-2mm)
52	+	+	Small mauve colonies (1mm)
58	+	+	Small mauve colonies (1mm)
59	+	+	pink colonies(1-3mm)
66	+	+	Typical mauve colonies (1-3mm)
67	+	+	Inhibited
77	+	+	Small mauve colonies (1mm)
78	+	+	Typical mauve colonies (1-3mm)
79	+	-	Inhibited
82	+	+	Pink colonies(1-2mm)



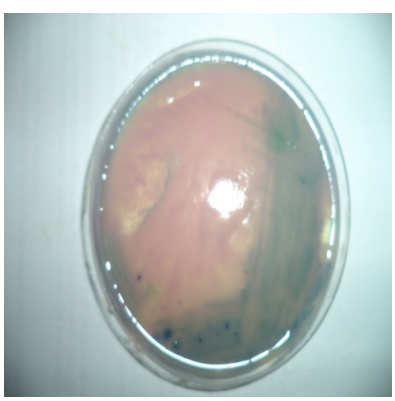
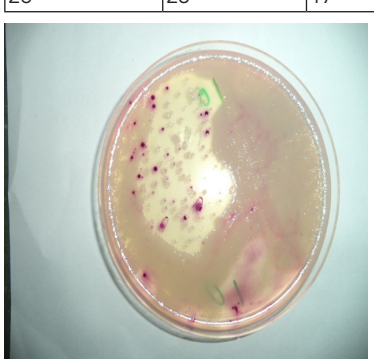


Fig. 1: Mauve colonies of *S. aureus* on CHROMagar *Staph. aureus*

The results for methicillin-resistant *S. aureus* on chromogenic medium showed clearly results and the MRSA colonies give rose color as positive identify (table 2) (Fig. 2).

Table 2:Iditified of MRSA by chromogenic medium

Total of *S.aureus* Biochemical tests	Total of *S. aureus* chromoagar	Total of MRSA Chromoagar	Colonies appearance
26	23	17	Rose



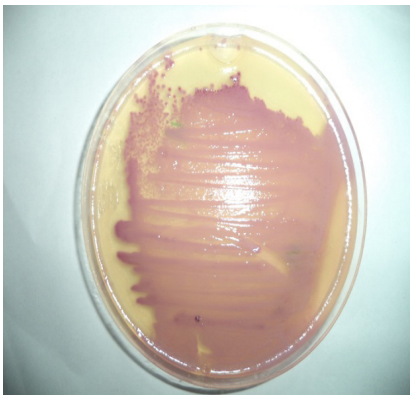


Fig.2: Rose colonies of MRSA on CHROMagar MRSA

4. DISCUSSION

Conventional methods for identification of pathogenic bacteria have a lot of false positive and negative results.

The results showed that chromoagar *Staph. aureus* gave the most accurate identify when compared with biochemical tests. this result confirms the findings reported by Samra et al. [9] , Stampi et al. [10] and Ten et al. [11].

CHROMagar *Staph. aureus* is a new chromogenic medium for as mauve colonies after 24 h of incubation [12].

Chromogenic media have many advantages like rapid detection, high sensitivity, highly specific, needles to further biochemical test in microorganism identification [13].

These techniques based on production substrate material for specific microorganism enzyme, according to the produced color by the microorganism can be identified easily [14].

S. aureus isolates was identified as a serious cause of nosocomial infections, and more recently, community-acquired MRSA was recognized as an emerging problem in a number of countries [15].

In the current study 17 out of 26 isolates (65.3 %) of *S. aureus* were methicillin- resistant *S. aureus*.

MRSA is a major human pathogen responsible for a wide spectrum of diseases [16-17].

Recently a shift in the epidemiology of MRSA infections have been documented, where by community associated methicillin-resistant *S. aureus* (CA-MRSA) infections have became more common. CA-MRSA may arise from the hospital origin clone that is carried into the community and then transmitted between the communities and then transmitted between or from de novo development of resistance through acquisition of resistance factor (mec A) by methicillin sensitive strains of *S. aureus* [18-19].

To detect MRSA in clinical samples ideally you should have and report the results within a short time [20].

In our results MRSA detected by chromogenic medium showed higher sensitivity , and this result agrees with many previous studies. [10,12, 21, 20]

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