

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS AUREUS USING STANDARD METHODS

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Abstract: A methicillin resistant *S. aureus* (MRSA) screen tests solely for the presence of MRSA and no other microbes. This test is used to screen for MRSA in patient in hospitals. The first strain of penicillin resistant *S. aureus* was isolated in London, England hospitals. *Staphylococcus* is a versatile organism with several virulent characteristics and resistance mechanisms. Multidrug resistance is now the norm among the Gram-positive bacteria pneumococci, enterococci and staphylococci. *S. aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections in humans and in various animal species, and its capacity to adapt to different environmental conditions. In the present study, a total of 36 isolates of MRSA were obtained. All the suspected colonies were gram stained and plated on manitol salt agar. Growth of golden yellow colonies of *Staphylococcus aureus* on manitol salt agar plate. On gram staining gram-positive cocci in clusters were observed on all the slides. On manitol salt agar, small yellow manitol fermenting colonies were obtained. On MacConkey agar small light pink lactose fermenting colonies were observed. In the present study, the efficiency of the tube coagulase test can be markedly improved by sequel testing of the isolates with Mannitol salt agar, DNase and Tube coagulase. There is no single phenotypic test (including tube coagulase) that can guarantee reliable results in the identification of *Staphylococcus aureus*.

Keywords: Gram-positive, MRSA, Resistant strain, *Staphylococcus*, Virulent

INTRODUCTION

Staphylococci are Gram-positive salt tolerant and spherical shaped bacteria. They are classified as coagulate positive (e.g. *Staph. intermedius* & *Staphylococcus aureus*) and coagulate negative (e.g. *S. epidermidis*, *S. hemolyticus*, *S. capitis*, *S. saprophyticus*) (Funmilola A. Ayeni, B.T Odumosu. 2016). The cocci are spherical, approximately 1µm in diameter, arranged in grape like clusters. They may be found singly, in pairs or in short chains especially in liquid culture. They are non-motile and non-sporing and some strains possess microscopically visible capsules (Anathanarayan, 2002). They grow readily on ordinary media within a temperature range of 10-42°C. Optimum temperature is 37°C and pH 7.4-7.6. On nutrient agar a typical 24hr *S. aureus* colonies are pigmented, smooth, entire, slightly raised, translucent and hemolytic on routine blood agar. Small colony variants (SCVs) of *S. aureus* produce colonies that are pinpoint in size, non hemolytic and non pigmented. In liquid medium, uniform turbidity is produced. Selective media used for isolating *S. aureus* contain 8-10% NaCl like salt-milk agar, ludlam's medium containing lithium chloride and tellurite (Bannerman, 2003).

The Objectives of the present study are collection of clinical specimens of ear pus, pus from wounds and post operative infections, boils, pimples and furuncles. The Isolation and identification of *Staphylococcus aureus* isolates using standard techniques.

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MATERIALS AND METHODS

Collection and processing of clinical samples

The clinical Microbiology specimen selected for this study were pus sample which included ear pus, pus from wounds and post operative infections, boils, pimples and furuncles.

The specimens were collected at the hospital aseptically within 2 h of collection these were transported to the laboratory for further processing. Sheep blood agar and mannitol salt agar was used for inoculation.

(i) Nutrient Agar Media

For the isolation of pure culture, Nutrient Agar Media (Hi-Media, Mumbai) was carefully weighed and dissolved in double distilled water properly thereafter pH was adjusted using pH meter and then melted on hot plate with magnetic stirrer (Remi). The medium was sterilized by autoclaving at 15 lbs for 15 minutes and allowed to cool down at approx. 45 to 50°C. Approximately 20 mL of the medium was aseptically poured in each sterile glass Petri dish. These were allowed to solidify under laminar flow. For the preparation of slants, the medium was prepared as described above and 5 mL of medium was poured in each 30 mL screw capped culture tube and then sterilized by autoclaving at 15 lbs for 15 min.

After autoclaving the tubes was gently shaken with a care not to allow any bubble, to mix the agar properly. These were placed at 45° angle in a slanting position to form the slants. These were stored in refrigerator until used.

(ii) Mannitol Salt Agar Media

For the isolation and identification of *S. aureus*, Mannitol Salt Agar Media (Hi-Media, Mumbai) was carefully weighed (111.02g) and dissolved in double distilled water (1000 mL) properly thereafter pH was adjusted using pH meter, then melted on hot plate with magnetic stirrer (Remi). The medium was sterilized by autoclaving at 15 lbs for 15 minutes and allowed to cool down at approx. 45 to 50°C. Approximately 20 mL of the medium was aseptically poured in each sterile glass Petri dish. These were allowed to solidify under laminar flow.

(iii) Blood agar media

For the isolation and identification of *S. aureus*, Blood Agar Media (Hi-Media, Mumbai) was carefully weighed (40.0 g) and dissolved in double distilled water (1000 mL) properly thereafter pH was adjusted to 7.2 using pH meter, then melted on hot plate with magnetic stirrer (Remi). The medium was sterilized by autoclaving at 15 lbs for 15 minutes and allowed to cool down at approx. 45 to 50°C. Approximately 20 mL of the medium was aseptically poured in each sterile glass Petri dish. These were allowed to solidify under laminar flow.

(iv) Coagulase Production

The ability to clot plasma is generally accepted criterion for the identification of *S. aureus*. Two different coagulase tests are performed: a tube test for detecting free coagulase and slide test for bound coagulase or clumping factor. While the tube test is definitive, the slide test may be used as a rapid screening technique to identify *S. aureus*. Coagulase test is carried out using rabbit plasma containing EDTA (Bannerman, 2003).

(v) Biochemical reactions of *Staphylococcus aureus* (Aneja, 2009).

They ferment sugar producing acid but no gas. Mannitol is fermented anaerobically only by *S. aureus*. They are catalase and urease positive. They reduce nitrates to nitrites, liquefy gelatin and are MR, VP positive but indole negative. They are lipolytic when grown on medium containing egg yolk. They produce phosphatase which can be demonstrated by growing on nutrient agar containing phenolphthalein diphosphate. In a medium containing potassium tellurite, tellurite is reduced and black colonies are produced (Humphreys, 2002; Anathanarayan, 2002). The characteristic clusters of gram positive cocci can be demonstrated by microscopy and the organism can be cultured readily on blood agar and most other media. Tube or slide coagulase is performed to distinguish *S. aureus* from coagulase negative staphylococci (Humphreys, 2002).

OBSERVATIONS

Fig 1.1. Gram positive cocci in clusters
Staphylococcus aureus.



Fig. 1.2. Whitish yellow pigmented colonies of
Staphylococcus aureus

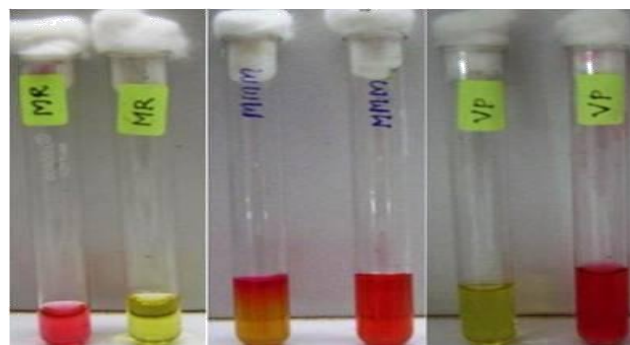


Fig. 1.3. Slide coagulase test for identification of *S. aureus*

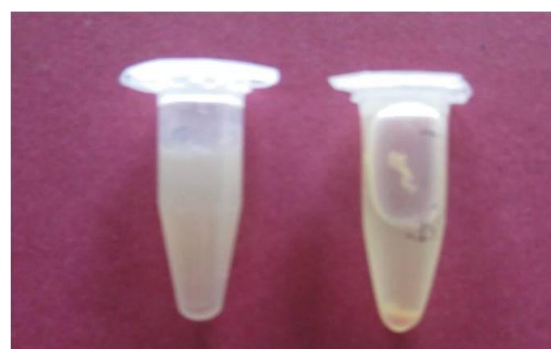


Fig. 1.4. Tube coagulase test for the identification of
S. aureus.



Fig. 1.5. Growth of *S. aureus* on Mannitol Salt Agar medium after 24 h, showing golden yellow pigmentation



Fig. 1.7 MR VP and motility test for the the identification of *S. aureus*.



Fig. 1.6. DNase tests for the identification of *S. aureus*.



Fig. 1.8 TSI, Citrate, Urease test for identification of *S. aureus*.

Table 1. Biochemical tests of clinical isolates, collected from clinical samples.

Isolate No.	Gram's Staining	Catalase	Coagulase	Oxidase	IMVIC Test			
					Indole	MR	VP	Citrate
DPC191	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC192	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC193	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC194	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC195	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC196	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC197	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC198	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC199	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1910	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve

DPC1911	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1912	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1913	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1914	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1915	+ve	+ve	+ve	-ve	Nd	+ve	+/-ve	+ve
DPC1916	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1917	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1918	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC1919	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC1920	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
DPC1921	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1922	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1923	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1924	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1925	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1926	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1927	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1928	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1929	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1930	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1931	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1932	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1933	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1934	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1935	+ve	+ve	+ve	-ve	-ve	+ve	+/-ve	+ve
DPC1936	+ve	+ve	+ve	-ve	-ve	Nd	+/-ve	+ve
+ve=positive; -ve=negative; Nd=not detected; +/-= variable								

RESULTS AND DISCUSSION

Identification of Isolates

The specimens selected for the study were pus cultures. All the specimens were inoculated on blood agar and nutrient agar and after overnight incubation at 37°C; the plates were screened for growth of *Staphylococcus aureus*. Large, circular, convex, smooth and opaque colonies were selected from nutrient agar (Fig. 1.2). Most of them were golden yellow coloured and a few were white in colour. On blood agar colonies with similar morphology were selected, most of them showed beta hemolysis. All the suspected colonies were gram stained and plated on manitol salt agar.

Identification of *S. aureus* by cultural characteristics

Growth of golden yellow colonies of *Staphylococcus aureus* on manitol salt agar plate is given in Figure 1.5. On gram staining gram-positive cocci in clusters were observed on all the slides. On manitol salt agar, small yellow manitol fermenting colonies were obtained as shown in Figure 1.5. On MacConkey agar small light pink lactose fermenting colonies were observed.

DNase, Catalase and Coagulase test

All the isolates were confirmed as *Staphylococcus aureus* by a positive catalase, coagulase and DNase test as given in Fig. 1.3, 1.4 and 1.6.

Biochemical reactions

The biochemical reactions of *S. aureus* isolates are given in Table 4.1b. All the sugars were fermented producing acid but no gas. On mannitol motility medium (MMM) fermentation of manitol was observed and the bacteria were non motile (Fig. 1.7). On TSI, glucose, sucrose, lactose and manitol were fermented resulting in acid slant and butt (A/A).

Indole was not produced and citrate was not utilized. So indole and citrate were negative. But they were MR and VP positive. When 5 drops of methyl red was added to the bacterial culture, incubated in glucose phosphate broth for 48 h, bright red color was observed indicating a positive MR reaction. Addition of 1ml of KOH and 3ml of 5% solution of α Naphthol to bacterial culture incubated in glucose phosphate broth for 48 h yielded deep red color after 30 min indicating a positive VP test.

CONCLUSION

The efficiency of the tube coagulase test can be markedly improved by sequel testing of the isolates with Mannitol salt agar, DNase and Tube coagulase. There is no single phenotypic test (including tube

coagulase) that can guarantee reliable results in the identification of *Staphylococcus aureus*.

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