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Original article

Conventional and rapid methods for identification of *Staphylococcus aureus* from clinical specimens

N.C.J. Anyanwu[a,*], W.C. John[b]
[a]*Department of Microbiology, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[b]*Federal College of Forestry, Jos, Plateau State, Nigeria.*

[*]Corresponding author; Department of Microbiology, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria.

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ABSTRACT

Staphylococcus aureus is a facultative anaerobic Gram-positive coccal bacterium whose incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. The purpose of this study was to identify Staphylococcus aureus from clinical specimens using routine conventional and rapid tests. Gram staining, catalase test, coagulase test, DNase test, haemolysis on blood agar and Microgen™ STAPH-ID kit tests were carried out. A total of 125 Gram positive cocci were tested. The Gram staining technique yielded 100 (80.00%) Staphylococcus spp (Gram positive cocci in clusters). 89(71.20%) isolates were positive to haemolysis on blood agar. Mannitol Salt Agar, DNase agar and Catalase test correctly identified 69 (55.2%) of the Gram positive cocci to be S. aureus as was confirmed by the Microgen™ STAPH-ID kit test. Coagulase test yielded 66 (52.8%) positive results. The Microgen™ STAPH-ID kit test identified three non-coagulase Staphylococcus aureus isolates. The Microgen™ STAPH-ID kit test was the most reliable of the tests, with accuracy comparable to any other rapid test. However, it is the most expensive of the tests. This study established that conventional tests can be used for direct identification of S. aureus to species level if the battery of tests is increased.

1. Introduction

Staphylococcus aureus is a facultative anaerobic Gram-positive coccal bacterium whose incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections (Menichetti, 2005).. Rapid and direct identification of S. aureus is crucial for proper management of patients with skin infections, abscesses, septicemia/bacteremia, gastroenteritis, endocarditis, toxic shock syndrome and certain food intoxications (Durack et al., 1994; Martineau et al., 1998).

In developing countries, phenotypic tests are routinely used in the diagnosis of staphylococcal infections, in which coagulase tests are usually confirmatory for S. aureus (Bello and Qahtani, 2006; Mugalu et al., 2006). Staphylococcus aureus is usually isolated on non-specific media (e.g. blood agar) and then presumptively identified before definitive overnight characterization (Kloos and Bannerman, 1995). In an attempt to achieve presumptive isolation in a single step, mannitol salt agar (MSA) was developed in 1945 for the selective isolation of pathogenic staphylococci in the clinical microbiology laboratory (Chapman, 1945). The growth and production of yellow colonies, due to the high salt content of the medium and fermentation of mannitol, is regarded as a presumptive tool in the identification of S. aureus. It is also described as a characteristic for the differentiation of coagulase-positive staphylococci from coagulase-negative staphylococci (CoNS) (Duguid, 1989). However, there are reports that some CoNS can also produce yellow colonies on MSA (Jayaratne and Rutherford, 1999; Simor et al., 2001; Zadik et al., 2001). Single phenotypic tests are inefficient for the identification of S. aureus. However, a combination of MSA and DNase improves the tube coagulase test (Najjuka et al., 2010).

The purpose of this study was to identify Staphylococcus aureus from clinical specimens using routine conventional and rapid tests.

2. Materials and methods

2.1. Specimens

A total of 100 clinical specimens (ranging from swabs to internal fluids except blood and cerebrospinal fluid) positive for Gram positive cocci (GPC) were included (Table1). Specimens were collected from patients in hospitals in Zaria, Nigeria, between April and October, 2012. Specimens collected were transported immediately to the postgraduate Clinical Microbiology laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, were the tests were performed.

2.2. Identification of isolates using conventional tests

Isolates were incubated at 37°C for 18-24 hours on blood agar (Oxoid, Cambridge, UK) and observed for haemolysis. Gram staining was carried out on presumptive isolates. Single colonies of Gram positive cocci were then tested with catalase test, coagulase test, DNase test and growth on MSA. Sequel testing of the isolates was further performed beginning with MSA, followed by DNase and finally Tube CoagulaseTest, to evaluate the performance of individual tests. S. aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228 were used as positive control.

2.3. Characterization of isolates using Microgen™ STAPH-ID system

The isolates suspected to be Staphylococcus spp were further identified with Microgen™ MID-69 microwell test strips. A single colony each, from 18-24 hours culture was emulsified in suspending medium supplied in the kit. The adhesive tapes sealing the microwell test strips were carefully peeled off and kept safe. Sterile Pasteur pipette was used to add 3-4 drops (approximately 100µL) of bacterial suspension to each well of the strips, after which wells 10 and 11 were overlaid with 3-4 drops of mineral oil. The microwell test strips were then sealed with the adhesive tapes removed earlier and incubated at 35-37 ºC for 18-24 hours. The results were read with the aid of the Microgen™ software database (containing the colour chart and substrate reference table) after adding the necessary reagents (PYR and Nitrate A and B).

3. Results

Table 1 shows the isolates screened in this study, Table 2 shows S. aureus isolated using conventional tests, and Table 3 shows Microgen STAPHYLOCOCCUS-ID 12 test result for some s. aureus isolates

Table 1
Isolates screened in this study.

Organism	Number of isolates obtained
Staphylococcus aureus	69
Staphylococcus epidermidis	31
Other Gram positive cocci	25
Total	125

Table 2
Isolation of *S. aureus* using conventional tests (including sequel testing).

Tests conducted	Number of isolates screened	Number of positive isolates	Percentage positive (%)
Gram Staining	125	100	80.00
Haemolysis on Blood Agar	125	89	71.20
Yellow colonies on MSA	125	69	55.20
Clear zone on DNase Agar	125	69	55.20
Catalase Test	125	69	55.20
Coagulase Test	125	66	52.80

4. Discussion

This study evaluated conventional and rapid methods for identification of S. aureus from clinical specimens. In our study, Microgen™ STAPH-ID test, Growth on MSA, DNase agar and catalase test had 100% sensitivity and specificity respectively. Haemolysis on Blood agar had 77.53% sensitivity and 64.29% specificity while Gram staining reported 69.00% sensitivity and 44.64% specificity. Coagulase test was 100% sensitive and 94.92% specific. Microgen™ STAPH-ID test also identified three coagulase negative S. aureus (CoNS) isolates that had been missed by coagulase test, although they were identified by other tests. Altered colonial morphology and negative reaction could have been due to subsequent sub-culturing before testing. This observation suggests that Microgen™ STAPH-ID test may be more specific than coagulase test for identifying S. aureus. Hence, to boost the specificity of coagulase test, sequel tests should be conducted on fresh isolates. Furthermore, it is conceivable that misleading results may occur if single tests such as haemolysis on Blood agar are used, particularly if there is a mixed growth, hence, the use of MSA and DNase agar are advised. Procop et al (2002) suggested that routine culture with standard agar plates continue to be used to identify cultures that contain mixtures of bacteria. Microgen™ STAPH-ID test, Growth on MSA, DNase agar and catalase test were the most reliable tests with 100% sensitivity and specificity. This is in line with Najjuka et al (2010) who reported that growth on MSA was the best at identifying S. aureus (though with lower sensitivity and specificity of 94% and 79% respectively) and recommended simultaneous use of all the three tests (beginning with growth on MSA, DNase and then Tube coagulase) for identification of S. aureus. Microgen™ STAPH-ID test was reliable in this study, but also relatively expensive; hence, its extensive use for routine identification of S. aureus in a developing country like Nigeria is a great challenge.

Table 3
Microgen *Staphylococcus-ID* 12 test result for some isolate.

Isolate Code	LAT	CPG	NIT	SUC	TRE	MAN	NAG	MNS	TUR	PHO	βGL	βGN	URE	ARG	PYR	Octal Code	Identification
P25	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-	47766	*S. aureus subsp. aureus*
ER05	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	57766	*S. aureus subsp. aureus*
W57	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	57764	*S. aureus subsp. aureus*
W120	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	77766	*S. aureus subsp. aureus*
AS103	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	67765	*S. aureus subsp. aureus*
W15	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	77766	*S. aureus subsp. aureus*
W11	-	+	+	+	-	+	+	+	+	+	+	-	+	-	-	35764	*S. aureus subsp. aureus*
P94	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	67766	*S. aureus subsp. aureus*
EY48	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	77766	*S. aureus subsp. aureus*
AS33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	77776	*S. aureus subsp. aureus*
AS67	+	-	+	+	-	-	-	+	-	-	+	-	-	+	-	54222	*S. epidermidis*
EY14	+	-	-	+	-	-	-	-	+	+	-	-	+	-	+	44145	*S. epidermidis*
ER78	-	-	+	+	-	+	-	+	-	+	+	-	+	-	-	15264	*S. epidermidis*
W32	-	+	-	+	-	-	-	+	-	+	+	-	+	-	-	24264	*S. epidermidis*
ER12	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	44222	*S. epidermidis*
EY17	-	+	-	+	-	+	+	+	+	+	+	-	+	-	-	25764	*S. aureus subsp. aureus*
W37	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	35766	*S. aureus subsp. aureus*
W72	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	77666	*S. aureus subsp. aureus*
ER01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	53565	*S. aureus subsp. aureus*
ER95	+	-	-	-	+	+	+	-	-	+	+	-	+	-	-	43464	*S. aureus subsp. aureus*

Key: LAT- Latex Agglutination Test, CPG- Colony Pigmentation, NIT- Nitrate, SUC- Sucrose, TRE- Trehalose, MAN- Mannitol, NAG- N-Acetyl Glucosamine, MNS- Mannose, TUR- Turanose, PHO- Alkaline Phosphate, βGL- βGlucosidase, βGN- βGlucuronidase, URE- Urease, ARG- Arginine, PYR- Pyrrolidonyl Arylamidase, W= Wound swab, EY=Eye swab, ER=Ear swab, P=Pus, AS= Aspirate.

5. Conclusion

This study reveals that routine conventional tests are reliable for identification of S. aureus in resource limited areas. There is however, no single test that can guarantee reliable results, hence the need for sequel testing using mannitol salt agar, DNase agar, then coagulase test.

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