Staphylococcus aureus – A Versatile Pathogen Biochemical Characterization and Antiibiogram

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Abstract

S. aureus is a major human pathogen capable of causing a wide range of infections and remains the second cause of nosocomial infections. To biochemically characterise the isolated coagulase-positive staphylococci, to determine its antimicrobial susceptibility and the MRSA status. 2220 samples were processed in the study period among which 150 were S. aureus, identified by Gram staining and coagulase test. Tests for characterisation included mannitol fermentation, demonstration of pigment production on milk agar, phosphatase test, DNase test, tellurite reduction test and urease production. Anti-microbial susceptibility was done using the commonly used antibiotics and MRSA (Methicillin-resistant Staphylococcus aureus) status was determined by cefoxitin disc diffusion method. Of the 150 tube coagulase positive S. aureus, only 145 (96.7%) were haemolytic, which were slide coagulase positive too in the first minute. The remaining 5 were slide coagulase positive after 1 minute. The positivity of 150 S. aureus to the other pathogenicity tests were mannitol fermentation (100%), DNase test (100%), pigment production on milk agar (100%), tellurite reduction (95.3%), phosphatase production (92%) and urease test (90.7%). Out of the 150 S. aureus, 54 % were MRSA. All the S. aureus isolates were resistant to ampicillin and cephallexin, partially resistant to other antibiotics and completely sensitive to vancomycin and linezolid. The MRSA isolates were resistant to cotrimoxazole, chloramphenicol, ciprofloxacin, tetracycline, gentamicin, erythromycin and clindamycin. The methicillin-sensitive strains were resistant to the above mentioned antibiotics but with a lower percentage; difference being about half the total percentage.

Keywords
S. aureus, Coagulase test, MRSA

Introduction

Staphylococcus aureus (S. aureus) is normally a ubiquitous, but relatively innocent commensal and coloniser of the skin and mucosa of humans and several animal species which is in apparent contrast to its infectious potential (Van Belkum et al., 2009). S. aureus has been demonstrated to be a major human pathogen capable of causing a wide range of infections, from relatively mild skin infections such as folliculitis and furunculosis to life-threatening conditions, including sepsis, deep abscesses, pneumonia, osteomyelitis, blood
stream infections and infective endocarditis through both toxin-mediated and non-toxin-mediated mechanisms (Van Belkum et al., 2009; Moreillon et al., 2005; Lowy, 2012).

*S. aureus*, has demonstrated its versatility by remaining a major cause of morbidity and mortality despite the availability of numerous effective anti-staphylococcal drugs (Lowy, 2012).

The first case of methicillin-resistant *S. aureus* (MRSA) was described in the United Kingdom in 1961. Currently, nosocomial MRSA rates approach 60% or more in many areas of the country (National nosocomial infections surveillance report, 2004).

The emergence of resistant strains represents a consequential response to selective pressures imposed by antimicrobial chemotherapy and once established, they are difficult to control and eradicate (Saikia et al., 2009).

Most isolates remain susceptible to glycopeptides (vancomycin, teicoplanin), oxazolidinones (linezolid), streptograminins (quinupristin-dalfopristin), and polycyclic compounds (tetracycline, tigecycline) (Moreillon et al., 2005; Deresinski, 2005). Low level resistance even to vancomycin is emerging at present (Assadullah et al., 2003). The prevalence of MRSA strains is reported to be increasing.

Interestingly, there appears to be significant variable in the epidemiology and prevalence of MRSA in different parts of the world and even in different regions of a country (Voss et al., 1994). Detection of mecA gene or its product, penicillin binding proteins (PBP 2a), is considered the gold standard for MRSA confirmation (Skov et al., 2006). Results of cefoxitin disc diffusion test is in concordance with the PCR for mecA gene, and thus the cefoxitin disc diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings (Anand et al., 2009). Constant monitoring of these strains is essential in order to control their spread in hospital environment and transmission to community (Naseer and Jayaraj, 2010). Hence, the present study is undertaken to know the cultural characteristics, and to study the antibiotic sensitivity pattern of *S. aureus* isolated from clinical samples in our hospital with special reference to methicillin-resistant *S. aureus*.

**Materials and Methods**

This study was conducted for a period of one and half year; from November 2011 to May 2013 in the Department of Microbiology, K.V.G Medical College and Hospital. *S. aureus* strains isolated from all clinical specimens received during the study period to microbiology laboratory were analysed.

**Specimen collection**

Pus, discharge from skin and soft tissue infections, swabs from ears, conjunctiva and umbilicus, urine and blood obtained from patients of K.V.G Medical College Hospital were included in the study Identification of *S. aureus* was done by standard procedure. Tests for characterisation included catalase test, coagulase tests- slide and tube, mannitol fermentation, demonstration of pigment production on milk agar, phosphatase test, DNase test, tellurite reduction test and urease production.

Antimicrobial susceptibility tests were carried out by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The following discs (Hi-Media, Mumbai) were used: Ampicillin (10µg), Cephalexin (30µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Pristinamycin (15µg),
Linezolid (30µg), Amikacin (30µg),
Tetracycline (30µg), Gentamicin (10µg),
Erythromycin (15µg), Clindamycin (2µg), and
Vancomycin (30µg).

Methicillin resistance was screened by disc
diffusion method using 30µg cefoxitin disc.
The diameter of the zone of inhibition was
measured and interpretation was done in
accordance with the CLSI guidelines. An
isolate was considered to be a MRSA strain if
cefoxitin inhibition zone diameter was < 21
mm.

Analysis of data

The data was analysed on IBM SPSS version
19. Chi-square test was applied to test the
difference between proportions, at 5% level of
significance.

Results and Discussion

A total of 2220 samples were processed in the
Department of Microbiology during the study
period, which included 965 urine samples, 531
pus and wound swabs, 372 blood samples, 287
sputum and 65 body fluids (Fig. 1).

Of the 150 tube coagulase positive S. aureus,
only 145 (96.7%) were haemolytic, which
were slide coagulase positive too in the first
minute. The remaining 5 were slide coagulase
positive after 1 minute.

The positivity of 150 S. aureus of other
pathogenicity tests were mannitol
fermentation (100%), DNase test (100%),
pigment production on milk agar (100%),
tellurite reduction (95.3%), phosphatase
production (92.%) and urease test (90.7%).

Of the 150 strains of S. aureus, 97 (65%) were
from male patients, 53 (35%) were from
female patients. Majority of the isolates of S.
aureus were from pus (87.3%). The infection
of other anatomical sites all put together was
only 12.7%.

Among the battery of antibiotics used, S.
aureus exhibited complete susceptibility to
vancomycin (100%) and linezolid (100%).
Next in the susceptibility order were
pristinamycin (84.7%), amikacin (66.7%),
clindamycin (56.7%), tetracycline (56.7%)
and cotrimoxazole (50.7%). Less than 50%
susceptibility was observed with erythromycin
(38%), chloramphenicol (38%), ciprofloxacin
(35.3%) and gentamicin (22.7%). A 100% resistance was shown to ampicillin and 1st
generation antibiotic, namely cephalaxin. All
the S. aureus were subjected to cefoxitin disc
diffusion for detection of methicillin
resistance (Fig. 2).

Sixty nine (46%) strains of the 150 isolates of
S. aureus were methicillin-resistant (MRSA)
by cefoxitin disc diffusion method

S. aureus isolated from pus was 88%, 50% being MRSA and 38% being MSSA (Fig. 3).

MRSA infections of the in-patients were
highest after the 4th day (25.2%) of
hospitalisation, which was highly significant
(p value 0.001). In contrast, MSSA were
highest on day 1 (26.2%) and by 4th day
reduced to zero (Fig. 4).

Figure 5 illustrates the pattern of susceptibility
of MRSA and MSSA isolates to other
antibiotics. While both MRSA and MSSA
were 100% sensitive to vancomycin and
linezolid, 83.9% and 85.6% respectively were
sensitive to pristinamycin. Next in the
susceptibility order were amikacin (69.2% and
63.7%), clindamycin (51.8% and 62.3%) for
MRSA and MSSA respectively. Less than
50% susceptibility was observed with
chloramphenicol (38.3% and 37.7%),
cotrimoxazole (29.6% and 49.3%),
erthyromycin (28.4% and 49.3%), and
ciprofloxacin (23.4% and 49.3%) for MSSA and MRSA respectively. A very high sensitivity pattern to tetracycline was observed for MSSA (92.8%) as compared to MRSA (25.9%). Both MSSA and MRSA were totally resistant to ampicillin and cephalexin.

Out of the 150 patients from whom S. aureus was isolated, 43 had the history of antibiotic use earlier (Fig. 6).

All the 43 S. aureus isolated from these patients were MRSA, which is highly significant (p value 0.001).

S. aureus is the most important nosocomial pathogen because of both the diversity and the severity of the infections it causes, including superficial, deep skin, and soft-tissue infections, endocarditis, and bacteremia, as well as a variety of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded-skin syndrome, and toxic shock syndrome.

Coagulase is an established pathogenicity test, done on spot for all staphylococci. Initially, the slide coagulase test is done which is followed by the tube coagulase for confirmation. In our study, all the S. aureus gave positive result for the coagulase test by both the methods. Earlier reports (Rajaduraiapandi et al., 2006; Brown and Ngeno; Shanthi and Sekar, 2009; Arora et al., 2010; Rahbar and Hajia, 2007; Han et al., 2007) too have established the high quality of coagulase test in establishing pathogenicity of S. aureus. Hence, the method is considered a ‘gold standard’ in the identification of S. aureus.

Mannitol fermentation, DNase and coagulase tests are the pathogenicity tests of importance for S. aureus. We observed that, both DNase production and mannitol fermentation were as sensitive as coagulase test in establishing the pathogenicity of S. aureus. (Han et al., 2007) reported only 99.6% positivity for S. aureus for mannitol fermentation test. Other pathogenicity tests for S. aureus such as production of pigment on milk agar (100%), phosphatase enzyme (92%) and reduction of tellurite to tellurium (95.3%), also showed high positivity though not 100%.

All the S. aureus have shown pigment production on milk agar but there was a slight variation in the tinge of golden-yellow colour. 86.7% of S. aureus demonstrated the production of golden-yellow pigment, the rest of the 13.3% produced a light-yellow pigment - which is definitely a feature of pathogenicity.

As compared to all the other characteristics, urease test shows the variation- 90% were positive, 10% were negative. Similar findings were observed by Udo et al., (2006), where urease positivity was 62%.

There is a significant difference between S. aureus amongst males (65%) and females (35%), which is almost similar to Tsering et al., (2011).

Our isolation rate of S. aureus from pus is also on the higher side of the spectrum (87%). We have isolated S. aureus from 4.7% of urine samples which is comparable to the studies done by Anupurba et al., (2003), Shanthi and Sekar (2009) and Velvizhi et al., (2011). S. aureus was isolated from only 3.3% of the blood samples which is in correlation with the reports of Rohani et al., (2000) and Velvizhi et al., (2011).

In our study, we have observed complete resistance (100%) to ampicillin and cephalexin. Similarly, Rohani et al., (2000) and Shanthi and Sekar (2009) have encountered 94% resistance to ampicillin and 82.5% resistance to ampicillin and cephalexin respectively.
**Fig.1** Distribution of *S. aureus* among various clinical samples

![Pie chart showing distribution of S. aureus among various clinical samples](image)

**Fig.2** Antibiotic sensitivity pattern of *S. aureus*

![Bar graph showing antibiotic sensitivity pattern of S. aureus](image)

**Fig.3** Percentage of MRSA and MSSA among the *S. aureus*

![Pie chart showing percentage of MRSA and MSSA](image)

**Fig.4** Association of MRSA and MSSA with number of days of hospitalisation

![Bar graph showing association of MRSA and MSSA with number of days of hospitalisation](image)
The resistance pattern to various other drugs varied from 15.3% to 61.3% which is similar to that of the other studies (Shanthi and Sekar, 2009; Verma et al., 2000; Vardhan et al., 2000; Naik and Deshpande, 2011; Dhanalakshmi et al., 2012.) IT was observed that a total susceptibility of S. aureus to vancomycin and linezolid which is in concurrence with other studies (Joshi et al., 2013). The slight variation in the resistance pattern of S. aureus to various antibiotics can be attributed to regional variation in the prescription pattern of antibiotics.

Because of the widespread use of antibiotics, especially in developing countries, the resistance profile of microorganisms is changing, evidenced by increasing occurrences of antibiotic resistance among bacterial populations. Additionally, resistance rates are typically higher in developing countries (with rates up to 99%) as compared to developed countries (where rates are less than 20%). Consequently, it is imperative that local surveillance of common pathogenic organisms and their antibiograms be implemented to advise the current use of antibiotics. This is essential to the formulation of prescribing policies based on local statistics.

Our study provides important data on current antimicrobial resistance, including methicillin resistance, for a collection of recent clinical isolates of S. aureus from various clinical samples in our hospital. MRSA is a major nosocomial pathogen causing significant morbidity and mortality. There appears to be a significant variable in the epidemiology and prevalence of MRSA in different parts of the world and even in different regions of a country. MRSA since their occurrence in 1961, the incidence has been increasing and a lot of variation in occurrence of MRSA in
various parts of the country and abroad. As it is causing lots of infections among hospitalised patients and community, MRSA has been targeted for intensive study by various authors.

Now coming to MRSA studies, studies from 1988 till date, range is varying from 6.9% to 57%. Our study has also shown 54% of MRSA among S. aureus. Few studies have shown exceptionally high levels of MRSA like Naseer and Jayaraj (2010), Gulbarga, whereas all others are around 50% for around 8-10 years. Our MRSA pattern is around 54% which is in comparison with most of the studies done during recent years. Hence, constant monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community. In this context, we have also observed about 50% of CoNS were methicillin-resistant.

All the pathogenic staphylococcal isolates are subjected to MRSA testing routinely. Such a high prevalence of MRSA in our study could be due to several factors. The indiscriminate use of antibiotics, lack of awareness and unethical treatment before coming to the hospital might have been the contributing factors.

There is a lot of variation in the MRSA resistance pattern to the common used antibiotics. In our study, though 100% resistance was observed to ampicillin and cephalaxin, for all the other antibiotics, the resistance pattern was within a limited zone. All these type of variable results need to be kept in mind before administration of any antibiotics in any of the hospital set ups or out-patient clinics. The above graph also indicated that a uniform antibiotic policy has not been followed strictly in India. Therefore, there is a need for formulation of a strict antibiotic policy towards these “notorious bugs” which are taking a lot of time, money and loss of working hours/ labour in the infected patients.

So formulation of a uniform antibiotic policy is the need of the hour. Also, a uniform quality control assessment program must be followed for accurate judgement of susceptibility testing techniques.

It is hoped that the results of this study may be useful to the clinicians in the management of hospitalised patients and out-patients with appropriate antibiotic advice. We expect such studies will also be helpful to the community at large.

Any amount of work on S. aureus is not sufficient to demonstrate their virulence factors, which includes resistance to various antibiotics. Maybe, repeated work has to be done in various regions for formulating antibiotic policy.

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