

Antibiotic susceptibility pattern among clinical isolates of *Staphylococcus aureus* with special reference to vancomycin

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Abstract

Introduction: *Staphylococcus* is a major pathogen of community and hospital acquired infections. Vancomycin is used in MRSA caused infections. Emergence of VISA and VRSA has been of great concern in clinical aspects.

Materials and Methods: All clinical samples were processed in the laboratory according to standard procedure. Inoculated plates were incubated at 37° C for 24-48 hours. Only *S. aureus* isolates were included in the present study. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method using a panel of drugs. Cefoxitin disc was used to identify methicillin resistance. The MIC of vancomycin for MRSA isolates was carried out by Agar dilution method and E-test according to standard methods. Heteroresistance to vancomycin was detected by using BHI screen agar.

Results: 190 *S. aureus* were isolated from various clinical samples. Most of the isolates were resistant to amoxycylav (96.2%) followed by ciprofloxacin (84.2%), erythromycin (33.2%), Clindamycin (31.2%), Cotrimoxazole (14.6%), Teicoplanin (4.2%), Mupirocin (2.1%) and none of the isolates were resistant to linezolid. Out of 190 *S. aureus* isolates, 97 (51.1%) were identified as MRSA. None of the isolates were resistant to vancomycin by agar dilution method and E-test method. Four out of 97 (4.1%) MRSA isolates showed intermediate susceptibility to vancomycin. Among the isolates with MIC of 2µg/ml, 5 (19.2%) showed heteroresistance to vancomycin by BHI screen agar method.

Conclusion: Rapid identification of patients harboring VRSA, VISA or hVISA and adherence to infection control protocols are very important in controlling the dissemination of these pathogens.

Keywords: MRSA, VISA, VRSA, Heterointermediate resistant *Staphylococcus aureus* (hVISA), Agar dilution method, E-test.

Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen and the most common cause of nosocomial infections [Seivert DM et al., 2008]. Infections caused by *S. aureus* are difficult to treat as they have the ability to destroy neutrophils and also show antibiotic resistance [Ng ST et al., 2011]. During 1970's, resistance to new semisynthetic penicillinase resistant antimicrobial agents (methicillin, oxacillin, nafcillin) developed [B Michael et al., 1996]. MRSA has been considered as a major nosocomial pathogen in health care facilities, but in the last decade it has also been observed to be an emerging pathogen in community acquired infections [Seralathan SE et al., 2017]. This led to the increased reliance on vancomycin for the treatment of Methicillin resistant *S. aureus* (MRSA) infections [Hageman JC et al., 2006]. In 1997, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan [Tenover FC et al., 2001 & Tenover FC et al., 1998]. Subsequently, Vancomycin intermediate *S. aureus* (VISA) were reported in the United States and around the world [Tenover FC et al., 2007]. In June 2002, a Vancomycin resistant *S. aureus* (VRSA) was identified [Chang S et al., 2003].

There is an increasing number of strains of *S. aureus* showing heterointermediate resistance to vancomycin (hVISA). hVISA strains are defined as

strains of *S. aureus* that contain subpopulation of vancomycin intermediate daughter cells [Song JH et al., 2004]. Hence, the present study was done to determine antibiotic susceptibility, possible presence of VISA and VRSA and also the presence of hVISA strains among vancomycin susceptible strains of *S. aureus*.

Materials and Methods

The present study was done after obtaining the institutional ethical committee clearance.

All clinical samples were processed in the laboratory according to standard procedure (samples were inoculated on blood agar and MacConkey agar except urine sample, which was inoculated on CLED medium).

Inoculated plates were incubated at 37° C for 24-48 hours aerobically. Identification of isolate as *S. aureus* was done according to standard methods [Baird D et al., 1996] and only *S. aureus* isolates were included in the present study.

One hundred and ninety *S. aureus* were isolated from various clinical samples like pus, sputum, blood, urine, ear swab, pleural fluid, CSF, nasal swab and suction tip etc.

Antibiotic susceptibility testing was done by using Muller-Hinton agar by Kirby-Bauer disc diffusion method using a panel of drugs like Amoxycylav (30µg), ciprofloxacin (5µg), erythromycin (15µg), Clindamycin

(2µg), Cotrimoxazole (25µg), Teicoplanin (30µg), Mupirocin (5µg), Linezolid (30µg). Cefoxitin disc (30µg) was used to identify methicillin resistance. *S. aureus* ATCC 25923 was used as a control strain.

The MIC of vancomycin for MRSA isolates was carried out by agar dilution method and E-test according to the standard methods.

Agar Dilution Method: Gradient plates of MHA plates were prepared with vancomycin (0.5-32µg/ml). 0.5 MacFarland matched culture suspension was 1 in 100 diluted with sterile saline and about 10µl was spot inoculated. Plates were incubated at 35° C for 24 hours. Lowest concentration of vancomycin that inhibited the visible growth of the strain was taken as MIC for that particular isolate.

E-test: E-test was done according to manufacturer's instructions. 0.5 MacFarland matched inoculum was swabbed on Muller-Hinton agar and E-strip was placed. Plates were incubated at 35° C for 24 hours. Vancomycin susceptible *S. aureus* ATCC 29213 was used as a control.

Isolate with MIC of 2µg/ml were considered for heteroresistance detection. Heteroresistance to vancomycin was detected by using BHI screen agar.

BHI Screen Agar Plates: BHI screen agar was prepared in-house by adding 4µg/ml of vancomycin and 16g/l pancreatic digest of casein. Four 10µl droplets from 0.5 MacFarland suspension were dropped by a pipette onto the BHI screen agar plates, allowed to dry for 5 min and incubated at 35°C. Plates were examined at 24 hours and 48 hours. An isolate was considered hVISA if at least one droplet had two or more colonies [Satola SW et al., 2011].

Results

190 *S. aureus* were isolated from various clinical samples like pus (126), blood (16), sputum (12), urine (12), nasal swab (08), suction tip (05), ear swab (03), endotracheal tube (03), bronchoalveolar lavage (02), pleural fluid (02) and CSF (01) (Table 1).

Most of the isolates were resistant to amoxycylav (96.2%) followed by ciprofloxacin (84.2%), erythromycin (33.2%), Clindamycin (31.2%), Cotrimoxazole (14.6%), Teicoplanin (4.2%), Mupirocin (2.1%) and none of the isolates were resistant to Linezolid (Table 2).

Out of 190 *S. aureus* isolates, 97 (51.1%) were identified as MRSA. None of the isolates were resistant to vancomycin by agar dilution method and E-test method. Four out of 97 (4.1%) MRSA isolates showed intermediate susceptibility to vancomycin by both agar dilution method and E-test.

Among the isolates with MIC of 2µg/ml, 5 (19.2%) showed heteroresistance to vancomycin by BHI screen agar method.

Table 1: Isolation of *S. aureus* from various clinical samples

Sample	Number of <i>S. aureus</i> isolates
Pus	126
Blood	16
Sputum	12
Urine	12
Nasal swab	08
Suction tip	05
Ear swab	03
Endotracheal tube	03
Pleural fluid	02
Bronchoalveolar lavage	02
Cerebrospinal fluid	01
Total	190

Totally 190 isolates of *S. aureus* from different samples were included. Samples from which bacteria other than *S. aureus* isolated were not included in the study.

Table 2: Antibiotic susceptibility pattern of *S. aureus* isolates

Antibiotic	Number of resistant strains Out of 190	Percentage of resistance
Amoxycylav	185	96.2
Ciprofloxacin	162	84.2
Erythromycin	64	33.2
Clindamycin	60	31.2
Co-trimoxazole	28	14.6
Teicoplanin	08	04.2
Mupirocin	04	02.1
Linezolid	None	None

Discussion

S. aureus is an important cause of community and hospital infections [Saravanan M et al., 2013]. Antibiotic resistant *staphylococci*, especially MRSA have become the most common cause of hospital acquired infections worldwide. MRSA is of concern, not only because of its resistance to methicillin, but also because of its general resistance to many other chemotherapeutic agents.

Therapeutic options for MRSA infections are limited to a very expensive and potentially toxic drugs like teicoplanin, vancomycin, linezolid and daptomycin etc [K Jayatilleke et al., 2012]. So, early detection of MRSA and formulation of effective antibiotic therapy, along with infection control is very important from an epidemiological view point. The present study showed the MRSA prevalence of 51.1% among clinical samples, which is comparable to the results of other studies [K Jayatilleke et al., 2012, Joshi S et al., 2013 & Bukhari SZ et al., 2011]. Cefoxitin disc diffusion method was done to screen MRSA strains because

cefoxitin is a potent inducer of *mecA* gene, gives clearer endpoints, less affected by the hyperproduction of penicillinase, requires no special medium or incubation temperature [TA Dhanalakshmi et al., 2012].

In our study, most of the isolates were resistant to amoxyclav (96.2%) followed by ciprofloxacin (84.2%), erythromycin (33.2%), Clindamycin (31.2%) etc. This high prevalence of drug resistance may be due to the irrational antibiotic usage, due to its easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries [Tiwari HK et al., 2006].

In view of this antibiotic resistance, vancomycin has been the drug of last resort. Transfer of glycopeptide and macrolide resistant genes by transconjugation among *Enterococci* and from *Enterococci fecalis* to *S. aureus* have been reported [Saha B et al., 2008]. The present study showed decreased susceptibility to vancomycin. Four out of 97 (4.1%) MRSA isolates showed intermediate susceptibility to vancomycin, but none of them showed resistance to vancomycin. In a study by Sharma P et al., 2012, 16 out of 156 (11.5%) *S. aureus* isolates showed intermediate susceptibility to vancomycin and no VRSA were detected. Study by Saderi H et al., 2005 showed that 3.5% of the isolates were VRSA and another study by T.A. Dhanalakshmi et al., 2012 did not report any case of VISA and VRSA. Though a very few instances only, VISA are a definite entity now. But the large scale development and subsequent spread of resistance to vancomycin is perceived as a fearsome threat to the already challenging therapy of MRSA [Assadullah S et al., 2003].

The present study revealed the waning susceptibility of *staphylococci* to vancomycin. This may be as a result of prolonged exposure of organisms to constant level of vancomycin in an opportune environment [Tenover FC et al., 1998].

Infections involving hVISA pose unique problem. Such strains are susceptible in vitro to vancomycin but contain subpopulation of $1 \text{ in } 10^6$ cells that can grow in the presence of $\geq 4 \mu\text{g/ml}$ of vancomycin. Because of the increased reports of vancomycin treatment failures and poor outcomes of patients infected with hVISA, an accurate and practical method for the detection of hVISA is very important [Satola SW et al., 2011].

E-test macromethod is expensive and hVISA testing by population analysis is labor-intensive, time consuming and unsuitable for routine use [Fitzgibbon MM et al., 2007]. Hence, BHI screen agar was easy to perform and is used in the detection of hVISA. In the present study, 19.2% were hVISA strains.

Limitation of the Study

MecA gene detection by PCR technique, which is considered the gold standard method for MRSA detection was not done in our study due to economic

constraints and also controls were not used in hetero resistance (hVISA) detection methods.

Conclusion

The present study reveals the emergence of *S. aureus* with decreased susceptibility to vancomycin and indicates the magnitude of antibiotic resistance in and around the study area. The major cause of this may be unawareness and indiscriminate use of broad-spectrum antibiotics. Rapid identification of patients harboring VRSA, VISA or hVISA as well as prompt isolation and adherence to infection control protocols are paramount in controlling the dissemination of these pathogens.

Abbreviations

- MRSA- Methicillin resistant *Staphylococcus aureus*
 VISA- Vancomycin intermediate *Staphylococcus aureus*
 VRSA- Vancomycin resistant *Staphylococcus aureus*
 hVISA- Heterointermediate resistant *Staphylococcus aureus*
 CLED- Cysteine lactose electrolyte deficient
 CSF- Cerebrospinal fluid
 ATCC- American type culture collection
 MIC- Minimum inhibitory concentration
 BHI- Brain heart infusion

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