

Original article

Antibiogram and Vancomycin Minimum Inhibitory Concentration (MIC) levels of *Staphylococcus* species isolated from clinical specimens

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Abstract:

Introduction: While the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) continues to increase worldwide, there is concern regarding increase in minimum inhibitory concentrations (MIC) of vancomycin amongst *S. aureus* strains. Coagulase Negative Staphylococcus (CoNS) which are part of normal flora of skin and mucous membrane, are incriminated in life threatening bloodstream nosocomial infections in critically ill newborn, critical care unit, patient receiving immunosuppressive therapy for malignant neoplasm, hematological malignancy and bone marrow transplant. An attempt was, therefore, made to study vancomycin susceptibility in 100 *staphylococcus* isolates from various clinical samples during the one and a half years from April 2013 to September 2014.

Materials and Methods: A total of 100 isolates of *Staphylococcus species* were collected from various clinical specimens and antibiotic susceptibility was performed by Kirby Baur disc diffusion technique. Vancomycin MIC of methicillin resistant strains was detected by agar dilution method and E tests.

Results: All 100 clinical isolates of staphylococcus were susceptible to vancomycin by disc diffusion method (CLSI 2008). By agar dilution method and E tests, all 30 MRSA isolates were susceptible to vancomycin.

Conclusion: In the present study, a shift to a higher MIC level of vancomycin (1.5-2 mcg/ml) amongst MRSA isolates was noted.

Keywords: MRSA, MIC.

Introduction:

Staphylococcus species can cause a wide variety of infections like boils, food poisoning, cellulitis, and toxic shock syndrome in humans through infection or the production of toxins. Because of the colonization of healthy people, it is almost impossible to prevent contact with these bacteria. Most staphylococcus species are transmitted by person-to-person contact, but viable staphylococcus on surfaces of clothing, sinks, and other objects can contact skin and cause infections.

Staphylococcus aureus (*S.aureus*), the major pathogenic species, is one of the main causes of community and hospital acquired infections,

leading to high morbidity and mortality. The treatment of the *S.aureus* infections has become problematic because of the emergence of resistance to methicillin, vancomycin and other antibiotics.¹ Of the methicillin-resistant strains, there are 2 types: community-acquired (CA-MRSA) and healthcare-associated (HA-MRSA). Those strains identified in the community among patients, who may or may not have the predisposing factor for nosocomial MRSA infections, or yield MRSA isolates within 48-72 h after hospital administration, are called community-acquired MRSA (CA-MRSA).²

Meanwhile, HA-MRSA are in long-term care facilities, have comorbidities (such as diabetes), are on dialysis, have prolonged hospitalization, and are ICU patients with indwelling percutaneous devices or catheters.³

Community-associated MRSA (CA-MRSA) strains differ from health care-associated *S. aureus* (HA-MRSA) strains in that they are more frequently recovered from skin and soft tissue sources and have at least two clones, designated USA300 and USA400 containing staphylococcal chromosome cassette *mec* (SCC*mec*) type IVa and produce the virulence factor Panton-Valentine leucocidin (PVL).⁴ Beta-lactam resistance alone tends to be common in CA-MRSA, whereas HA-MRSA is more multidrug resistant. In CA-MRSA, the strains may be susceptible to trimethoprim-sulfamethoxazole and clindamycin, unlike healthcare-associated, which may be susceptible to trimethoprim-sulfamethoxazole but not so susceptible to clindamycin.

The determination of antimicrobial susceptibility is therefore crucial for optimal therapy, epidemiological purposes and for infection control measures⁵. The routinely used methods cannot accurately detect the methicillin and the vancomycin resistance⁶.

Coagulase Negative Staphylococcus (CoNS) are known commensals of the skin & anterior nares of human beings. Infections caused by CoNS are difficult to treat because of the coexisting risk factors and the multiple drug resistant nature of the organisms⁷.

Hence, this study was undertaken to know the antibiogram of the isolates from our tertiary care hospital, to the commonly used antibiotics & to estimate the prevalence of methicillin and vancomycin resistance among *S.aureus* and CONS.

Aims & Objectives:

1. Speciation of Staphylococcus isolates including Coagulase Negative Staphylococcus (CoNS) recovered from clinical samples.
2. Antibiogram of the isolates by Kirby Bauer Disc diffusion method.
3. Determination of minimum inhibitory concentrations (MIC) of vancomycin by Estrip/ agar dilution method

Materials and methods:

A total of 100 isolates of *Staphylococcus* species recovered from 1300 clinical samples including pus, wound swabs, ear swab, conjunctival swab, blood culture, plural fluid and urine received in the microbiology laboratory, both outdoor and inpatients of the hospital, were included during the study period. The isolates collected were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase test and anaerobic acid formation from mannitol. The identification of *S.aureus* was done by standard methods.⁶

Speciation of CoNS was done by Novobiocin and urease activity, ornithine decarboxylase, aerobic acid production from mannose and susceptibility to polymixin B.⁸

(Chart- 1)

The antimicrobial susceptibility pattern of *Staphylococcus* isolates was determined by Kirby – Bauer disk diffusion method on Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI 2008) guidelines to penicillin (10U), ampicillin (10µg), erythromycin (15µg), gentamicin (10µg), tetracycline (30µg), amoxicillin-clavulanic acid (30 µg), clindamycin (2 µg), cefazolin (30 µg), linezolid (30µg), linezolid (30µg), netilmycin (30µg) vancomycin (30µg). Standard strains of methicillin sensitive *S.aureus*

(ATCC 25923) and methicillin resistant of *S.aureus* (ATCC 43300) were used as controls.^{8,9}

The screening for methicillin resistance was done by the cefoxitin disc diffusion method (30µg).

Vancomycin MIC was determined by the E-test method (Hi Media Ezy MIC™ Strip EM 060 with vancomycin range 0.016-256 mcg/ml) & it was rechecked by the agar dilution method. All the plates were incubated at 35⁰C for 24-48 hours.

This study was approved by the Ethics committee of the institution.

Results:

Out of the 100 *Staphylococcus* isolates, 50 MSSA, 30 MRSA, 20 CoNS were identified. Majority of the MSSA i.e. 88 % (44/50) were recovered from pus and wound swab. 68% isolates were isolated from indoor patients and 32% were from outdoor patients. 66% were recovered from surgical specialties followed by 16% each from Dermatology and Pediatrics. (Table 1).

All 50 isolates of MSSA were susceptible to cefazolin, vancomycin, teicoplanin, netilmycin, and linezolid but resistant to ampicillin and penicillin.47(94%) were susceptible to gentamicin

and only 19 (38%) were susceptible to amoxycylavulanic acid.

Out of 30 MRSA isolates 93.33% were recovered from pus and wound swabs, and 96.66% were isolated from surgical specialties. Methicillin resistant strains were more frequently isolated from patients admitted to General surgery (50%).(Table1). All 30 isolates (100%) were susceptible to vancomycin, teicoplanin, netilmycin, but resistant to cefoxitin, cefazolin, amoxycylavulnic acid, ampicillin and penicillin. Sensitivity to other antibiotics ranged from 76.66% in gentamicin, 36.6% in clindamycin & 33.3% in erythromycin. (Table 2)

Amongst the CoNS, *S.haemolyticus* was the most commonly isolated species (30%) followed by *S. lugdunensis* (20%) and *S. warneri* (20%). All the CoNS isolates showed susceptibility to cefoxitin, cefazolin, vancomycin, teicoplanin, netilmycin and linezolid. No vancomycin intermediate *Staphylococcus aureus* (VISA) and vancomycin resistant *Staphylococcus aureus* (VRSA) were identified by both methods- the vancomycin agar dilution method and E-test. (Table 3)

Table 1 :Distribution of MRSA and MSSA from different clinical samples :

Clinical Samples	MSSA (n=50)		MRSA (n=30)	
	Number percentage	percentage	Number	Percentage
Pus and Wound swab	44	88 %	28	93.33%
Urine	4	8%	-	-
Conjunctival swab	2	4%	-	-
Plural fluid	-	-	1	3.33%
Ear swab	-	-	1	3.33%

Table 2 : Vancomycin MIC of MRSA (n=30)

Vancomycin (mcg/ml)	MIC	Number of isolates with MIC with Agar dilution method	Number of isolates with MIC with E-test
0.25		2 (6.66%)	3 (10%)
0.5		1 (3.33%)	1 (3.33%)
0.75		8 (26.66%)	8 (26.66%)
1		11(36.66%)	10 (33.33%)
1.5		6 (20%)	6 (20%)
2		2 (6.66%)	2 (6.66%)

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Antibiotic	MSSA(n=50)		MRSA(n=30)		CoNS(n=20).	
	Number susceptible	Percentage	Number susceptible	Percentage	Number susceptible	Percentage
Cefoxitin	50	100%	0	100%	20	100%
Cefazolin	50	100%	0	100%	20	100%
Amoxyclavulanic acid	19	38%	0	100%	16	80%
Ampicillin	0	0	0	100%	5	25%
Penicillin	0	0	10	33.3%	2	10%
Erythromycin	40	80%	11	36.6%	12	33.3%
Clindamycin	42	84%	23	76.66%	17	36.6%
Gentamicin	47	94%	30	100%	19	94%
Vancomycin	50	100%	30	100%	20	100%
Teicoplanin	50	100%	30	100%	20	100%
Netilmycin	50	100%	30	100%	20	100%
Linezolid	50	100%	30	100%	20	100%

Table: 3: Antibiotic susceptibility pattern of MRSA and MSSA, CoNS

Discussion:

Staphylococcus aureus is becoming increasingly resistant to routinely used antibiotics. In the last two decades not only has the frequency of hospital acquired staphylococcal infections increased steadily, but MRSA has become the most prevalent

and important antimicrobial resistant pathogen, causing serious nosocomial and community associated infections⁹.

In the present study, prevalence rate of MSSA was 50%; this finding was similar to a study by Anupurba, *et al* from Uttar Pradesh (45.17%)¹⁰ and

Dibah *et al* from Iran (53.6%).¹¹ However, Sharma *et al* from Mangalore reported prevalence of 74.25% of MSSA.¹² The prevalence rate of MRSA in the present study was 30%. Recent studies with similar results have been reported from Mangalore (29.1%)¹³ and Coimbatore (31.1%)¹⁴ while a study conducted in Mangalore, India by Bhat *et al*, in 1997 had found the prevalence of MRSA to be lower (21%).¹⁵

In the present study, a greater proportion of MRSA isolates were recovered from surgical specialties, i.e. patients admitted in General surgery 50% followed by Orthopedics 20%, Pediatric surgery 16.66%, Obstetrics & Gynecology 10% with only 3.33% from Medicine. Tyagi *et al*. showed high occurrence of MRSA from Neurosurgery and Orthopedics (26% and 24% respectively).¹⁶ This could be due to higher risk of cross contamination with *Staphylococcus aureus* or other nosocomial pathogens in these areas.

All the MSSA isolates were sensitive to vancomycin, teicoplanin, linezolid, netilmycin but resistant to penicillin and ampicillin in this study. Besides this, the isolates showed maximum susceptibility to gentamicin (94%), followed by clindamycin (84%), and erythromycin (80%). The low sensitivity of MSSA to amoxycylav(38%) is a cause for concern. Sharma *et al* showed 87.7% of MSSA were resistant to penicillin, 80.7% to amoxycylav, 30.5% to erythromycin, 13.9% to clindamycin, 1.56% to gentamicin.¹⁷

In contrast, all MRSA strains were comparatively more resistant to antibiotics & showed 100% resistance to penicillin, ampicillin, cefazolin, amoxycylavulanic acid, and 66.66% to erythromycin, and 63.33% clindamycin.

MSSA showed higher sensitivity than MRSA strains to gentamicin (94% vs. 76.66%), clindamycin (84% vs. 36.6%) and erythromycin (80% vs. 33.3%).

Mir *et al* showed that MRSA isolates had high resistance to ampicillin and clindamycin. This study found 3.5% resistance to vancomycin while in the present study, MSSA and MRSA isolates were 100% sensitive to vancomycin.¹⁸

CoNS, once considered to be normal flora of the human body, are now known to be a major cause of opportunistic infections. In the present study, CoNS are isolated from various clinical specimens which includes pus, wound swab, blood culture, urine, ear swab and pleural fluid. In the present study, *S.haemolyticus* was the most frequently isolated species (30%), followed by *S.lugdunensis* and *S.warneri* (20% each) and *S.schleiferi* and *S.saprophyticus* (15% each). In comparison, Parashar¹⁹ noted *S. epidermidis* as their most commonly isolated species followed by *S.haemolyticus*(16.75%).

In the present study, only one isolate of MRSA showed constitutive clindamycin resistance. Inducible clindamycin resistance was detected by D-test in 60% isolates of MRSA and 16% of MSSA. This was higher as compared to Yilmaz *et al* (24.4% MRSA, 14.8% MSSA).²⁰

Vancomycin intermediate *Staphylococcus aureus* (VISA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) strains are not detected by the disk diffusion method. Acceptable methods used to detect these strains are nonautomated and include broth or agar dilution and the E-test. Also, the Clinical Laboratory Standards Institute (CLSI) has recently lowered breakpoints for vancomycin and presently, strains with minimum inhibitory concentration (MIC) of 4–8 µg/ml are considered VISA and with MIC ≥ 16 µg/ml are considered VRSA.²¹

In the present study, two MRSA isolates had an MIC of 2mcg/ml i.e on the higher side whereas maximum number (12 MRSA) showed MIC <1 mcg/ml. This may show a shift in MIC towards the

higher level.²² Wide spread use of vancomycin among MRSA has been reported to result in reduced susceptibility to vancomycin. Vancomycin is used sparingly to treat infections in our set-up. This could possibly be the reason why vancomycin resistance was not detected among MRSA isolates in the present study.²³

Agar dilution and E-tests methods were equally efficacious in detecting MIC.

Conclusion:

Though all isolates were susceptible to vancomycin in the present study, a shift to a higher MIC level (1.5-2 mcg/ml) amongst MRSA isolates was noted, suggesting that misuse and overuse of the drug may

lead to development of VISA and VRSA in the future. Accurate identification of Methicillin resistance in *S.aureus* is of considerable clinical importance. Ideally, a combination of phenotypic and genotypic methods would yield the most accurate results. The emergence of VISA and VRSA reports from India also necessitate the detection of such strains especially in clinical non responders. Routine testing for Vancomycin MIC is recommended to know the ability trends of Vancomycin susceptibility in the hospital. This would help in instituting adequate barrier and control measures.

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