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A Prospective Study Showing the Incidence of Resistant Strains of Staphylococcus Aureus to Methicillin and Vancomycin in Various Clinical Infections along with Some Major Risk Factors, in a Tertiary Care Centre of Bareilly

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Abstract: ***Background: S. aureus has been recognized as continuously challenging the clinicians despite the availability of antibiotics from nearly 70 yrs and emergence of various types of antibiotic resistance mechanisms especially to methicillin and vancomycin, which was the theme of this study. Methods: This study was prospective in design and conducted in the Department of Microbiology, Bareilly. All S. aureus strains were isolated and screened for methicillin and vancomycin resistance by disc diffusion test, further intermediate susceptibility to vancomycin was detected by E-strip test., as there is no intermediate criteria for vancomycin by disc diffusion test. Results: Out of 505 Staphylococcus aureus isolates, we found that MRSA, VISA, and VRSA as 80.8%, 0.6% and 0.0% respectively. Maximum number of MRSA isolates were found with septicaemia, UTI & pneumonia i.e. 20.5%, 19.6%, 11.3% respectively followed by rest clinical diagnosis. As far as VISA is concerned, all three isolates were isolated from the patients who had all four risk factors, mentioned below. Conclusion: This report is a pointer towards emerging low level vancomycin resistance in S.aureus in India. The possible reason given for this development is inappropriate & injudicious use of vancomycin especially in situations where an alternate antimicrobial like β-lactam in reasonably higher dose can be more beneficial.***

1. Introduction

Staphylococcus aureus is the most clinically significant species of *Staphylococci* has been recognized as an important cause of human disease for more than 100 years[1]. It is one of the pathogens of greatest concern because of its intrinsic virulence factors, its ability to cause diverse array of life threatening infections, it's competency to adapt to different environmental conditions and its nasal carriage, which accounts for possible spread and re infection[2].It is one among the top three major potential pathogens responsible for community and hospital acquired infections causing diseases ranging from relatively minor skin and soft tissue infections primarily to life-threatening systemic infections which can be either toxin/non-toxin mediated, leading to high morbidity and mortality throughout the world[3] [4].

The rate of nosocomial MRSA approximately doubled from 30% in 1990s to 80% in current scenario for many countries including India [5] .The incidence of MRSA varies from 25% in Western India to 50% in South India[6]. In 1996, a clinical MRSA strain, Mu50 was isolated from pus of sternal incision, by Hiramatsu et al, in Japan with decreased vancomycin susceptibility [7].From India, Tiwari and Sen were the first to report VISA from Northern part [8].Similarly the first isolate of vancomycin resistant *Staphylococcus aureus* with the MIC>128µgm/ml was reported from United States in June 2002 [9].However the first isolate of VRSA from India was in the year 2005, by Tiwari and Sen from Banarus Hindu University, Varanasi with van gene – negative[10] and that with *van* gene – positive was from Kolkata by Biswajit Saha[10]. Subsequently VISA and VRSA strains were reported to have been isolated from, United States[11],[12],[13], Brazil[14], Germany [15], Belgium [16]andother countries of the world, hence now it has become a definite entity.

2. Methods

This study was prospective in design of complete one year from 1st January 2016 to 31st December 2016 and conducted in the Department of Microbiology, SRMSIMS, Bareilly. Out of 2639 samples, 505 were isolated as *S. aureus* from various clinical specimens like that pus, wound or vaginal swabs, blood, body fluids (CSF, pleural fluid, ascitic fluid), urine, sputum, endotracheal secretion etc. were included. Institutional Ethical clearance was obtained.

Streak culture method was employed for sample by inoculation on Blood agar (HiMedia M073) and MacConkey's agar (HiMedia M082) after receiving samples. Culture plates were incubated at 370C aerobically for 24-48 hours. Plates were observed for typical colony characteristics of Staphylococcus aureus on Blood agar (β-hemolysis). Gram's staining was performed, and observed for GPC in clusters, under oil immersion lens of microscope. *S. aureus* was confirmed by Catalase test (3% H2O2),slide coagulase test, tube coagulase test and by mannitol fermentation test.

Additional biochemical test-Hugh leifson's Oxidative Fermentative test was set up as reference procedure to differentiate from*Micrococcaceae,* in addition to resistance to Bacitracin disc (0.04 units).

Laboratorydetection of Methicillin Resistance:

Cefoxitin disc diffusion test: A direct colony suspension of each *Staphylococcus aureus* isolate is prepared to a 0.5 McFarland standard and plated on Mueller-Hinton agar. A cefoxitin (30µg) is placed on the surface and incubated at 35°C for 18 hours. The zone must be measured in reflected light. The following tables show the breakpoints for defining methicillin resistance [17].

Interpretative criteria for cefoxitin disc diffusion test

Susceptible*	†	Resistant**
Staphylococcus	≥ 22	≤ 21 *aureus*

* Report as Oxacillin susceptible
** Report as Oxacillin resistant
† There is no intermediate category with the cefoxitin disc diffusion test.

Laboratory Detection of Vancomycin Resistance:
Following 2 methods were used , modified Kirby – Bauer disc diffusion method using 30µg Vancomycin disc and was done keeping in view E-test as gold standard for detection of vancomycin resistance.

a) **Disc diffusion by Modified Kirby Bauer's Method [18]**
Vancomycin susceptibility by modified Kirby – Bauer disc diffusion was performed using Vancomycin 30µg disc. The diameter of zone of inhibition was measured and interpreted according to CLSI guidelines 2007[18].*Staphylococcus aureus* ATCC 25923 were used as a vancomycin susceptible control strains and *Enterococcus. faecalis* ATCC 51299 as vancomycin resistant control strain.

Prior to 2009, the CLSI guidelines were as follows

Interpretative criteria for Vancomycin disc diffusion test [18]:

Susceptible*	†	Resistant**
Staphylococcus aureus	≥ 15	≤ 14

* Report as Vancomycin susceptible.
** Report as Vancomycin resistant.
† There is no intermediate category with the Vancomycin disc diffusion test.

urthermore, in 2009, the CLSI altered the guidelines for Staphylococci such that the disk diffusion was no longer an acceptable means for testing Vancomycin susceptibility in these organisms.

b) **Determination of Minimum Inhibitory Concentration (MIC) value [17]**
The MIC value of vancomycin was determined by E-test [Epsilometer-test]. A suspension that matches the turbidity of a 0.5 McFarland standard and a lawn culture was prepared by pouring the growth suspension on the surface of the BHI agar plate. After drying the surface for half an hour, the E-strips were placed over the surface and incubated over night at 35^0C.The plates were read only when sufficient growth was seen and the MIC values were recorded where the ellipse intersects the MIC scale on the strip. If the ellipse intersects the strip in between 2 dilutions MIC was recorded as the value which is nearest to the intersection. *Staphylococcus aureus* ATCC 25923 were used as a control strain.

For classifying isolates of *Staphylococcus aureus* with reduced susceptibility to vancomycin based on the laboratory breakpoint published bythe clinical and laboratory standards institute [CLSI guidelines] [17]:-
1) Vancomycin sensitive *Staphylococcus aureus* [VSSA]: ;≤2 µg /ml.
2) Vancomycin intermediate *Staphylococcus aureus* [VISA]: 4-8µg /ml.
3) Vancomycin resistant *Staphylococcus aureus*[VRSA]:- ≥16µg/ml

Statistical analysis
Data collected were cleaned, filled in the excel sheet and analyzed. Percentages and proportions were used to express data.

Exclusion criteria
Cases of wound infection which did not yield the growth of *staphylococci*, but yielded growth of other bacteria, fungal, commensal growth and mixed infection.

3. Results

Out of 2639 clinical specimens, only Gram positive cocci were observed in 1389 (52.6%) samples followed by 685 (25.6%) samples were shown both *Gram negative bacilli* and *Gram positive cocci*, 152 (5.8%) samples fungal growth, 230 (8.7%) samples commensal growth & 183 (6.9%) samples had shown no growth of any bacteria. In these 183 cases, there was no visible discharge or collection but a clinical suspicion of wound infection was made clinically because of their non-healing nature.

Out of 1389 samples had shown GPC, 505 were confirmed as *S. aureus* by above mentioned characteristic biochemical tests. Our study were shown MSSA 18.6%, MRSA 80.8%, VISA 0.6% and VRSA 0.0% whereas rest of 884 gram positive isolates as commensal/contaminant flora like Micrococci and Coagulase negative staphylococci (CoNS) that has been excluded from study.

By taking the clinical diagnosis in consideration of total ,the **Incidence of MRSA & VISA** shown in **Table I**, maximum number of MRSA isolates were found with septicaemia, UTI & pneumonia i.e. 20.5%, 19.6%, 11.3% respectively followed by post-op infection, traumatic wound & rest clinical diagnosis.

Table 1: Incidence of MRSA & VISA according to clinical diagnosis

S. NO	Clinical diagnosis	MSSA		MRSA		VISA	
		No.	%	No.	%	No.	%
1.	Abdominal pain	1	1.0	1	0.2	0	0.0
2.	Abscess	6	6.3	12	2.9	0	0.0
3.	BPH	0	0.0	1	0.2	0	0.0
4.	Burn	1	1.0	9	2.2	0	0.0
5.	Cellulitis	1	1.0	9	2.2	0	0.0
6.	Diabetic foot	2	2.1	13	3.1	0	0.0

7.	Folliculitis	0	0.0	4	0.9	0	0.0
8.	Gangrene	0	0.0	9	2.2	0	0.0
9.	Infertility	2	2.1	15	3.6	0	0.0
10.	Mastitis	1	1.0	10	2.4	0	0.0
11.	Meningitis	1	1.0	3	0.7	0	0.0
12.	Non healing ulcer	3	3.1	11	2.7	0	0.0
13.	Osteomyelitis	4	4.2	14	3.4	0	0.0
14.	Otitis media	1	1.0	1	0.2	0	0.0
15.	Paronychia	1	1.0	5	1.2	0	0.0
16.	Pharyngitis	1	1.0	10	2.4	1	33.3
17.	Pneumonia	17	18.0	42	11.3	0	0.0
18.	Post op infection	10	11.8	30	7.3	0	0.0
19.	Septicaemia	9	9.5	84	20.5	0	0.0
20.	Tonsillitis	0	0.0	1	0.2	0	0.0
21.	Traumatic wound	4	4.2	24	5.8	0	0.0
22.	Umbilical discharge	0	0.0	1	0.2	0	0.0
23.	UTI	20	21.2	81	19.6	1	33.3
24.	Vaginitis	9	9.5	19	4.6	1	33.3
Total		94	100.0	408	100.0	3	100.0

Another analysis of MRSA & VISA isolates were done again according to the total number of individual clinical isolates which showed, the six predominant lesions are UTI, septicemia, pneumonia, post-op infection, Vaginitis & traumatic wounds showing the number of 101, 93 ,49, 30, 29 & 28 in descending order. The incidence of MRSA in these predominant lesions are 79.2%, 90.3%, 85.8%,100.0%, 65.5% & 85.7% respectively.

As the number of samples in rest of the lesions are very less. So the exact inference regarding the incidence of MRSA cannot be drawn. Analysis of MRSA & VISA isolates according to the total number of individual clinical isolates shown in Table II.

Table 2: Incidence of MRSA & VISA according to the total number of individual clinical isolates

s. no	Clinical diagnosis	Total no	MSSA	percent	MRSA	Percent	VISA	Percent
1.	Abdominal pain	2	1	50.0	1	50.0	0	0.0
2.	Abscess	25	3	12.0	22	88.0	0	0.0
3.	BPH	1	0	0.0	1	100.0	0	0.0
4.	Burn	10	1	12.5	9	87.5	0	0.0
5.	Cellulitis	10	1	10.0	9	90.0	0	0.0
6.	Diabetic foot	15	2	13.3	13	86.7	0	0.0
7.	Folliculitis	4	0	0.0	4	100.0	0	0.0
8.	Gangrene	9	0	0.0	9	100.0	0	0.0
9.	Infertility	17	2	11.7	15	88.2	0	0.0
10.	Mastitis	14	1	7.1	13	92.9	0	0.0
11.	Meningitis	4	1	25.0	3	75.0	0	0.0
12.	Non healing ulcer	24	3	12.5	21	87.5	0	0.0
13.	osteomyelitis	18	4	22.2	14	77.8	0	0.0
14.	Otitis media	2	1	50.0	1	50.0	0	0.0
15.	Paronychia	6	1	16.7	5	83.3	0	0.0
16.	Pharyngitis	12	1	8.3	10	83.3	1	8.3
17.	Pneumonia	49	7	14.2	42	85.8	0	0.0
18.	Post op infection	30	0	0.0	30	100.0	0	0.0
19.	Septicemia	93	9	9.7	84	90.3	0	0.0
20.	Tonsilitis	1	0	0.0	1	100.0	0	0.0
21.	Traumatic wound	28	4	14.3	24	85.7	0	0.0
22.	Umbilical discharge	1	0	0.0	1	100.0	0	0.0
23.	UTI	101	20	19.8	80	79.2	1	0.9
24.	Vaginitis	29	9	31.0	19	65.5	1	3.4
	Total	505	71	14.1	430	85.1	3	0.6

The 4 major risk factors, to be included in our study, were > 72 hrs of admission, previous hospitalization/ surgery, Presence of i.v. line/ indwelling devices, H/o intake of broad spectrum antibiotics.

As far as **Association of Staph. aureus with various risk factors** is concerned, the rate of isolation of Staph. aureus is shown in **Table III.**

Table 3: Association & isolation of staph. aureus along with risk factors

S No.	Risk Factors	Total		MSSA		MRSA		VISA	
				NO.	PERCENT	NO.	PERCENT	NO.	PERCENT
1.	>72 hrs of admission	P	225	4	1.8	218	96.9	3	1.3
		N	280	67	24	213	76	0	0
2.	Previous hospitalization/ surgery	P	233	4	1.7	226	97	3	1.3
		N	272	67	24.6	205	75.4	0	0
3.	Presence of iv line/ indwelling devices,	P	281	8	2.8	270	96.2	3	1
		N	224	63	28	161	72	0	0
4.	H/o intake of broad spectrum antibiotics	P	433	1	0.2	429	99	3	0.8
		N	72	70	97.2	2	2.8	0	0

Soon analysis of various Staph. aureus isolates, we have found that as far as MSSA is concerned, the incidence of isolation of MSSA, from various patients in various risk factors/groups, fall in the range of 0.2- 2.8%. But in case of MRSA, the number is quite high, if all the risk factors were taken in consideration, they fall in a range between 96. 2 – 99.0%.

4. Discussion

Increasing prevalence of MRSA, lead to the extensive use of Vancomycin. This inturn lead to the decreased susceptibility to Vancomycin all over the World including India, this was soon followed by strains of *Staphylococcus aureus* that were totally resistant to vancomycin [8],[9]. Such resistance resulted in serious clinical and public health consequences because currently a very few licenced alternatives are available to treat vancomycin resistant *Staphylococcus aureus* infections [19].

To tackle this grave situation constant monitoring of these isolates is important, for this purpose,this study was undertaken to determine the incidence or MRSA, VISA, VRSA in our tertiary care centre.

In the present study, maximum MRSA were isolated from pus & swab samples from various clinical lesions [abscess, burn , cellulitis, diabetic foot, folliculitis, mastitis ,non healing ulcer, otitis media, paronychia, post op infections, traumatic wound, vaginitis umbilical discharge & osteomyelitis] were collected [42.9%] . followed by UTI [20%], blood [18.4%], respiratory secretions [12%]. This pattern correlates with studies conducted by Vidya Pai *et al* in 2010 [20] and Nitish Kumar Sharma *et al* 2013 [21]. This is due to the reason that *Staphylococcus aureus* accounts for most of the skin and soft tissue infections, septicemia and also respiratory tract infections.

None of one literature is conducted including all various clinical lesions. On analysis of clinical lesions individually. Among the different clinical conditions included in the present study, the MRSA wound infection rate among diabetic foot infections as monomicrobial organism was 86.7%., which is corroborative with the 71.4% MRSA infection, done by Mohammed et al [22].A lower rate of 42.8% & 4.16% is observed by Murugan et al [23] & Jasmine et al [24], respectively.

A number of studies from India have investigated the causative organisms of VAP. Up to 40% of these infections can be polymicrobial. *Staphylococcus aureus* were identified as the common VAP pathogens in monomicrobial isolates, with varying incidence. Our study showed 85.8% of MRSA infection in VAP,which is corroborative with 85.71% incidence of MRSA in study of Saroj Golia et al[25]. Lower incidence of MRSA infection26.7% & 43% were observed by Alok Gupta et al [26] & Sujata et al [29] respectively.

In present study, out of 30 isolates of post op infections, 30 i,e.100% were found as MRSA strains.but none of the srains was resistant to vancomycin. Inappropriate use of antibiotics and consequent selective antibiotic pressure has been incriminated in the genesis of the antibiotic resistant strains in the literature.

Surgical site infections are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections. They have been responsible for the increasing cost, morbidity and mortality related to surgical operations and continue to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis. The most common isolate was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* . Many studies have reported *Staphylococcus aureus* as the commonest isolate from the postoperative wound infection[28],[29],[30]. In the present study, predominance of *Staphylococcus aureus* in surgical site infection is consistent with reports from other studies. Number of studies in the literature indicate gradual increase in the emergence of antibiotic resistant microorganisms in surgical patients[31],[32],[33],[34].

Special interest in *Staphylococcus aureus* surgical site infection is mainly due to its predominant role in hospital cross infection and emergence of virulent antibiotic resistant strains. In the present study, all *Staphylococcus aureus* strains from the infected wound were resistant to penicillin. Ineffectiveness of penicillin in *Staphylococcus aureus* has been reported in other studies also

In our analysis of bacterial isolates from UTI patients, incidence MRSA infection showed 79.2% .The ratio of MRSA to all Staph. aureus isolated were 68.3%, 87.9%, 82.8%, 82.5%, 75.0%, & 70.2% from 2000-2005, respectively, a study done by Katsumi et al [35].

Among the *Staphylococcus aureus* 28.6% (10/35) were sensitive to methicillin, implying the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) to be 71.4% in one study . Other studies in India have quoted the prevalence of MRSA ranging from 54.8% Anupurba S. et al [36] to 80.89% Verma S, et al[37].

Our study showed the incidence of staph. aureus infection in cases of septicemia were18.4% (93/505), out of which 90.3% (84/93)were found as MRSA.. another study I Roy et al[38], also showed incidence of *Staph. aureus* (14%) & the antimicrobial susceptibility testing revealed that resistance to penicillin was frequent in Staph. *aureus* (95.9%)

In the present study, only 3 isolates [0.6%] of Staphylococcus aureus showed the intermediate zone of susceptibility by E-test ,so they were termed as Vancomycin intermediate *Staphylococcus aureus* [VISA] . Widespread use of vancomycin to treat infections caused by MRSA has been reported to result in the emergence of low level resistance.

VISA strains have been reported by many other researchers- Hiramatsu et al in Japan [9] , Tenover et al [39] in NewYork. Isolates of vancomycin resistant *Staphylococcus aureus* have emerged in many parts of the world. These isolates appear to achieve clinically relevant levels of resistance to vancomycin that leads to treatment failure. At

present, the proportion of MRSA with reduced susceptibility to vancomycin is well known. VRSA and VISA isolates have been reported by several researchers like Tiwari and Sen et al[8] ,Biswajit Saha et al [42], Venubabu Thati [40] &G. A. Menezes et al [41] who stated that it was mainly due to excessive use of antibiotics in intensive care units and in other health care sectors.The emergence of VRSA is a critical concern to the therapeutic dilemma caused by the presence of multi drug resistant organisms in recent years [42].

Failure with Vancomycin occurs due to its slow bactericidal activity, low penetration in tissues and it's increasing MICs.

5. Conclusion

For preventing the emergence of multidrug-resistant organisms require a comprehensive, systematic approach that integrates the health care and public health systems. We need to encourage and facilitate adherence to recommended prevention and control guidelines, conduct active surveillance by screening of MRSA and VRSA, to detect the emergence of these organisms, and ensure vigorous antibiotic stewardship by health care providers.

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