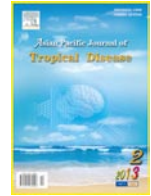




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Surveillance of infection status of drug resistant *Staphylococcus aureus* in an Indian teaching hospital

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PEER REVIEW

ABSTRACT

Peer reviewer

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Comments

Surveillance of *S. aureus* is a coveted work of any hospital, since this bacterium is regarded as the superbug in the health domain for its resistance to all most all antibiotics of the time, i.e., pandrug resistance. The present study described resistance of the pathogen to 16 antibiotics, including vancomycin in an Indian hospital.

(Details on Page 141)

Objective: To access nosocomial and community accounts of multidrug resistant strains of *Staphylococcus aureus* (*S. aureus*) isolated by surveillance in a teaching hospital, over a period of 30 months. **Methods:** Clinical samples from nosocomial sources, i.e., wards and cabins, intensive care unit (ICU) and neonatal intensive care unit (NICU) sources, as well as community or outpatient department (OPD) sources of a hospital were used for isolating strains of *S. aureus* resistant to methicillin/oxacillin and vancomycin, over a period, November 2009–April 2012. **Results:** Of a total of 1507 *S. aureus* isolates, 485 strains from community and 1022 isolates were from nosocomial sources; Out of 485 (100%) OPD *S. aureus* isolates, 390 (80.41%) were MRSA strains. Similarly, from wards and cabins of 564 (100%) isolates, 461 (81.73%) strains were MRSA; whereas of 458 (100%) isolates obtained from ICU and NICU, 363 (79.25%) strains were MRSA. It was ascertained with χ^2 -tests of independence that MRSA strains were equally distributed in "community" or "wards and cabins" or "ICU and NICU" sources, alike rest other drug-resistant *S. aureus* strains. Antibiotic sensitivity patterns of isolated strains with 16 antibiotics were ascertained. Out of 390 (100%) MRSA strains isolated from OPD, 80 (20.51%) were vancomycin resistant (VRSA) and 173 (44.35%) strains were moderately sensitive to vancomycin or called, vancomycin intermediate strains (VISA). Similarly, from nosocomial sources, out of 461 (100%) MRSA isolates obtained from wards and cabins, 110 (23.86%) strains were VRSA and 208 (45.11%) were VISA strains, whereas out of 363 MRSA isolates obtained from ICU and NICU, 61 (16.8%) VRSA strains and 164 (45.17%) VISA strains were found. A progressive increase of percent values of drug resistance to 16 antibiotics used for antibiotic profiling revealed its subtle infection dynamics. **Conclusions:** This study revealed the appalling state of occurrence of MRSA and VRSA in a resource-limited setting. A progressive increase of percent values of drug resistance to 16 antibiotics used revealed its subtle infection dynamics.

KEYWORDS

Staphylococcus aureus, Methicillin resistant *S. aureus*, Vancomycin resistant *S. aureus*, Nosocomial infection, Antibiotic sensitivity, Community infections

1. Introduction

Staphylococcus aureus (*S. aureus*) colonizes asymptotically at interior nares of nose, on skin and soft tissues

of healthy individuals. But, when it finds a way to bloodstream, a spectrum of idiosyncratic ailments are triggered on, ranging from casual skin reactions, rhinitis, otitis media infection and mastitis, through

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serious suppurative wounds, osteomyelitis, septic arthritis and severe urinary tract infections (UTI), to life-threatening invasions, pneumonia, septicemia, bacteraemia and endocarditis, as well^[1]. Particularly, several clonal variants of *S. aureus* are resistant to the penicillin group of antibiotics, methicillin/oxacillin that are known, methicillin resistant *S. aureus* (MRSA), causing surgical site infections and are found at any wound. Moreover, in a German study, it was reported that a majority of MRSA strains were from wound infections (56.9%), with pneumonia cases being the second most common (21.0%), followed by bloodstream infections (BSI) (15.1%) and UTI (6.9%), being the third and the fourth most-common cases, respectively^[2]. The most striking situation is that MRSA strains have emerged with concomitant resistance to many commonly used antibiotics of groups, aminoglycosides, macrolides, fluoroquinolones and others, chloramphenicol and tetracycline^[3]. In addition, MRSA isolates are often found resistant to cephalosporins, cefems and other β -lactams, ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenem, imipenem^[3]; thus, MRSA isolates are multidrug resistant (MDR). Thus, MDR-MRSA is the new or rather a continually evolving paradigmatic pathogen.

MDR-MRSA strains, the silently violent incarnations of *S. aureus* widespread in community and hospital environments have posed serious clinical imbroglio^[3], with glycopeptides as the only antibiotic group of choice for the control. Only vancomycin (glycopeptide), but not teicoplanin (glycopeptide) or linezolid (oxazolidinone), as used elsewhere, are in use in India. The incidences of moderately sensitive vancomycin strains or vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) strains are increasing in India^[4], and in other countries including the US^[5].

Prima facie, indiscriminate uses of antibiotics could be regarded as the cause of emergence of MDR-MRSA, as in regions where the availability of antibiotics is limited, the prevalence of MRSA was low^[6]. Generally, the origin of MDR pathogens is multi-factorial, as discussed^[7]. Thus, the problem of saturnine emergence of the gamut of MDR pathogens didn't happen to us obliviously, but those are welcomed by prosaic legislations and commissions in the odyssey of the antibiotic development from the last century, despite information on mechanism of bacterial mutations and their rates.

Further, nosocomial infections are reported from intensive care units (ICUs), because of the severity of infection by one or other pathogen in patients with pestiferous wounds. This situation causes spreads of several infectious bacteria at a time by cross-infections. Frequently, device associated nosocomial infections have been reported from many hospitals due to human errors, despite better cleanliness of general hospital environments^[7]. Moreover, community-acquired *S. aureus* infections are also too often reported^[8], wherein, poverty remains an obvious

determinant in developing countries, at least. Indeed, nosocomial/community spreads of infections could be attributed to the lack of general awareness among public, an indurate attitude, or the lack of specific awareness among paramedical staff, and at least to the cornucopia of physiological and genetic survival mechanisms of the chicaning MDR strains of pathogens.

However, when local factors of spread of infections could be identified and quantified under an effective medical surveillance system, due steps could be initiated for the abatement of pathogen spreads, as the continual wave of the emergence of armored MDR pathogens create a frightening or rather an exacerbating state of affairs in clinical managements. The present study records hospital- and community- acquired accounts of antibiotic resistance of egregious *S. aureus* strains, isolated from clinical samples of a hospital, over a period of 30 months as an incitement for this most common or rather iconic MDR wound pathogen, labeled as a "superbug" in the heath domain. This study gives information on antibiogram of MDR strains of MRSA in a typical Indian hospital, a systematic study reported never before for it, from India. This Indian epitome should strengthen the epidemiological database and would help fixing facilitation of quality improvement in hospital management and for the reduction in cost of hospitalization, as well as in the reduction of morbidity and mortality due to this Gram-positive MDR pathogen. The pharmacy world too is anticipated to be benefitted by this and similar studies on subtle MDR pathogens all over, for finesse in dovetailing suitable drugs of non-microbial origin even, as antimicrobials. Consequently, it would help prevent, *a priory*-the use of some lowbrow antibiotic regimen, for the desperate patients in quandary, dabbling with a MDR infection that might prove as the terminal bacteremia.

2. Materials and methods

2.1. Bacterial isolation and biochemical identification

Clinical samples were collected for isolation of *S. aureus* strains from patients attending outpatient department (OPD or community source), as well as patients admitted into wards, cabins, ICU and neonatal intensive care unit (NICU) of Sum Hospital, Bhubaneswar. Over a period of 30 months (November 2009–April 2012), only 7234 samples were found to have one or other bacterial pathogen. Gram-positive cocci in clusters were cultured on nutrient agar and blood agar media; when cultured on nutrient agar, butyrous, glistening, round, elevated, medium-sized colonies with golden colour (due to presence of triterpenoids or carotenoids in cell membrane), and on blood agar with yellow coloured, round and elevated colonies with β -haemolysis were seen; those were taken to be *S. aureus*. The strain of Microbial Type Culture Collection (MTCC) *S. aureus* 7443 was used as the

reference control. For pure-culture samples of these Gram-positive cocci, catalase and coagulase tests were performed, as detailed elsewhere^[9]. For confirmation, the colonies were streaked on gelatin mannitol salt agar medium and were incubated at 37 °C, for 48 h for the growth of yellow colonies^[10].

2.2. Antibiotic sensitivity and detection of MRSA

The standard MTCC number 7443 strain and all the isolated *S. aureus* strains were subjected to antibiotic sensitivity tests with 16 antibiotics, by the Kirby–Bauer’s method (disc diffusion) detailed previously^[7]. For the detection of MRSA, chromogenic agar media test was used; pure clinical isolates of *S. aureus* were streaked onto MRSA-agar media (Hichrome–MeReSa agar media, HiMedia, Mumbai) and were incubated for 24 h at 37°C; MRSA strains had blue colonies (Figure 1) and non-MRSA strains had white colonies. Further, for the “cefoxitin disc diffusion test”, all the isolates were subjected to cefoxitin 30 µg/disc. A 0.5 McFarland standard suspension of an isolate was made and lawn culture was done on Muller–Hinton agar (MHA) plate. Plates were incubated at 37°C for 18 h and inhibition-zone diameters were measured. A value of inhibition-zone diameter less than 22 mm was reported as oxacillin resistant and that more than 21 mm was considered as oxacillin sensitive^[3,11].

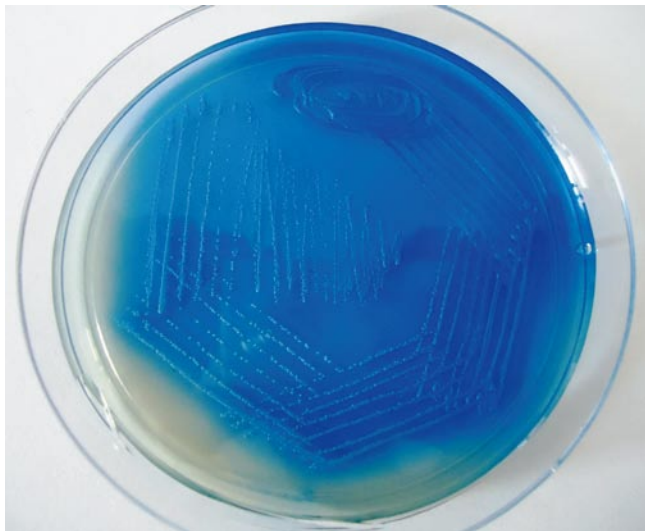


Figure 1. Blue coloured MRSA colonies on chromogenic agar medium.

2.3. Detection of vancomycin resistant and intermediate strains

Vancomycin screen agar plate was prepared by addition of 6 µg/mL vancomycin to brain heart infusion agar (HiMedia). Inoculum suspension was prepared for the turbidity of a 0.5 McFarland standard. Then, an aliquot of 0.1 ml of this suspension was spread on vancomycin–screen agar plate and was incubated for 24 h at 35 °C in ambient air. Any visible growth indicated the vancomycin resistance^[3,12]. In addition, the *S. aureus* MTCC 7443 strain was used as the vancomycin–susceptible control.

2.4. Confirmation and quantitative analysis of MRSA and VRSA

A 96-well micro-titer plate was used to determine the MIC (minimum inhibitory concentration) values of two antibiotics of oxacillin and vancomycin in broth cultures, for 25 selected strains, of each MRSA, MSSA, VRSA and VISA. An exponential culture of a test strain of *S. aureus* in Muller–Hinton (MH) broth (HiMedia) was suitably diluted with the normal saline solution to obtain the level of equivalent to the 0.5 McFarland standard. TTC (2, 3, 5–triphenyltetrazolium chloride) was used as an indicator of bacterial growth. To an aliquot of 20 µL overnight grown test culture, an aliquot of 100 µL of the antibiotic stock solution of concentration 512 µg/mL and an aliquot of 100 µL of MH broth were added to the second well of the micro-titer plate. This solution was serially diluted at each successive well till the final concentration of 0.25 µg/mL antibiotic in the 12th, the last well was obtained. Finally, an aliquot of 5 µL 0.5% TTC was added to all wells and the micro-titer plate was incubated at 37 °C for 18 h. Wells were examined for the development of the pink colour that in a well indicated the bacterial growth, and the absence of the pink colouration was taken as the growth inhibition and that was the MIC value^[13]. Obviously, the first well of the micro-titre plate was the control without any antibiotic.

3. Results

In the surveillance period of 30 months, out of 7234 samples, a total of 1507 isolates were detected as *S. aureus* and strains were isolated to pure cultures. Of these 1507 (100%) isolates, 485 (32.18%) were from community sources (OPD); while 1022 (67.81%) were from hospital sources (ICU and NICU, as well as wards and cabins) (Table 1).

Table 1

Occurrence of *S. aureus* isolates according to source of isolation.

Period	ICU and NICU	Wards and cabins	Nosocomial total	OPD	Grand total
Nov 2009–Jan 2010	53	61	114	40	154
Feb 2010–Apr 2010	26	54	80	47	127
May 2010–Jul 2010	32	59	91	45	136
Aug 2010–Oct 2010	47	55	102	50	152
Nov 2010–Jan 2011	44	56	100	52	152
Feb 2011–Apr 2011	35	59	94	45	139
May 2011–Jul 2011	56	55	111	48	159
Aug 2011–Oct 2011	59	51	110	50	160
Nov 2011–Jan 2012	55	41	96	50	146
Feb 2012–Apr 2012	51	73	124	58	182
Total	458	564	1022	485	1507

ICU, intensive care unit; NICU, neonatal intensive care unit; OPD, outpatient department.

Of community or OPD sources of 485 (100%) isolates, staphylococcal infections were reported in decreasing order

from: pus 242 (49.89%), 166 swabs (34.22%), 68 urine samples (14.02%), other 5 body fluids (1.03%) and least from 4 blood samples (0.008%). Likewise, from sources of wards-and-cabins of 564 (100%) isolates, majorities of staphylococcal infections were reported from pus 277 (49.11%) and 193 swabs (34.04%), followed by 50 urine (0.0%), 22 blood (3.9%) and least from other 22 body fluids (3.9%); of ICU-and-NICU sources of 458 (100%) isolates, majorities of staphylococcal infections were reported from 229 pus (50.0%) and 154 swabs (33.62%), followed by 35 urine samples (7.64%), 32 blood samples (6.98%) and least from 8 body fluids (1.74%). Out of 485 (100%) OPD

S. aureus isolates, 390 (80.41%) were MRSA strains. Similarly, from wards and cabins of 564 (100%) isolates, 461 (81.73%) strains were MRSA; whereas of 458 (100%) isolates obtained from ICU and NICU, 363 (79.25%) strains were MRSA (Figure 2).

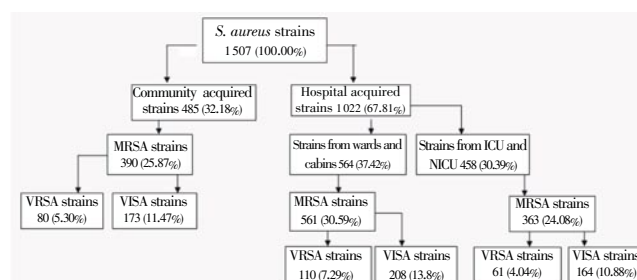


Figure 2. Distribution patterns of MRSA and VRSA colonies in nosocomial and community settings.

The χ^2 -test of independence was used to test whether MRSA strains were more distributed other than antibiotic resistant strains in “community” or “wards and cabins” or “ICU and NICU” sources, using a rxc contingency table; the computed χ^2 -value was 0.024 and the tabulated χ^2 value is

Table 2

Occurrence of *S. aureus* strains in different clinical samples from community and nosocomial sources.

Community sources (OPD)							
Period	Pus	Swabs	Urine	Body fluids	Blood	Total samples	Moving averages
Nov 2009–Jan 2010	23 (19)	14 (08)	2 (2)	1 (1)	–	40 (30)	45.5 (38.5)
Feb 2010–Apr 2010	28 (25)	16 (14)	3 (2)	–	–	47 (41)	
May 2010–Jul 2010	21 (21)	17 (14)	5 (5)	1 (1)	1 (1)	45 (41)	
Aug 2010–Oct 2010	27 (24)	16 (13)	6 (4)	–	1 (1)	50 (42)	48.7 (38.4)
Nov 2010–Jan 2011	30 (27)	16 (15)	5 (4)	–	1 (1)	52 (47)	
Feb 2011–Apr 2011	23 (21)	15 (11)	6 (5)	–	1 (–)	45 (37)	
May 2011–Jul 2011	21 (14)	19 (12)	7 (5)	–	1 (–)	48 (32)	51.5 (38.0)
Aug 2011–Oct 2011	19 (13)	20 (20)	9 (3)	2 (1)	–	50 (37)	
Nov 2011–Jan 2012	22 (18)	17 (14)	11 (6)	–	–	50 (38)	
Feb 2012–Apr 2012	28 (23)	6 (13)	14 (9)	–	–	58 (45)	
Total	242 (205)	166 (134)	68 (45)	4 (3)	5 (3)	485 (390)	48.5 (38.3)
Nosocomial sources (wards and cabins)							
Nov 2009–Jan 2010	32 (30)	16 (15)	7 (4)	3 (2)	3 (3)	61 (54)	57.2 (49.5)
Feb 2010–Apr 2010	23 (19)	21 (18)	5 (4)	1 (0)	4 (3)	54 (44)	
May 2010–Jul 2010	29 (28)	17 (13)	7 (5)	3 (2)	3 (3)	59 (51)	
Aug 2010–Oct 2010	24 (21)	22 (21)	5 (4)	3 (3)	1 (0)	55 (49)	56.2 (48.0)
Nov 2010–Jan 2011	28 (27)	25 (22)	3 (3)	–	–	56 (52)	
Feb 2011–Apr 2011	31 (28)	21 (19)	4 (3)	1 (0)	2 (2)	59 (52)	
May 2011–Jul 2011	30 (23)	20 (14)	5 (2)	–	–	55 (39)	55.0 (39.8)
Aug 2011–Oct 2011	28 (25)	16 (14)	4 (2)	2 (–)	1 (–)	51 (41)	
Nov 2011–Jan 2012	23 (19)	14 (08)	2 (2)	2 (1)	–	41 (30)	
Feb 2012–Apr 2012	29 (28)	21 (13)	8 (3)	7 (2)	8 (3)	73 (49)	
Total	277 (248)	193 (157)	50 (32)	22 (10)	22 (14)	564 (461)	56.1 (45.7)
Nosocomial sources (ICU and NICU)							
Nov 2009–Jan 2010	17 (14)	10 (08)	4 (3)	19 (0)	1	53 (25)	39.5 (28.8)
Feb 2010–Apr 2010	10 (08)	11 (09)	2 (2)	3 (2)	–	26 (21)	
May 2010–Jul 2010	15 (14)	8 (07)	5 (4)	3 (3)	1	32 (28)	
Aug 2010–Oct 2010	28 (25)	16 (14)	3 (2)	–	–	47 (41)	45.5 (39.3)
Nov 2010–Jan 2011	24 (22)	15 (12)	3 (1)	2 (1)	1	44 (36)	
Feb 2011–Apr 2011	18 (15)	12 (10)	2 (1)	1 (1)	2 (1)	35 (28)	
May 2011–Jul 2011	28 (27)	25 (22)	3 (3)	–	–	56 (52)	55.3 (46.0)
Aug 2011–Oct 2011	31 (28)	21 (19)	4 (3)	1 (0)	2 (2)	59 (52)	
Nov 2011–Jan 2012	30 (23)	20 (14)	5 (2)	–	–	55 (39)	
Feb 2012–Apr 2012	28 (25)	16 (14)	4 (2)	2 (–)	1 (–)	51 (41)	
Total	229 (201)	154 (129)	35 (23)	32 (7)	8 (3)	458 (363)	46.7 (38.0)

Number in parenthesis is number of isolated MRSA strains; χ^2 -test of independence was used; – means the clinical samples had no MRSA isolate.

9.210 for degree of freedom=2 and at $P=0.001$; so, the null hypothesis was not rejected, confirming MRSA strains were equally distributed in “community” or “wards and cabins” or “ICU and NICU” sources, alike rest other drug-resistant *S. aureus* strains (Table 2).

Table 3

Occurrence of vancomycin resistant and vancomycin intermediate *S. aureus* strains among MRSA strains from community and nosocomial sources.

Community sources (OPD)			
Period	MRSA	VRSA (VISA)	Moving averages
Nov 2009–Jan 2010	30	8 (12)	
Feb 2010–Apr 2010	41	9 (18)	9.3 (15.0)
May 2010–Jul 2010	41	12 (16)	
Aug 2010–Oct 2010	42	8 (14)	
Nov 2010–Jan 2011	47	13 (15)	8.5 (15.8)
Feb 2011–Apr 2011	37	8 (18)	
May 2011–Jul 2011	32	5 (16)	
Aug 2011–Oct 2011	37	4 (21)	5.5 (20.0)
Nov 2011–Jan 2012	38	8 (19)	
Feb 2012–Apr 2012	45	5 (24)	
Total	390	80 (173)	7.8 (16.9)
Nosocomial sources (wards and cabins)			
Nov 2009–Jan 2010	54	12 (23)	
Feb 2010–Apr 2010	44	16 (18)	12.3 (21.5)
May 2010–Jul 2010	51	9 (26)	
Aug 2010–Oct 2010	49	12 (19)	
Nov 2010–Jan 2011	52	8 (26)	10.5 (21.0)
Feb 2011–Apr 2011	52	13 (18)	
May 2011–Jul 2011	39	9 (21)	
Aug 2011–Oct 2011	41	12 (24)	10.0 (19.5)
Nov 2011–Jan 2012	30	11 (13)	
Feb 2012–Apr 2012	49	8 (20)	
Total	461	110 (208)	10.9 (20.7)
Nosocomial sources (ICU and NICU)			
Nov 2009–Jan 2010	25	4 (12)	
Feb 2010–Apr 2010	21	2 (11)	4.25 (15.2)
May 2010–Jul 2010	28	4 (17)	
Aug 2010–Oct 2010	41	7 (21)	
Nov 2010–Jan 2011	36	4 (15)	6.5 (17.0)
Feb 2011–Apr 2011	28	6 (11)	
May 2011–Jul 2011	52	9 (21)	
Aug 2011–Oct 2011	52	7 (23)	8.5 (19.2)
Nov 2011–Jan 2012	39	11 (12)	
Feb 2012–Apr 2012	41	7 (21)	
Total	363	61 (164)	6.4 (17.2)

In community sources, occurrence of MDR *S. aureus* strains were computed for moving averages with data-set of 10 quarters in 30 months. It was seen that moving average values were 45.5, 48.7 and 51.5 at the first, middle and the last quarter, respectively. This signifies that there was progressive increase in occurrence of MDR *S. aureus* strains in each month/quarter. Similarly, MRSA strains occurred almost unchanged in first four, middle four and the last four quarters of the study (Table 2). In wards-and-cabins sources, moving averages were almost unaffected, but in ICU-and-NICU, there were significantly high values

in moving averages from value of 39.5, through 45.5 to 55.3. From the moving average values of MRSA strains from wards-and-cabins, it found to be decreasing from first four, to last four quarters of the study, whereas in ICU and NICU their occurrence increased from first four, to last four quarters.

Further, MRSA strains obtained from both the sources were tested for sensitivity against vancomycin. Out of 390 (100%) MRSA strains isolated from OPD, 80 (20.51%) were vancomycin resistant (VRSA) and 173 (44.35%) strains were moderately sensitive to vancomycin or called, vancomycin intermediate strains (VISA) (Table 3), and the rest 137 (35.12%) strains were sensitive to vancomycin. Similarly from nosocomial sources, out of 461 (100%) MRSA isolates obtained from wards and cabins, 110 (23.86%) strains were VRSA and 208 (45.11%) were VISA strains (Table 3), while 143 (31.01%) strains were totally sensitive to vancomycin; whereas out of 363 MRSA isolates obtained from ICU and NICU, 61 (16.8%) VRSA strains and 164 (45.17%) VISA strains were found (Table 3), whereas the rest 138 (38.01%) isolates were vancomycin sensitive.

In community sources, occurrence of VRSA and VISA strains were computed for moving average values with data set of 10 quarters in 30 months. It was seen that moving averages of the first four quarters were 9.3, and 8.5 in the middle four quarters, and was 5.5 in the last four quarters. This signifies that there was decrease in the occurrence of VRSA strains. However, the occurrence of VISA strains increased through first four, middle four and the last four months of the study (Table 3).

Likewise in wards-and-cabins sources and in ICU-and-NICU sources, moving averages for VRSA strains decreased, but the occurrence of VISA strains increased through the first four, the middle four and the last four quarters of the study, both in wards and cabins as well as in ICU and NICU (Table 3).

Table 4

Percentages of resistance of *S. aureus* to individual antibiotics of aminoglycoside group.

Period	Aminoglycosides			
	Ac		Ge	
	N	C	N	C
Nov 2009–Jan 2010	66	61	69	51
Feb 2010–Apr 2010	72	66	72	53
May 2010–Jul 2010	76	69	77	64
Aug 2010–Oct 2010	81	73	83	69
Nov 2010–Jan 2011	87	76	87	76
Feb 2011–Apr 2011	89	84	85	81
May 2011–Jul 2011	57	78	88	56
Aug 2011–Oct 2011	66	81	73	62
Nov 2011–Jan 2012	72	58	69	77
Feb 2012–Apr 2012	59	69	72	81
mean±SD	72.5±10.9	71.5±8.5	77.5±7.5	67.0±11.5

Antibiotic in µg/disc. Ac, amikacin 30; Ge, gentamicin 10; Ch, chloramphenicol 30; Lz, linezolid 30; Tc, tetracycline 30; N, nosocomial; C community.

Percent values of resistance of *S. aureus* to different

groups of antibiotics are presented in Tables 4, 5, 6 and 7. Under amikacin, in Table 4, there are subheadings, N (nosocomial) and C (community) sources. In the first quarter, as evident from the samples from community, a total of 40 *S. aureus* strains were isolated, of which 61% were resistant to amikacin, 84% were resistant to oxacillin, so on for other antibiotics presented in other tables (Tables 4 to 7). On the other hand, for the first quarter of study, 94 strains were isolated from all the 4 nosocomial sources (Table 2), of which 66% of these strains were resistant to amikacin, 79% were resistant to oxacillin and 73% were resistant to ampicillin and so on so forth, for the rest antibiotics. Three stand-alone antibiotics, chloramphenicol, linezolid and tetracycline had higher resistant percent values in strains of nosocomial sources compared to abundance in community settings. But linezolid is used as the most preferred antibiotic for *S. aureus*.

In each progressive quarter, it was seen that percentage of resistance to an antibiotic steadily increased. For amikacin, the resistance percentage of community acquired pathogenic *S. aureus* strains increased from 61% in first quarter and

66% in the second quarter to 59% in last quarter. Similarly in nosocomial section, it increased from 66 to 72% and then to 76% in first three quarters and ultimately to 72% in the last (tenth) quarter (Table 4). Moreover, progressive increase of percent values of drug-resistant strains from “community” or “wards and cabins” or “ICU and NICU” sources, for each antibiotic had been seen for the rest other antibiotics (Tables 4 to 7).

Among the two aminoglycosides antibiotics used against *S. aureus* isolates, the maximum resistance values were recorded against amikacin of 89% in nosocomial isolates and 84% in community isolates. But, gentamicin had drug resistance to a comparative level in the community sector, but both antibiotics had similar resistant percent values in nosocomial sources (Table 4). Again of 4 β -lactam antibiotics used, penicillin was found as the most resistant antibiotic with 97% and 95% resistant values in nosocomial and community isolates, respectively. Further, out of the 7 antibiotics to 5 different individual groups of antibiotics used, nosocomial isolates had the maximum value of resistance for erythromycin of 85% and in community isolates

Table 5

Percentages of resistance of *S. aureus* to individual antibiotics of β -lactam group.

Period	β -lactams							
	Am		P		Ak		Ox	
	N	C	N	C	N	C	N	C
Nov 2009–Jan 2010	73	47	89	79	16	11	79	84
Feb 2010–Apr 2010	76	53	91	81	23	18	81	86
May 2010–Jul 2010	81	57	94	84	27	22	84	82
Aug 2010–Oct 2010	84	63	94	87	32	27	87	85
Nov 2010–Jan 2011	86	67	95	90	37	30	90	85
Feb 2011–Apr 2011	88	73	97	95	45	32	95	87
May 2011–Jul 2011	78	77	93	93	19	15	92	89
Aug 2011–Oct 2011	82	79	89	89	34	23	87	82
Nov 2011–Jan 2012	59	56	86	81	41	34	78	69
Feb 2012–Apr 2012	85	63	83	79	33	42	69	67
mean \pm SD	79.2 \pm 8.5	63.5 \pm 10.6	91.1 \pm 4.4	85.8 \pm 5.8	30.7 \pm 9.4	25.4 \pm 9.5	84.2 \pm 7.7	81.6 \pm 7.5

Antibiotic in μ g/disc. Am, ampicillin 10; P, penicillin 10; Ak, amoxycyclav 30; Ox, oxacillin 1; N, nosocomial; C, community.

Table 6

Percentages of resistance of *S. aureus* to individual group of antibiotics.

Period	Fluoroquinolones		Glycopeptides				Lincosamide		Macrolides				Sulfonamides	
	Gf		Te		V		Cd		Azm		E		Cot	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C
Nov 2009–Jan 2010	56	47	63	33	47	32	65	31	47	32	67	61	47	44
Feb 2010–Apr 2010	61	55	67	39	51	38	67	36	51	38	69	66	55	49
May 2010–Jul 2010	67	59	75	47	56	44	71	47	56	44	74	69	59	52
Aug 2010–Oct 2010	72	67	77	54	63	51	74	54	63	51	77	73	67	59
Nov 2010–Jan 2011	76	71	79	61	67	57	77	61	67	57	83	76	74	66
Feb 2011–Apr 2011	78	75	80	65	72	62	81	65	72	62	85	84	78	69
May 2011–Jul 2011	59	59	78	56	50	59	79	67	77	59	89	88	81	73
Aug 2011–Oct 2011	53	67	73	77	52	69	82	60	76	67	81	59	69	69
Nov 2011–Jan 2012	76	77	83	59	59	67	87	59	82	72	68	77	64	54
Feb 2012–Apr 2012	69	49	59	62	62	71	79	67	57	69	62	73	59	59
mean \pm SD	66.7 \pm 9.0	62.6 \pm 10.4	73.4 \pm 7.9	55.3 \pm 12.9	57.9 \pm 8.1	55.1 \pm 3.4	76.2 \pm 6.9	54.7 \pm 12.8	64.8 \pm 11.9	55.1 \pm 13.6	75.5 \pm 8.9	72.6 \pm 9.3	65.3 \pm 10.7	59.4 \pm 9.7

Antibiotic in μ g/disc: Gf, gatifloxacin 5; Te, teicoplanin 10; V, vancomycin 30; Cd, clindamycin 2; Azm, azithromycin 15; E, erythromycin 15; Cot, co-trimoxazole 25; N, nosocomial; C, community.

it was 84%; the second antibiotic was clindamycin (87% nosocomial and 67% community-acquired). Similarly, among the 3 individual antibiotics used, a maximum resistance value was recorded against both linezolid and tetracycline of 89 % in nosocomial and 73% resistance to linezolid in community isolates, respectively.

Table 7Percentage of resistance of *S. aureus* to stand-alone antibiotics.

Period	Ch		Lz		Tc	
	N	C	N	C	N	C
Nov 2009–Jan 2010	49	33	84	14	84	14
Feb 2010–Apr 2010	56	37	86	16	86	16
May 2010–Jul 2010	59	46	82	19	82	19
Aug 2010–Oct 2010	63	53	85	24	85	24
Nov 2010–Jan 2011	68	59	85	27	85	27
Feb 2011–Apr 2011	72	64	87	32	87	32
May 2011–Jul 2011	79	73	89	22	79	39
Aug 2011–Oct 2011	69	54	82	29	89	47
Nov 2011–Jan 2012	57	51	54	32	84	32
Feb 2012–Apr 2012	47	39	49	19	75	19
mean±SD	61.9± 10.2	50.9± 12.6	78.3± 14.3	23.4± 6.5	83.6± 4.1	26.9± 10.7

Antibiotic in µg/disc: Ch, chloramphenicol 30; Lz, linezolid 30; Tc, tetracycline 30; N, nosocomial; C, community.

With a cohort of 25 MRSA strains, the MIC range was 16 to 512 µg/mL for oxacillin and for the 25 MSSA strains the MIC range was 1 to 4 µg/mL; similarly, for 25 VRSA strains, the MIC range was 8 to 512 µg/mL, and for 25 VISA strains the MIC range was 1 to 4 µg/mL. These MIC values confirmed the presence of MRSA and VRSA strains, as the break point for being resistant to both oxacillin and vancomycin was ≥ 4 µg/mL (Table 8).

Table 8

Detection of MIC values in MRSA and VRSA.

Concentration of antibiotic solution (µg/mL)	Oxacillin		Vancomycin	
	MRSA (n=25)	MSSA (n=25)	VRSA (n=25)	VISA (n=25)
0	25	25	25	25
≤ 0.25	–	–	–	–
0.5	–	–	–	–
1	–	3	–	5
2	–	13	–	9
4	–	9	–	11
8	–	–	3	–
16	3	–	2	–
32	2	–	3	–
64	5	–	4	–
128	6	–	4	–
≥ 256	9	–	9	–

Each antibiotic stock solution, 512 µg/mL was serially diluted at each successive well, from the 12th well for final concentration of 0.25 µg/mL antibiotic at the second well was obtained; –, nil.

4. Discussion

From a careful inspection of the infection scenario of

a hospital, one would be overwhelmed of accounts of pathogenic *S. aureus* strains imbued in wards and ICUs at least, as found here. Not unless one is a clinician, strive to control its pernicious infection would never come to mind with proclivity and tenacity. Nor would it occur in mind unless one is a clinical microbiologist that clonal nexuses of this original commensal-avatar has become insidiously pathogenic^[14], and the evolved strains have spiraled to a unbridled notorious standard, due to the emergence of multidrug resistance in them; surprisingly, one would hardly find a more vivid illustration of a commensal, transforming into a perilous pathogen with an armamentarium of multidrug resistance, in the last few decades. In a study, MRSA strains mostly occurred as a result of surgical procedures and the insertion of urinary catheters, which accounted for 51% and 39% of the MRSA, respectively; it was further reported that the time period of having exposures to conditions favouring the spread of MRSA for a period was greater than 7 or 7.4 days^[15] that confirmed that these strains can spread easily and quickly in a hospital setting, and a long term hospitalization could lead to increased susceptibility to MRSA, at least because patients admitted to ICUs are multi-morbid or have surgical wounds^[15]. In another study, it was found that 51.5% of infected MRSA patients had already been infected at their time of admission to hospitals, which cause the introduction of new MRSA strains to hospitals from community, in reality^[16]. In England and Wales, less than 2% *S. aureus* strains were reported methicillin-resistant in 1990, but by 2002, a fiery figure, 42% *S. aureus* strains were methicillin-resistant; approximately 300000 cases of nosocomial MRSA infections were estimated each year leading to 5000 deaths^[17]. Now 10 years, the present work from a less developing country, describes 85.3% of MRSA in community sources; and 85.4% of MRSA alone in nosocomial sources.

Origin of MRSA has many possibilities: 1. Bacterial mutation rates being high, the development of clonal nexuses of *S. aureus* is fast^[18]. 2. “Positive selection pressure”, the accepted/viable concept of evolution is apparently valid for bacteria, not least because of the availability of antibiotics and their degraded products readily in nature, such as in the untreated hospital and community drains, an altered influx potential in disallowing an antibiotic at the plasma membrane level could often be the mechanism required for resistance, as exemplified in an American study^[19]. 3. Genetic recombination mechanisms—conjugation and transformation should occur more likely than expected in untreated hospital sewage system, because all sorts of bacteria with grading levels of antibiotic resistance are physically together, thereby inducing cell to cell contact or in taking DNA from some lysed pathogenic strain^[20]. In many developing countries, the scientific hospital waste disposition should be expected to be in a developing state, unwittingly. 4. Horizontal transfer of resistant strains of pathogens to both community, indoor and outdoor

hospital settings from surroundings is expected because of the crowding effect of patients and their attendants, in developing countries. Further, in developed countries the horizontal transfer of pathogens from surroundings to hospitals would definitely be the minimum, but the possibility cannot be ruled out. 5. Lastly, DNA restriction enzyme polymorphism of t-6 ribosomal RNA gene is reported to be distributed throughout *S. aureus* chromosome, which has the methicillin resistant determinant (*mec*) as an episome (or even as a plasmid); the *mec* sequences could enter into the bacterial sequences at 3 points in the genome of *S. aureus*, A, B and C, as illustrated from Japan[21]. Unfortunately, the plasmid/episome confers rapid resistance to a lot of antibiotics of different classes/generations. Gene transfers through conjugation involving the transposon, “Tn-1546” with the gene *mecA*, encoding a modified penicillin binding protein confers resistance to the methicillin and other penicillin derivatives was reported. The *mecA* gene encodes the penicillin-binding protein PBP2a, which cannot be bound by β -lactam antibiotics and in turn prevents the disruption of cell wall formation by these antibiotics. This *mec*-gene is located on mobile genetic element called the, “staphylococcal cassette chromosome-*mec*” (SCC-*mec*)[22]. In fact, when an antibiotic binds to the protein that prevents the synthesis of peptidoglycan in the bacterial cell wall, the resistance is conferred. Some bacteria can produce a “modified penicillin binding protein” that ceases to bind to the antibiotic, which eventually prevents the targeted effects of the antibiotic[16]. Indeed, resistance of *S. aureus* to β -lactam antibiotics is attributed to the presence of the *mecA* gene.

Each year approximately, two million cases of hospitalizations are recorded in nosocomial spread of BSI by both *P. aeruginosa* and *S. aureus*; and particularly, excessive stay in hospital is attributed to the increase in the mortality in US hospitals[5]. Further, treatment options are limited to vancomycin, linezolid and tigecycline for MRSA[23], and MRSA is reported to be resistant to macrolides, lincosamides, aminoglycosides, β -lactams, as well as penicillin, cephalosporin and their derivatives. Thus, these strains create a tremendous pressure for the control of MRSA in Asia, Europe and America[23–25]. *S. aureus* strains are reported to be resistant to vancomycin through *vanA* gene; resistance to vancomycin as well as β -lactam antibiotics would pose much difficulty to control its infection than that seen for MRSA alone. Further, the MRSA control with the latest generation of antibiotics could be detrimental to infections by other pathogens of a hospital, due to transfers by genetic recombination of characters of resistance[26].

Expression of methicillin resistance was reported to be affected by many factors, *viz.*, incubation temperature and time, inoculum size, salt concentration and a few more[27]. Coagulase negative *Staphylococci* are generally nonpathogenic, but sometimes behave as opportunistic pathogen under a highly immunocompromised condition,

which is not recorded here. Interestingly, a soil bacterium *Streptomyces* sp. had been reported to have a biological control activity on MRSA[28].

Nosocomial and community acquired infections from an Indian hospital of MRSA had been reported to be prevalent[29]. Vancomycin has always been taken as the antibiotic as the last resort for MRSA[30], but in this study VISA and VRSA had been reported, which were fully resistant or partially resistant to vancomycin that suggests that this antibiotic could be resistant to MRSA in a short. The development of VISA is suspected due to combination therapy of vancomycin with an aminoglycoside (gentamicin) for a synergistic action of the two antibiotics[31]. Vancomycin resistant *enterococci* have too been long reported from the US[5]. Analysis of aminoglycoside resistance in *S. aureus* revealed that 60 to 95% of isolated strains in this study were resistance to both amikacin and oxacillin. It is reported that the PCR detection of aminoglycosides modifying enzyme have been found in both *P. aeruginosa* and *S. aureus* as plasmid mediated[7].

At all the time, the full range of antibiotics is the vanguard party of the health-care system, with due inherent technically controlling fixes. But, an utterly sad fact today is that more than 95% MRSA worldwide do not respond to the first line antibiotics[8], due to artifices in bacterial genomes confirming the law that simple genomes evolve faster than complex genomes. First reported in 1960, MRSA has become endemic today in hospitals covertly worldwide, and 30% of *S. aureus* isolates are MDR, two decades ago, as conjectured from surveillance in the US[32]. However, the fear of pandrug-resistance (resistance to all antibiotics and drugs in present use), as cautioned in Gram-negative pathogens[33], cannot be ruled out in *S. aureus*. But the future recourse to the profusion of antibiotics of *Streptomyces* would never be rhetoric[34], and our return to an post-antibiotic era, as raised[40], is a remote possibility. Anyway, search of a dove-tailed control module is the call of the situation for avenging or estranging this appalling pathogen. Moreover, an American study showed the cost of hospitalization with *S. aureus* infections was serious; its infections caused an average 3 times costlier than any other ailment within 20 days and death rates were too high[15].

In conclusion, it could be stated that the wide-spread of nosocomial infections stems from ineptitude and the lack of awareness of severity of the problem among medical and paramedical staff. Further, over-use of devices time and again, in an Indian hospital for incoming patients everyday is the maximum and that could be the glitch leading to nosocomial spreads of subtle and pernicious pathogens. For example, the blood pressure monitoring machine, the x-ray unit, catheters, ventilators or oxygenating devices are in frequent use and too should have significant roles in the spread of raging MDR pathogens. Ipso facto, services by hands of careless health-care workers become migratory determinants of many infectious diseases.

Secondly, general hospital wastes, offal and clinical

samples are mismanaged, by and large, in substandard hospitals both at the site of waste generation and during their disposal. Big waste holders without lid cause spreads of pathogens, if time gap of waste collection is more. Inorganic solid wastes along with domestic wastes of hospitals are used as landfills, sometimes unwittingly. In many Indian hospitals shoes are left out religiously, but the question is, whether it is sufficient for the logistic requirement of deterring microbial spores and cells, a minor fraction do not use a waste-bin religiously.

Further, it would not be rhetoric to state that the unmindful letting of space/chance for the spread of pathogenic bacteria is the lack of due antiseptic care of everywhere, always in a hospital.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Staphylococcus aureus was originally a commensal on human body. However, because of the emergence of its multidrug resistant strains it causes problems of OPDs and ICUs as nosocomial spread. Thus it was worthwhile to have surveillance of a hospital that is attended by patients of all ages and economic status. Today this bacterium is emerged as methicillin resistant *S. aureus* (MRSA) and this strain has become a superbug in health domain.

Research frontiers

Studies are being performed for assessing the ability of MDR *S. aureus* and MRSA in community and hospital settings in a hospital of a developing country. This study would

provide epidemiological database for effective means of control.

Related reports

A recent avalanche of reports on *S. aureus* from hospitals: 1. Kinnevey PM *et al.* 2013. *Antimicro Agent Chemother* 57: 524–531; they have stated that surveillance of MRSA strain is warranted and will require updating of currently used SCC–mec typing methods for identifying resistance markers. 2. López–Aguilera S *et al.* 2013. *Enfermed Infec Microbiol Clinica* (in press); in Spain 56.4% students almost never washed their hands before to attending to the first patient, and only 38.6% always washed after examining patients. More than a third (35.7%) ignored the hand hygiene protocol, and 38.6% had not received specific formation, in a study. 3. Han JH *et al.* 2013. *Epidemiol Infect* 141: 165–173; they found that 134 (34.2%) and 73 (18.6%) patients had *S. aureus* isolates with reduced vancomycin susceptibility ascertained by E–test and micro broth dilution, respectively.

Innovations & breakthroughs

MRSA, VRSA, and VISA strains are reported in this study. These would be clear cases of concern to a clinician, as resistance to beta–lactam group and vancomycin would cause problems in the control of this suppuration causing bacterium.

Applications

Avant–garde antimicrobials could be planed for MRSA, VRSA and VISA strains. These could be synergistic applications involving antibiotic–antibiotic or antibiotic–other source of antimicrobials. Furthermore, this study gives a picture of an infectious status of a hospital for this bacterium.

Peer review

Surveillance of *S. aureus* is a coveted work of any hospital, since this bacterium is regarded as the superbug in the health domain for its resistance to all most all antibiotics of the time, *i.e.*, pandrug resistance. The present study described resistance of the pathogen to 16 antibiotics, including vancomycin.

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