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Prevalence and Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Isolates at a Tertiary Care Hospital in Chandigarh

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ABSTRACT

Introduction: The inherent capability of acquiring resistance and increased mortality rates has made methicillin-resistant *S.aureus* (MRSA) a significant contributor towards the rising community and health care burden. The study aims to determine the prevalence and antibiotic susceptibility profile of *S.aureus* isolates from various clinical samples at a tertiary care hospital, Chandigarh. **Methods:** A total of 563 *S.aureus* was isolated from various clinical samples over one year (January-December, 2018). *S.aureus* was identified by characteristic growth on Blood and MacConkey agar. Antibiotic susceptibility testing of the isolates was done by Kirby‑Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) 2018 guidelines. **Results:** Among 563 *S.aureus* isolates, the prevalence of methicillin resistance was 20.2% and 13.85% were D-test positive.*S.aureus* isolates were majorly isolated from patients with skin and soft tissue infections (86.6%) followed by bloodstream infections (6.5%) and urinary tract infections (2.5%). While MRSA isolates showed higher resistance towards ciprofloxacin (76.3%), erythromycin (68.4%), clindamycin (45.4%), co-trimoxazole (33.6%), and gentamicin (31.5%), they were highly susceptible to Vancomycin (100%), Teicoplanin (97.2%) and linezolid (95.6%). Moreover, among MRSA isolates, Linezolid resistant *S.aureus* (LRSA) was as high as 4.4%. None of the isolates were found to be resistant to mupirocin. **Conclusion:** While the MRSA prevalence has decreased over a decade, due to strict adherence to antimicrobial stewardship programs, the MRSA resistance pattern across various classes of antibiotics especially linezolid has increased. Therefore, the current anti-MRSA agents such as vancomycin and linezolid should be used judiciously to avoid more resistance against them.

INTRODUCTION:

In India, infectious diseases are escalating exponentially thereby posing themselves as a national health threat leading to an increased mortality rate along with increased disability and hospitalization cost. The crude mortality rate of infectious disease in India is 416.75 per 100,000 persons [1]. Moreover, aberrant usage of antibiotics coupled with poor antimicrobial stewardship over the years has contributed to developing 'superbugs' which is an army of multi-drug resistant pathogens in both community and health care settings.

Gram-positive pathogens, especially *Staphylococcus aureus* express various virulence factors that establish infectious diseases ranging from bacteraemia, skin, and soft tissue to endocarditis, pneumonia, and device-related infections [2]. 13-74% of global *S.aureus* infections have been reported to be Methicillin-resistant *S.aureus* (MRSA) [3]. MRSA is a life-threatening nosocomial pathogen, associated with worse outcomes than patients infected with methicillin-sensitive strains (MSSA) [4]. Due to its high prevalence of resistance, mortality rate, and ever-rising burden on community and health care settings, WHO in 2016 has listed MRSA as a 'high' priority pathogen [5]. Even in the national ICMR-AMRSN study, the MRSA prevalence was 37.3% which rose to 38.6% in 2018 with north India reporting the highest MRSA rates of 52.8% [6].

Vancomycin and Linezolid are the first choice of drugs for MRSA infections; however, some reports emphasize the presence of multi-drug resistant (MDR) pathogens that are also resistant towards vancomycin and linezolid, thereby making it a perplexing task for the clinicians to manage MRSA infections. Therefore, the rising MDR pathogens across India intrigued us to investigate the prevalence and the antibiotic sensitivity pattern of *S.aureus* in various infections in our hospital in Chandigarh.

MATERIAL AND METHODS:

Study design: This retrospective cross-sectional study was carried out from January to December 2018 at the Department of Microbiology, Government Medical College Hospital, Chandigarh, India. *S.aureus* isolates were isolated from various clinical samples which were processed using standard laboratory techniques and were further cultured overnight on sheep blood agar (CBA) (HiMedia, India) and MacConkey's agar (HiMedia, India). After colony characterization and gram staining, the isolates were further processed via various biochemical tests, e.g. catalase test, free and bound coagulase test, and anaerobic mannitol

fermentation. The antibiotic susceptibility testing was reported as per the Clinical and Laboratory Standards Institute (CLSI) criteria, 2018[7].

Oxacillin screen agar test: A bacterial inoculum of each strain was made and turbidity was adjusted to 0.5 McFarland. One drop of this suspension was inoculated on Mueller–Hinton agar containing 4% NaCl and 6 µg oxacillin ml^{-1} (HiMedia, India). Plates were incubated at 35°C for 24 h. Any strains showing growth on the plate containing oxacillin were considered to be resistant to methicillin [8].

Cefoxitin disk diffusion test: All strains were tested with 30 mg cefoxitin discs (HiMedia, India) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16–18 h incubation at 35°C. Zone size was interpreted according to CLSI (2018) criteria.

Antimicrobial susceptibility testing: The Kirby Bauer disc diffusion method was used routinely to detect the sensitivity of *S. aureus* isolates and interpretations were made according to CLSI (2018) guidelines [7]. The following antibiotics were used – penicillin (10 µg), oxacillin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), doxycycline (30 µg), gentamicin (10 µg), norfloxacin (5µg), nitrofurantoin (300µg), cotrimoxazole (1.25/23.75 µg), chloramphenicol (30 µg) and Quinopristin-Dalfopristin (15 µg). The linezolid resistance ranges used are S: ≥21mm and R: ≤20mm (CLSI, 2018).

Detection of MRSA with reduced susceptibility to vancomycin was performed using vancomycin screening agar with vancomycin concentrations 6 µg/ml. Susceptibility to vancomycin (S:≤2µg/ml; R: ≥16µg/ml) and teicoplanin (S:≤8µg/ml; R: ≥32µg/ml) was defined based on the MIC breakpoints according to the CLSI (2018) guidelines.

Detection of Mupirocin susceptibility: Kirby –Bauer disk diffusion method was used by employing a 5 or 10µg of mupirocin to detect low-level resistance and a 200µg disk for high-level resistance detection. [7]

D test: All erythromycin-resistant and clindamycin-sensitive *Staphylococcus* strains were further tested by D-test for finding inducible clindamycin resistance. On Mueller Hinton agar, standard recommendations for inoculum preparation and inoculation were followed. ERY disc was placed at a distance of 15 mm (edge to edge) from the CLI disc. Following overnight incubation at 37° C, the appearance of the CLI zone close to the ERY disc was noted[9]. The interpretation was done as follows: [10]

- Growth up to CLI and ERY discs indicates resistance to both ERY and CLI (cMLS B phenotype).

- Demonstration of flattened CLI zone between ERY and CLI disc shows inducible clindamycin resistance, (iMLS B phenotype).

- No flattening of CLI zone - negative for inducible clindamycin resistance (MS phenotype) i.e. resistant to ERY but susceptible to CLI.

RESULTS:

A total of 563 *S. aureus* isolates were analyzed from January-December, 2018. The majority of *S.aureus* (86.6%) were isolated from patients with skin and soft tissue infections (SSTIs) followed by bloodstream (6.5%), urinary tract (2.5%), and respiratory tract (1.4%) infections. Table no 1 highlights the infection where *S.aureus* isolates are the major causative organism.

Table no 1: Prevalence of *S.aureus*isolates in various infections

Infections	***S.aureus* isolates (%)**
Skin and Soft Tissue Infection (SSTI)	86.6%
Blood Stream Infection (BSI)	6.5%
Urinary Tract Infection (UTI)	2.5%
Respiratory Tract Infection (RTI)	1.4%
Bone and Joint Infection (BJI)	0.7%
Ear Infection	0.7%
Eye Infection	0.7%
Intra-Abdominal Infection	0.3%
CNS Infections	0.3%

While the MRSA prevalence was 20.2%, the MSSA isolates formed 79.7%of the total *S.aureus* isolates in the year 2018. Table 2 depicts the MRSA and the MSSA (Methicillin sensitive *S.aureus*) prevalence in the year 2018. Further, the MRSA prevalence in SSTIs was observed to be 19% while in BSI it was as high as 29.7%.

Table No. 2: MRSA and MSSA prevalence in *S.aureus* isolates

Total *S.aureus* isolates	(n=563)	Total Prevalence
MRSA	114	20.2%
MSSA	449	79.7%

MSSA: methicillin-sensitive *S.aureus;* MRSA: Methicillin-resistant *S.aureus*

Antibiotic susceptibility pattern:

An antibiotic sensitivity pattern for penicillin, ciprofloxacin, doxycycline, gentamycin, erythromycin, chloramphenicol, clindamycin, co-trimoxazole, mupirocin, linezolid, vancomycin, daptomycin, and teicoplanin was determined. Among the total *S.aureus*isolates, the penicillin resistance was the highest (91.2%) followed by ciprofloxacin (62.8%), erythromycin (45.9%), co-trimoxazole (34.9%) and clindamycin (26.7%). None of the isolates were found to be resistant tovancomycin, mupirocin, and quinopristin- dalfopristin.

As compared to MSSA isolates, MRSA showed much higher resistance towards penicillin (95.6%), ciprofloxacin (76.3%), erythromycin (68.4%), clindamycin (45.4%), co-trimoxazole (33.6%), gentamycin (31.5%), and doxycycline (10.8%). **Fig1** highlights the resistance pattern of both MRSA and MSSA isolates.

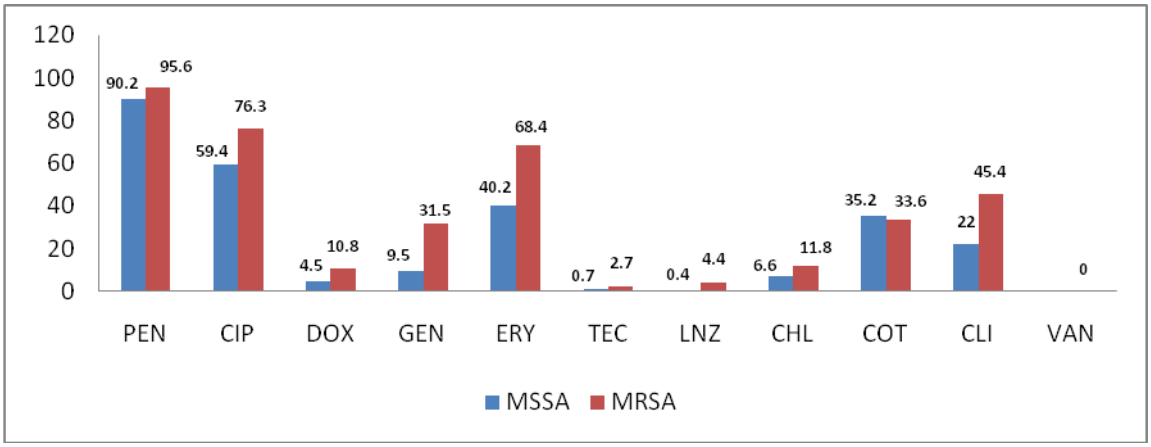

Figure No. 1: Resistance pattern amongst MRSA and MSSA isolates

Further, the MRSA isolates were highly susceptible to linezolid (95.6%) and Teicolpanin (97.2%). Both MRSA and MSSA isolates were shown to be sensitive to vancomycin (100%) Apart from these tested antimicrobials, nitrofurantoin and norfloxacin were also tested against *S.aureus,* isolated from urinary tract infection. MSSA isolates showed 100%

susceptibility to both antibiotics. However, out of 3 MRSA isolates, one isolate showed resistance towards both, nitrofurantoin and norfloxacin.

Out of 563 *S. aureus* isolates, 78 (13.85%) were D-test positive. Table 3 shows the percentage of MRSA and MSSA showing inducible resistance to clindamycin (iMLSB phenotype), MS phenotype (resistant to erythromycin and sensitive to clindamycin but no induction), and strains showing combined resistance and sensitivity to erythromycin and clindamycin.

Table No. 3: Percentage of MRSA and MSSA showing different resistant phenotypes

Isolates	iMLSB Phenotype (%)	cMLSB Phenotype (%)	MS Phenotype (%)	Sensitive to both Ery and CLI (%)
MRSA (114)	18 (15.78)	52 (45.61)	08 (7.01)	36 (31.57%)
MSSA (449)	56 (12.47)	99 (22.04)	114 (25.38)	180 (40.08)
Total (563)	74	151	122	216

MRSA isolates showed higher inducible resistance (15.78%) and constitutive resistance (45.61%) as compared to MSSA which showed 12.47% inducible and 22.04% constitutive resistance.

DISCUSSION:

India was the leading antibiotic consumer among the low middle-income countries with an antibiotic consumption increase of 103% between 2000 and 2015. During this period, the average antibiotic consumption per day also escalated by 63% [11]. With easy availability, rampant use of antibiotics, and poor anti-microbial stewardship programs, the MDR pathogens had an ample opportunity to thrive and become a national health burden today.

In our country, the MRSA prevalence ranges from 17.6% to 80.4% depending upon the geographical location and the antibiotic regimen being used [12,13]. Our center had previously reported MRSA prevalence of 38% from 2008-09 [14]. However, with a robust and strict antimicrobial stewardship program implied at our hospital, we observed an MRSA prevalence of as low as 20.2% in 2018.

Further, both national studies, INSAR and ICMR-AMRSNhave highlighted*S.aureus*to be the most dominant pathogen in SSTIs [14, 15]. Similarly in our study too, we noticed a similar

trend where *S.aureus*was isolated from 86.6% of SSTI patients. Apart from SSTIs, *S.aureus* has had also been recently recognized as an important causative pathogen in bloodstream infections with a study reporting a crude mortality rate of 31% amongst MRSA bacteremia patients [16]. Moreover, MRSA prevalence in various reports, have shown to be as high as 48-54% in BSIs [14,17]. Interestingly in our study also, the MRSA prevalence (29.7%) was highest in patients with BSI.

The growing incidence of MDR pathogens has made the treatment and management of various infections, a perplexing task for clinicians. In our study, though the MRSA prevalence was low, the MRSA resistance pattern across various classes of antimicrobials (Ciprofloxacin, Erythromycin, Gentamycin, Chloramphenicol, Co-trimoxazole, and Clindamycin) was much more pronounced than the MSSA isolates. Various studies have noted similar trends of higher antimicrobial resistance for MRSA isolates [14,15,18] which could further contribute to its rising mortality rates in India.

Glycopeptides such as vancomycin and teicoplanin are the first-line drug for the treatment of MRSA infections. While various reports have shown VRSA (Vancomycin-resistant *S.aureus*) prevalence to range from 2-7.1% [19, 20], however at our hospital, none of the isolates were resistant to vancomycin.

Linezolid is the only oral antibiotic available amongst its counterparts, which is widely used for treating resistant *S.aureus* infections. Lower cost price and overuse of linezolid in an easy to treat gram-positive infections have given rise to linezolid resistant strains. In 2012, our group was the first to report linezolid resistance in CoNS[21] after which there were many reports that very well document this resistance pattern in CoNS species [22-24]. In 2015, Rai et al for the first time reported linezolid resistance in MRSA to isolate from India [25]. However, there are not many reports, in India, on linezolid resistance which is <1%. Interestingly, in our study, we show that 4.4% of the MRSA isolates were linezolid resistant. Though >95% of the MRSA isolates were sensitive to linezolid, however, such rising resistant rates amongst the commonly used anti-MRSA agents indicate the upsurge in the MRSA resistant pattern. Apart from vancomycin and linezolid, the other two anti-MRSA agents are Daptomycin and Ceftaroline. We are currently in the process of establishing their susceptibility pattern in our department. We also found that none of the isolates were resistant to mupirocin in our study. This result is similar to a study by Rajkumari*et al* in 2014 which also showed no resistance in MRSA as well as MSSA strains to mupirocin. [26]

D-test has turned out to be a vital part of routine antimicrobial susceptibility tests for all clinical isolates of *S. aureus* as clindamycin has become an excellent antimicrobial to treat skin and soft tissue infections. Failure to identify iMLS B resistance may lead to clinical failure of clindamycin therapy. In our study, 78 (13.85%) *S.aureus* isolates were D-test positive. MRSA isolates showed higher inducible resistance (15.78%) as compared to MSSA which showed 12.47% inducible resistance. A previous study by Gadepalli*et al* has shown increased inducible resistance in MRSA strains (30%) [27]. Similarly, our previous study in 2008, had also shown 20% inducible resistance in MRSA and 17.3% inducible resistance in MSSA isolates [28]. A significant reduction in inducible clindamycin resistance in our present study as compared to the previous studies highlight the strict antimicrobial stewardship programs as well as infection control guidelines at our institute.

In conclusion, the strict antibiotic guidelines at our hospital have helped in reducing the MRSA prevalence from 38% in 2009 to 20.2% in 2018. However, even though the MRSA prevalence has decreased, the data provides a holistic snapshot of the changing antibiotic paradigm of the resistant *S.aureus* isolates especially against the currently available anti-MRSA agents like linezolid. This will not only assist the physician in choosing an appropriate antibiotic regimen but will also further emphasize the need for rational antibiotic use.

Conflicting Interest:

The authors declare no conflicts of interest.

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