Prevalence of community-associated *Staphylococcus aureus* strains among university students

Shil A.[1,A,B,C,D], Bishayi B.[2,A,C,F,], Sikdar (ne'e Bhakta) M.[*1,A,C,D,E, F]

1. Microbiology, Nutrition and Dietetics Laboratory, Physiology Unit, Department of Life Sciences, Presidency University, Kolkata, India
2. Department of Physiology, Immunology Laboratory, University of Calcutta, University Colleges of Science and Technology, Kolkata, India

A- Conception and study design; **B -** Collection of data; **C -** Data analysis; **D -** Writing the paper;
E- Review article; **F** - Approval of the final version of the article; **G** - Other (please specify)

ABSTRACT

Purpose: Colonization of multiple antibiotic resistant *Staphylococcus aureus* (*S. aureus*) in nasal cavity is associated with heightened risk of infections. The emergence and spread of multiple antibiotic resistant community-associated (MAR-CA) *S. aureus* strains has worsened the situation. The aim of this study was to assess the rate of prevalence and patterns of antibiotic resistance in *S. aureus* strains isolated from members of the student community in Presidency University, Kolkata, India,.

Materials and methods: *S. aureus* isolates from university students were subjected to phenotypic and genotypic identification, construction of phylogenetic tree and submission of 16S rRNA sequences to GeneBank. Statistical analysis was done using Chi-square test to evaluate the significance of risk factors on the prevalence of community- associated (CA) and multiple antibiotic resistant *S. aureus* strains.

Results: Outcome of this study discloses the highest nasal colonization rate is that of CA- *S. aureus* strains (51.11%), followed by CA-MRSA strains (13.08%). 9.66% of the colonized strains are MAR (Multiple Antibiotic Resistant) CA- *S. aureus*.

Conclusions: High nasal carriage rates of CA and MAR *S. aureus* strains point to increased risk of development of life threatening infections whenever these commensal microorganisms come in contact with carrier's blood. They can raise mortality rates by damaging cardiovascular and respiratory systems by causing endocarditis and pneumonia respectively, which are difficult to treat using antibiotics. This study conveys an alarming message, since it points to the insufficiency of antibiotics for the treatment of infectious diseases. Awareness about the prudent use of antibiotics, restricted and judicious antibiotic use and alternative therapeutic measures can help to keep the situation under control.

Keywords: *Staphylococcus aureus*, Community-associated methicillin-resistant, Multiple antibiotic resistant, Nasal carriage.

DOI: 10.5604/01.3001.0015.6400

[*]**Corresponding author:**
Mausumi Sikdar (née Bhakta)
Associate Professor, Department of Life Sciences
Presidency University, Kolkata-73; E-mail: mausumi.dbs@presiuniv.ac.in

Received: 20.08.2021
Accepted: 20.10.2021
Progress in Health Sciences
Vol. 11(2) 2021 pp 65-75

INTRODUCTION

Staphylococcus aureus, one of the most prevalent commensal microorganisms, frequently causes a wide range of sickness starting from simple skin infections to life threatening diseases. Though a member of the normal microbial flora, it plays a key role in the spreading of hospital as well as community acquired infections. Due to its ability to colonize on human skin, it is commonly found in the nasal mucosa. The pathogen is persistently colonized in the nasal cavities of 20% to 80% of the population, and such individuals can transmit it to other people, ultimately raising the possibilities of them developing infectious diseases [1]. Multiple antibiotic resistances in *S. aureus* now seem to be the rule. Nasal colonization of *S. aureus* has now been recognized as a major risk factor for the development of infections in patients, particularly those undergoing surgery, hemodialysis, HIV infected and also admitted in intensive care units. *S. aureus* has been identified as the cause of recurrent furunculosis, on the basis of the fact that, 60% of patients suffering from furuncles and impetigo having nasal colonization with this bacterial strain. Besides, it is also the causative agent for bacteremia, endocarditis and pneumonia [2]. The phenomenon of multiple antibiotic resistances demonstrated by *S. aureus* strains has made the treatment of such infections extremely difficult and expensive.

The skin, nasal nares, gastrointestinal tract, axilla, ear lobes and rectum are the different parts of the human body that serve as common colonization sites for *S. aureus*, but the anterior nares are main reservoirs. The microbes can be transmitted through hands from cutaneous sites to nasal mucosa and then gradually to the upper respiratory tract and so on. Studies revealed that individuals of the same household carry genetically identical strains of *S. aureus.* Healthcare workers, who are asymptomatic nasal carriers, play key roles in *S. aureus* transmission in the community and sometimes become the reason of methicillin-resistant *S. aureus* (MRSA) outbreaks [3].

Acquisition of antibiotic resistance is a common phenomenon in the microbial world. In order to combat against penicillin resistant *S. aureus* strains, methicillin was developed and now MRSA (Methicillin Resistant *Staphylococcus aureus*) strains are a global threat. Antibiotic resistance has spread not only in *S. aureus*, but has also spread among other bacterial species such as *Klebsiella pneumoniae, Escherichia coli, etc.* [4] Previously, researchers have demonstrated that shuttle plasmid is the carrier of antibiotic resistance gene and experimental evidence supports that these plasmids are responsible for the intraspecies transfer of chloramphenicol resistance gene among *S. aureus* [5,6]. For therapeutic purposes, there are now only a few selective antibiotics to which the strains are still sensitive, vancomycin being one of them, although emergence of vancomycin resistant *S. aureus* strain has also been reported [7].

Among the two major varieties of MRSA, hospital- acquired *S. aureus* strains (HA-MRSA) are those that can be identified in patients after 72 h of admission to any health care facility centers, and on the contrary, if the isolates are found to be present in patients within 72 h of admission are identified as community associated (CA-MRSA) strains. MRSA strains can be identified by the presence of the mecA gene. MRSA strains possess staphylococcal cassette chromosome mec (SCCmec) gene having different subtypes. CA-MRSA contains type IV and V genes, while HA-MRSA contains type I, II and III genes. Panton-Valentine-Leukocidin (PVL), a cytotoxin, produced by CA-MRSA, can destroy leukocytes due to its pore forming ability [8]. PVL producing CA-MRSA can cause skin and soft tissue infections, [9] osteomyelitis [10] and necrotizing pneumonia [11]. These *S. aureus* mediated infections may damage different organs of human body and ultimately increase the mortality rate.

The ease of transmission in the population makes CA-MRSA more virulent and epidemio-logically significant than HA-MRSA [12]. While reviewing literature, we found that students of different age groups, starting from the school level to the college level are carriers of CA-MRSA and may transmit it within the community. According to a study report in India, the prevalence of *S. aureus* in children within the 5-15 years age group is 16%-52% [13]. The rate of prevalence and antibiotic susceptibility patterns of *S. aureus* shows a wide variation in different community settings. Though the presence of carriers of resistant bacterial strains in the community may be of a very negligible percentage, but this is enough to transmit the bacteria in whole community, as its spread occurs through skin contact. Thus educational institutions are among those places bearing high chances of transmission, as people gather here from different surroundings.

Prevalence of a high percentage of antibiotic resistant *S. aureus* strains in the community is one of the important reasons for increasing morbidity and mortality rates due to infectious diseases. With this background, the focus of this study was to assess the rate of prevalence and patterns of antibiotic resistance in *S. aureus* strains isolated from members of the student community in Presidency University, Kolkata, India, so that awareness can be generated among them regarding this alarming situation, which has arisen due to misuse of antibiotics.

MATERIALS AND METHODS

Study design

A total of 558 students (239 males and 296 females) of Presidency University, located at central Kolkata, India, participated in this study. The study protocol was approved by Human Ethical Committee of Presidency University, Kolkata (PU/IEC-H/MSCS04, dated 22nd March 2011). After explaining the procedure of sample collection, a duly signed consent form was obtained from each volunteer before any sampling.

Inclusion and exclusion criteria

The criteria of subjects, to be fulfilled, for participating in this study is given in Table 1.

Table 1. Criteria required for being the subjects in this study

Inclusion criteria	Exclusion criteria
Healthy students	History of hospitalization
Both male and female	Required antibiotic therapy in last one year
Age should be within 18-24 years	Age above 24 years

Data collection

The names of volunteers were first enlisted. Data, relevant to this study, such as gender, details of antibiotic consumption, previous and present medical history were then collected by questionnaire method. Based on the exclusion and inclusion criteria, eligible subjects were identified and then *S. aureus* strains were collected from them.

Collection of community associated strains

Swab collection

In order to isolate *S. aureus* strains, nasal swabs were collected from the anterior nares of volunteers using sterile cotton buds, moistened with normal saline and streaked immediately on nutrient agar (Himedia, Mumbai, India) plates containing 5% sodium chloride (5% NaCl plates). The plates were incubated overnight at 37º C in aerobic condition [14].

Biochemical identification of collected strains

The colonies growing on 10% NaCl plates were cultured on mannitol salt agar plates. Based on the morphological characteristics of colonies and their ability to ferment mannitol, *S. aureus* colonies were isolated and were subjected to further steps of confirmation by Gram staining and biochemical methods such as catalase and coagulase tests.

Molecular identification of isolated *S. aureus* strains

DNA Extraction

Extraction of DNA from the isolated strains was carried out using HiPurA™ Bacterial Genomic DNA Purification Kit, MB505, (HiMedia, Mumbai, India) and its concentration was measured by Nanodrop Spectrophotometer (BioRad).

Gene amplification by PCR

S. aureus strains, identified by their phenotypic characteristics, were further tested for presence of species specific 16S rRNA, *nuc, pvl and SCCmec* subtype genes. Polymerase chain reaction (PCR) amplification of those genes was carried out with the extracted DNA of the sample along with forward and reverse primers of respective genes (Table 2) according to the protocol followed by Karmakar et al [15]. *S. aureus* ATCC 25923 was used as positive control.

Construction of Phylogenetic tree

The PCR products of 16S rRNA gene from ten selected CA-*S. aureus* strains were sequenced at Xcelris laboratory (Ahmedabad, Gujarat, India), followed by BLAST analysis and submission of data to GeneBank (www.ncbi.nlm.nih.gov).To determine the manner in which, the CA-*S. aureus* strains collected in this study were genetically related to the globally prevalent strains of the same species, phylogenetic analysis of genetic distance was carried out with the help of MEGA software (Molecular Evolutionary Genetics Analysis) (version 10) [16].

Antibiotic susceptibility profiling

The antibiotic susceptibility patterns of all confirmed *S. aureus* strains was determined by Kirby-Bauer disc diffusion method and the interpretative criteria for zone inhibition were set as per recommendations of Clinical and Laboratory Standards Institute (CLSI) [17]. The commercially available antibiotic discs such as ampicillin (10µg/disc), amikacin (30µg/disc), aztreonam (30µg/disc), cefexime (5µg/disc), chloramphenicol

(30µg/disc), gentamycin (10µg/disc), kanamycin (30µg/disc), methicillin (5µg/disc), norfloxacin (10µg/disc), tetracycline (30µg/disc), and vancomycin (30µg/disc) were used. A strain was considered as multidrug resistant if it was resistant to at least one antibiotic, belonging to three or more groups [19].

Table 2. Primers and their sequences used in PCR

Gene	Primers	Sequences (5' ⟶ 3')	References
16S rRNA	27-F	AGAGTTTGATCGTGGCTCAG	[12]
	1492-R	CGGTTACCTTGTTACGACTT	
Nuc	nuc-F	GGCATATGTATGGCAATTGTTTC	[8]
	nuc-R	CGTATTGCCCTTTCGAAACATT	
Pvl	pvl-F	AAATGCTGGACAAAACTTCTTGG	
	pvl-R	TTTGCAGCGTTTTGTTTTCG	
SCC *mec* I	Type I-F	GCTTTAAAGAGTGTCGTTACAGG	[18]
	Type I-R	GTTCTCTCATAGTATGACGTCC	
SCC *mec* II	Type II-F	CGTTGAAGATGATGAAGCG	
	Type II-R	CGAAATCAATGGTTAATGGACC	
SCC *mec* III	Type III-F	CCATATTGTGTACGATGCG	
	Type III-R	CCTTAGTTGTCGTAACAGATCG	
SCC *mec* IVa	Type IVa-F	GCCTTATTCGAAGAAACCG	
	Type IVa-R	CTACTCTTCTGAAAAGCGTCG	
SCC *mec* IVb	Type IVb-F	TCTGGAATTACTTCAGCTGC	
	Type IVb-R	AAACAATATTGCTCTCCCTC	
SCC *mec* IVc	Type IVc-F	ACAATATTTGTATTATCGGAGAGC	
	Type IVc-R	TTGGTATGAGGTATTGCTGG	
SCC *mec* IVd	Type IVd-F	CTCAAAATACGGACCCCAATACA	
	Type IVd-R	TGCTCCAGTAATTGCTAAAG	
SCC *mec* V	Type V-F	GAACATTGTTACTTAAATGAGCG	
	Type V-R	TGAAAGTTGTACCCTTGACACC	
*mec*A	mecA-F	CAATGCCAAAATCTCAGGTAAAGTG	[8]
	mecA-R	AACCATCGTTACGGATTGCTTC	

Statistical analysis

Two tail Chi-square test was performed for testing significant differences. Statistical significance was considered at the level of $p< 0.05$.

RESULTS

Identification of *S. aureus*

Maximum number of collected strains from each batch were found to grow on NaCl plates and were both catalase positive as well as Gram positive, while, a discernible reduction in their number was noticed when subjected to coagulase test and then allowed to grow on blood agar plates followed by growth on mannitol salt agar (MSA) plates (Fig. 1).

Rates of prevalence of *S. aureus* strains

This study was conducted on 535 students, 44.67% (239/535) of whom were males and 55.32 % (296/535) were females over a period of four years. The total number of *nuc* positive, confirmed *S. aureus* strains collected from students were 207 (38.69%), out of which 13.04% (27/207) were MRSA and 9.66% (20/207) were multiple antibiotic resistant (MAR) strains. Among the confirmed *S. aureus* strains, 76 (36.71%) strains were CA-*S. aureus*. The rate of prevalence of CA-*S. aureus* was higher 51.11% (23/45) in the first batch of students compared to the other batches. Among the total number of 239 screened males, 32 (13.38%) and 44 out of the total 296 screened females (14.86%) were positive for CA-*S. aureus*. Only 25% strains (8 out of 32) isolated from males and 27.27% strains (12 out of 44) isolated from females are multiple antibiotic resistant.

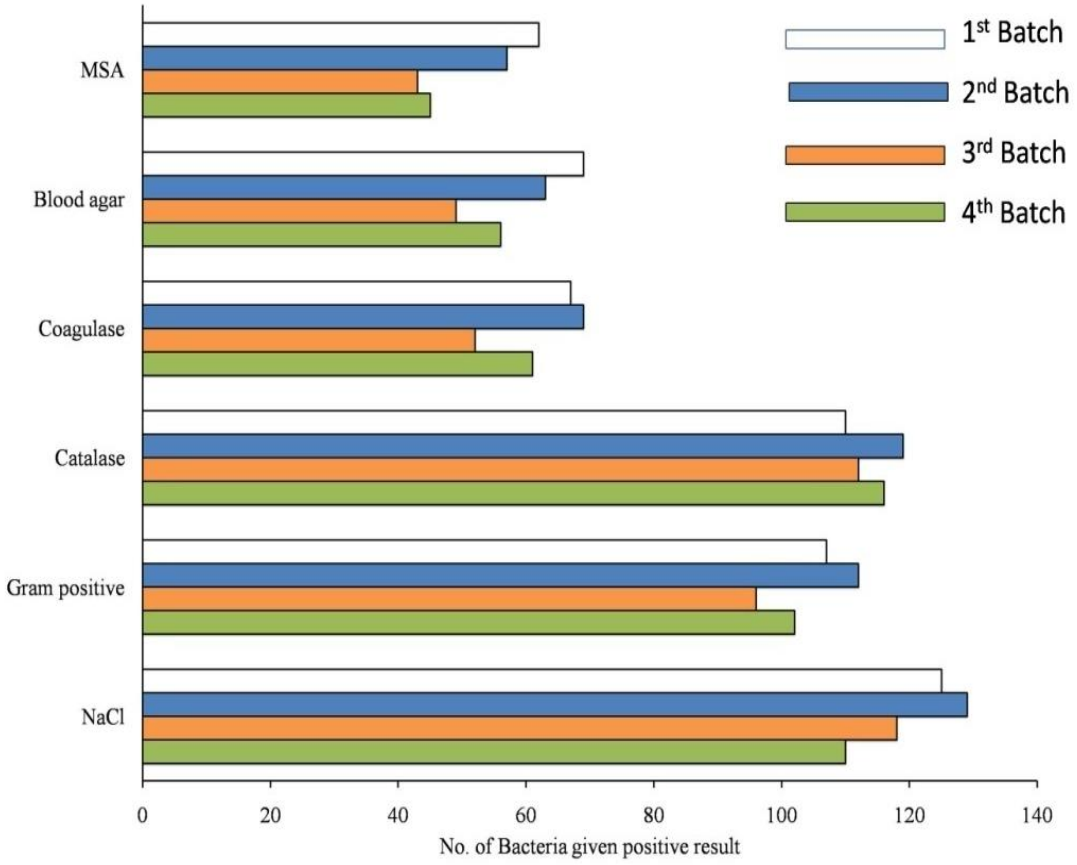

Figure 1. Results of confirmation of *S. aureus* strains by biochemical tests (MSA= Mannitol salt agar)

Effect of different variables on the prevalence of *S. aureus*

The rates of prevalence of *S. aureus* and the occurrence of MAR strains do not significantly vary with the gender of participants. Similar results were also observed in case of other parameters i.e., frequency of antibiotic consumption, visit to healthcare centers, attachment of any family members with hospitals ($p > 0.05$) (Table 3).

Table 3. Association of the rate of nasal carriage of *S. aureus* and MAR *S. aureus* strains with various risk factors such as (a) gender (b) rate of antibiotic consumption (c) history of visit to hospitals/clinics and (d) the involvement of family members with health care facilities

a) Association of gender of participants with the rate of nasal carriage of *S. aureus* and MAR *S. aureus* strains			
Presence of *S. aureus* strains	Gender		p-value
	Male (n= 256)	Female (n= 279)	
S. aureus (SA)	86	121	0.122^{NS}
MAR *S. aureus* (MSA)	9 out of 86	11 out of 121	0.802^{NS}
b) Association of the rate of antibiotic consumption of subjects with the rates of nasal carriage of *S. aureus* and MAR *S. aureus*			

Presence of *S. aureus* strains	Incidences of Antibiotic consumption in the previous year		p-value
	Once (n= 265)	More than once (n= 270)	
S.aureus (SA)	97	110	0.513^{NS}
MAR *S. aureus* (MSA)	8 out of 97	12 out of 110	0.45 ^{NS}
c) Association of the history of visit to hospitals/clinics with the nasal carriage rate of *S. aureus* and MAR *S. aureus.*			
Presence of *S. aureus* strains	History of visit to hospitals/clinics (last 1 year)		p-value
	Visited (n= 265)	Not visited (n=270)	
S. aureus (SA)	108	99	0.744 ^{NS}
MAR *S. aureus* (MSA)	8 out of 108	12 out of 99	0.341 ^{NS}
d) Association between the rates of nasal carriage of *S. aureus* and MAR *S. aureus* with the involvement of family members with health care facilities			
Presence of *S. aureus* strains	Association of family members with health care facilities		p-value
	Associated (n=265)	Not associated (n=270)	
S. aureus (SA)	91	116	0.526^{NS}
MAR *S. aureus* (MSA)	6 out of 91	14 out of 116	0.144^{NS}

Molecular identification

Batch wise data regarding availability of *nuc*, *pvl*, *mec* positive strains of *S. aureus* has been presented in Table 4.

Highest number of strains harbouring all the three genes, were found in the third batch. The presence of IVa and IVb subtypes of SCCmec gene is comparatively higher than other subtypes among the strains, whereas, I, II, and III subtypes were not detected in any strain.

Table 4. Genetic characteristics of community associated *S. aureus* strains isolated from students

Batch of Study	**No. of Students Screened**	***nuc* positive *S. aureus***	**mecA positive CA-*S.aureus***	**SCC*mec* subtypes in CA-*S. aureus***						
				IVa	**IVb**	**IVc**	**IVd**	**V**	**Unidentified**	**Total CA-*S. aureus***
1st	133	45	8	6	8	-	-	3	5	23
2nd	128	43	5	5	-	4	3	2	3	17
3rd	135	57	4	6	5	4	2	2	6	25
4th	139	62	10	3	4	-	-	1	2	11

Neighbour joining tree indicates that strains collected from the student community are not distantly related to the other global strains of *S. aureus* in terms of partial sequence of 16S rRNA gene (Fig.2).

The accession numbers of identified CA-*S. aureus* strains given by Gene bank are listed in Table 5.

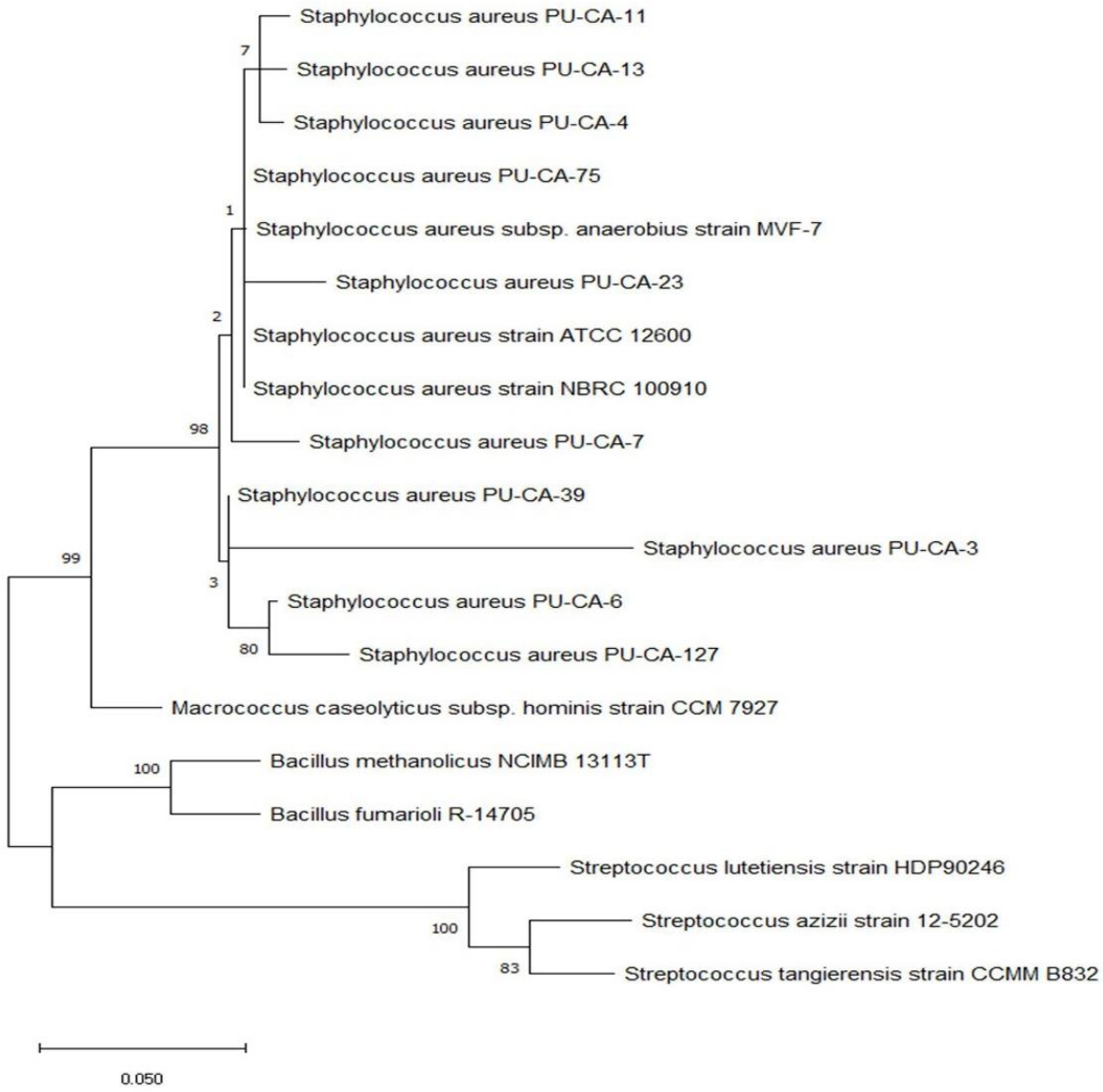

Figure 2. Neighbour joining tree of community associated *S. aureus* strains collected from students

Table 5. List of community associated strains identified.

Sl. No.	Strain No. of CA - *S. aureus*	GeneBank Accession number
1.	PU-CA-3	MT-131357
2.	PU-CA-4	MT-111844
3.	PU-CA-6	MT-154266
4.	PU-CA-7	MT-133036
5.	PU-CA-11	MT-133030
6.	PU-CA-13	MT-111915
7.	PU-CA-23	MN-843752
8.	PU-CA-39	MT-568666
9.	PU-CA-75	MT-568664
10.	PU-CA-127	MT-154268

Antibiotic susceptibility profiling

The batch wise distribution patterns of resistance to different groups of antibiotics of all confirmed *S. aureus* strains, obtained during the study period are presented in Fig. 3 and 4. All strains collectively showed the highest resistance to the beta-lactam group of antibiotics, followed by aminoglycosides, chloramphenicol, tetracycline and floroquinolones, whereas, most of them were sensitive to glycopeptides. During the whole study period, spread over four batches, 9.66% (20 out of 207) were MAR (multiple antibiotic resistant) *S. aureus* strains.

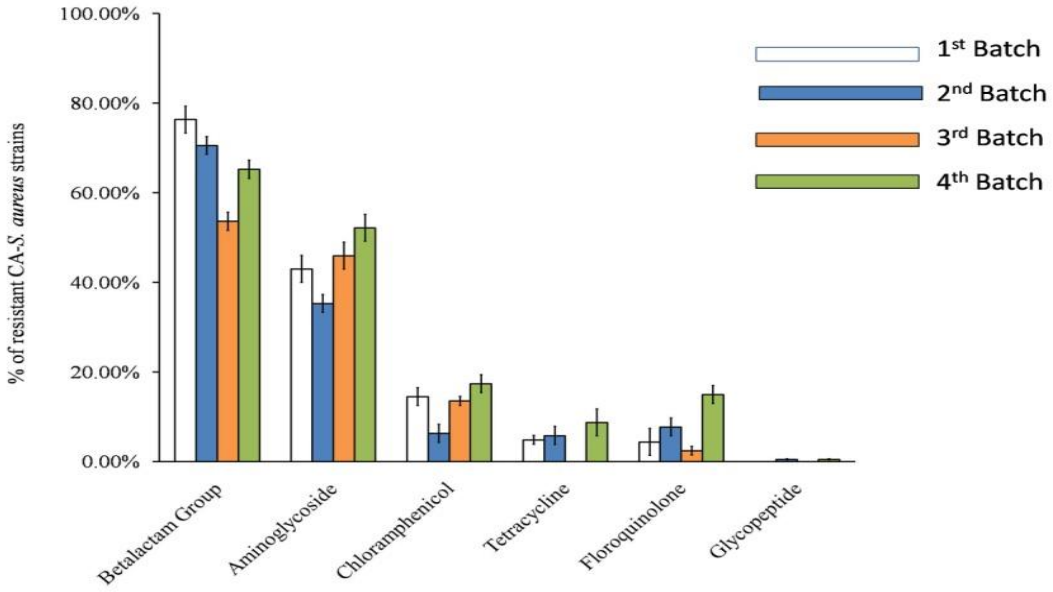

Figure 3. Antibiotic resistance patterns of community associated *S. aureus* strains.
All data are expressed in terms of mean ± SD. Results are a mean of three separate experiments

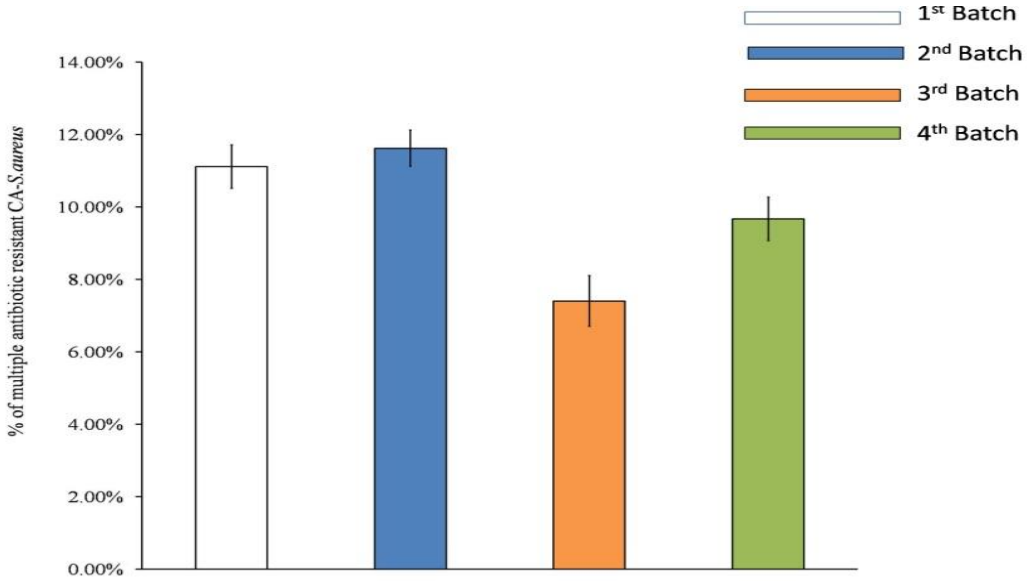

Figure 4. Batch wise prevalence of multiple antibiotic resistant community associated *S. aureus* strains.
All data are expressed in terms of mean ± SD

DISCUSSION

Positive reaction to catalase and coagulase tests, positive Gram stain reaction, along with demonstration of hemolytic and fermentation ability on blood agar and MSA plates are the essential markers for the phenotypic identification of *S. aureus*. Our study reveals higher number of catalase containing Gram positive cocci, which is correlated with the work done by Karmakar et al [20].

Extensive review of literature suggests that this study is the first of its kind done in this part of world. There are no previous reports on the prevalence of community associated *S.aureus* strains in university students in the eastern part of India. We find the highest prevalence rate (51.11%) of CA- *S. aureus* in the first batch of students. A similar finding was also noticed in a study conducted by Singh et al [13] in Uttar Pradesh, where the *S. aureus* colonization rate was 46.67% in children. Nasal carriers basically play the role of asymptomatic reservoir, from where the infections can be transmitted to others in propinquity [21]. Nasal carriage of *S. aureus* plays an imperative role in the spread of its pathogenicity, by elevating the risk of infections in pre or postoperative patients, where they can be infected by the pathogens present on their own skin before admission to hospital [22]. Alongside being a potent cause of skin and soft tissue infections in the community, nasal carriage of *S. aureus* can also be considered as a persuasive risk factor for emergence and spread of nosocomial infections [23].The situation is extremely alarming, considering the high population density of this region, which can lead to the rapid spread of antibiotic resistance genes. Therefore, the increasing prevalence of this pathogen in the community has now become a hidden threat, as it has the potential to weaken our young generations by infecting them.

To prevent the spread of disease, infection control policies must be implemented rapidly after detecting the causative organism. Two conventional genes, namely nuc and mecA were identified for detection of *S. aureus* and MRSA respectively. Both CA-MRSA and HA-MRSA can be isolated from either hospital or community, as coexistence of both of them has also been reported. In order to reduce the possibility of getting HA-MRSA, possession of SCCmec type IV and V were considered as CA-MRSA, while other subtypes such as I, II, III are typically restricted to HA-MRSA [9].The outcome of our experiments also supports this statement. All isolates of CA-*S. aureus* is PVL positive, indicating that they all secrete a potent cytotoxin, which enables them to kill host leukocytes.

Results of our study indicate that there is no significant difference in the distribution of MAR strains of CA- *S. aureus* among male and female participants. Similar observations were reported by Patil et al. [24], supporting our report that gender is not at all a notable factor for colonization of *S. aureus* and its ability of acquiring resistance genes. On the other hand, frequent antibiotic consumption is a significant factor, which endorses the phenomenon of multiple antibiotic resistance in microorganisms. So, the absence of a significant association between groups on the basis of antibiotic consumption, either once or more than once in a year, is supported by the findings of Tigabu et al. [25]. The remaining factors are not significantly associated with the prevalence of CA-*S. aureus* and MAR-CA-*S. aureus* strains.

Generally CA-MRSA strains are resistant to the beta-lactam antibiotics, but sensitive to non β-lactam group [26], due to production of beta-lactamase enzyme. But according to our results, most of the MRSA strains are also resistant to aminoglycosides and chloram-phenicol, followed by other groups of antibiotics, indicating that these are MAR strains. Similar observations were also reported by Lee et al. [27] and Rojaset al. [28] i.e., MRSA strains have a very high tendency to acquire resistance to a wide variety of antibiotics, commonly used for remedial interventions. These events, in turn, create difficulties in the way of management of infectious diseases, although herbal extracts of medicinal plants such as *Catharanthus roseus* can be used as an alternative to conventional antibiotics for curative purpose against multiple antibiotic resistant community associated *S. aureus* strains [29].

CONCLUSIONS

The high prevalence rate of nasal colonization of *S. aureus* among University students, according to the results of the present investigation, points to the enhanced risk of its transmission in the rest of the community. Emergence of MRSA along with MAR-*S. aureus* strains in the community openly challenges the therapeutic world for its incapability in the management of diseases, which ultimately leads to uncontrolled mortality rate. The present study warns about the consequences of unrestricted use of antibiotics, which ultimately leads to serious health issues. Therefore, the present study suggests that lack of information regarding the proper use of antibiotics may cause antibiotic resistance in microorganisms; hence misuse of antibiotics must be prevented to control the spread of antibiotic resistance gene to reduce the chances of emergence and spread of new antibiotic resistant pathogenic strains, and reduce the morbidity and mortality due to multiple antibiotic resistant *S. aureus* strains.

ORCID
Aparna Shil
https://orcid.org/000-0001-5550-5549
Mausumi Sikdar (née Bhakta)
https://orcid.org/000-0001-5050-2875

Acknowledgement
Authors acknowledge Faculty Research and Professional Development Fund (FRPDF) grant of Presidency University, Kolkata for providing financial assistance in the completion of this work.

Disclosure statement
The authors declare no conflict of interest regarding this work.

Ethical approval:
This study was performed in accordance with the principles put across in the declarations of Helsinki and approved by the Human Ethical Committee of Presidency University, Kolkata (PU/IEC-H/MS-CS04, dated 22nd March 2011).

REFERENCES

1. Ugwu MC, Mokwe NM, Ejikeugwu PC, Enemor EC, Eze CO, Ugwu BC, Gugu TH. Antibiogram of *Staphylococcus aureus* from healthy school pupils in Agulu, Southeastern Nigeria. Int. J Res. 2015 May;4(5):5-9.
2. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. *Staphylococcus aureus* nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. Front. Microbiol. 2018 Oct;8(9):2419.
3. Lamanna O, Bongiorno D, Bertoncello L, Grandesso S, Mazzucato S, Pozzan GB, Cutrone M, Chirico M, Baesso F, Brugnaro P, Cafiso V. Rapid containment of nosocomial transmission of a rare community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) clone, responsible for the Staphylococcal Scalded Skin Syndrome (SSSS). Ital. J Pediatr. 2017 Dec;43(1):1-6.
4. Saha S, Deb B, Sikdar M.B, Bhattacharyya RN, Mandal SN. An effective, complete and non-toxic dose of biogenic mangrove plant synthesized silver nanoparticles that can revive the susceptibility of resistant antibiotics against *Klebsiella pneumoniae*. Biomed. (India) 2018 Jan;38:497-504.
5. Bhakta M, Bal M. Identification and characterization of a shuttle plasmid with antibiotic resistance gene from *Staphylococcus aureus*. Curr. Microbiol. 2003 Jun 1;46(6):0413-7.
6. Bhakta M, Arora S, Bal M. Intraspecies transfer of a chloramphenicol-resistance plasmid of staphylococcal origin. Indian J Med Res. 2003 Apr;117:146-51.
7. Hasan R, Acharjee M, Noor R. Prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in methicillin resistant *S. aureus* (MRSA) strains isolated from burn wound infections. Tzu Chi Med. J. 2016 Jun 1;28(2):49-53.
8. Galia L, Ligozzi M, Bertoncelli A, Mazzariol A.: Real-time PCR assay for detection of *Staphylococcus aureus*, Panton-Valentine Leucocidin and Methicillin Resistance directly from clinical samples. AIMS Microbiol. 2019; *5*(2):138 -46..
9. Hanitsch LG, Krüger R, Hoppe PA, et al. Outpatient decolonization after recurrent skin infection with Panton-Valentine leukocidin (PVL)-producing *S.aureus*—The importance of treatment repetition. PloS One 2020 Apr 21; 15(4):e0231772.
10. Gijón M, Bellusci M, Petraitiene B, et al. Pediatric Community-Acquired bone and joint *Staphylococcus aureus* infections in Europe: severe infections are associated to Panton-Valentine Leucocidin presence. The Pediatr Infect Dis J. 2020 Jun;1;39(6):e73-6(4).
11. Papanikolaou A, Kotsifas KA, Balis E. Community acquired pneumonia due to Panton-Valentine leukocidin producing methicillin resistant *Staphylococcus aureus* in Greece. Med Res Arch. 2018 Jul;17:6(7);2-10.
12. Maalej SM, Trabelsi JJ, Claude-Alexandre G, Boutida I., mastouri M., Besbes S., Barguellil F., Laurent F., Hammami A. Antimicrobial susceptibility and molecular epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Tunisia: results of a multicenter Study. J Infect Dis Epidemiol. 2019;5:071.
13. Singh AK, Agarwal L, Kumar A, Sengupta Ch., Pal Singh R. Prevalence of nasal colonization of methicillin-resistant *Staphylococcus aureus* among school children of Barabanki district, Uttar Pradesh, India. J Family Med Prim Care 2018 Jan-Feb;7(1):162–6.
14. Kumar H, Zahoor U, Rana R, Mahajan T, Devi MP, Garg RK, Kumar R, Khanna S, Laskar HN, Thakur P, Tongbram K. Prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* among tribal population of north-western Himalayas, India. J. Envt. Biol. 2018 Jul 1;39(4):419-25.

15. Karmakar A, Jana D, Dutta K, Dua P, Ghosh C. Prevalence of Panton-Valentine leukocidin gene among community acquired *Staphylococcus aureus*: a real-time PCR study. J. Pathog. 2018 Sep 2;ID 4518541.
16. Saleh RO, Raheema RH, Jameel ZJ. Phylogenetic tree and submission of *Staphylococcus aureus* isolate from skin infection. J Pure Appl Microbiol. 2018 Dec 1;12(4):2199-204.
17. Wayne, P. A. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Second Informational Supplement. CLSI Document M100-S22. 2012.
18. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. Journal Clin Microbiol. 2005 Oct;43(10):5026-33.
19. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar 1;18(3):268-81.
20. Karmakar A, Dua P, Ghosh C. Biochemical and molecular analysis of *Staphylococcus aureus* clinical isolates from hospitalized patients. Can J Infect Dis Med Microbiol. 2016;2016:9041636.
21. Munjal R, Mudey G. Nasal carriage of *Staphylococcus aureus* among undergraduate medical students: Prevalence and antibiogram including methicillin resistance, inducible clindamycin resistance, and high-level mupirocin resistance. J Datta Meghe Inst Med Sci Univ. 2018 Apr 1;13(2):91-4.
22. Choo EJ. Community-associated methicillin-resistant *Staphylococcus aureus* in nosocomial infections. Infect Chemother. 2017 Jun 1;49(2):158-9.
23. Kateete DP, Bwanga F, Seni J, Mayanja R, Kigozi E, Mujuni B, Ashaba FK, Baluku H, Najjuka CF, Källander K, Rutebemberwa E. CA-MRSA and HA-MRSA coexist in community and hospital settings in Uganda. Antimicrob Resist. Infect. Control. 2019 Dec;8(1):1-9.
24. Patil AK, Namineni S, Cheruku SR, Penmetsa C, Penmetcha S, Mallineni SK. Prevalence of community-associated methicillin-resistant *Staphylococcus aureus* in oral and nasal cavities of 4 to 13-year-old Rural School Children: A cross-sectional study. Contemp Clin Dent. 2019 Jan;10(1):99.
25. Tigabu A, Tiruneh M, Mekonnen F. Nasal carriage rate, antimicrobial susceptibility pattern, and associated factors of *Staphylococcus aureus* with special emphasis on MRSA among urban and rural elementary school children in Gondar, Northwest Ethiopia: A comparative cross-sectional study. Int J Prev Med. 2018 Dec; 11:2018.
26. Kong EF, Johnson JK, Jabra-Rizk MA. Community-associated methicillin-resistant *Staphylococcus aureus*: an enemy amidst us. PLoS Pathog. 2016 Oct 6;12(10):e1005837.
27. Lee GC, Dallas SD, Wang Y, Olsen RJ, Lawson KA, Wilson J, Frei CR. Emerging multidrug resistance in community-associated *Staphyloco ccus aureus* involved in skin and soft tissue infections and nasal colonization. J. Antimicrob. Chemother. 2017 Sep 1;72(9):2461-8.
28. Rojas I, Barquero-Calvo E, van Balen JC, Rojas N, Munoz-Vargas L, Hoet AE. High prevalence of multidrug-resistant community-acquired methicillin-resistant *Staphylococcus aureus* at the largest veterinary teaching hospital in Costa Rica. Vector-Borne and Zoonotic Dis. 2017 Sep 1;17(9):645-53.
29. Shil A, Mukherjee S, Bishayi B, Sikdar (Nee) Bhakta M. A comparison of antibacterial effects of *Catharanthus roseus* and *Camellia sinensis* (Black Tea) and their synergistic effect along with antibiotic against multiple antibiotic resistant strains of *Staphylococcus aureus*. J Herbs Spices Med Plants 2021 Apr 3;27(2):135-48.