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Prevalence of Nasal Carriage of MRSA among University Students at National Ribat University, 2022

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Background

Staphylococcus aureus is considered to be a persistent member of the human endogenous microbiota and has historically been associated with important and serious cases of infection. It has the ability to rapidly develop resistance to antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) is considered to be a paradigm for bacterial infections, since it is associated with high rates of morbidity and mortality [1-3]. In assisting carriers of bacterial infections or colonized or infected patients, or in handling contaminated objects, healthcare workers' hands can become contaminated and university students in communities. These personnel may subsequently transmit the microorganism to other patients. However, this situation is not exclusive to the hospital environment. Clinically manifested diseases in the community or in professionals and/or patients may lead to situations in which some individuals are asymptomatic carriers, also called colonized individuals or simply carriers, when the disease is present in the host organism without causing apparent manifestations [1, 4]. In the United States and Taiwan, the prevalence of strains acquired in the community is 52%, thus exceeding the proportion of strains acquired in hospital environments. There have also been reports of cases of MRSA acquired in the community [5-7]. Healthcare students play an important role in the epidemiology and pathogenesis of Staphylococcus aureus infection and can act as a source of dissemination both in the community and in hospital environments, and for carrying bacteria from one of these environments to another [1].

In Brazil, this topic has been little addressed, but it is known that the presence of MRSA among students has been gradually spreading [1]. Hence, it has become relevant to summarize the knowledge of MRSA that has resulted from research on this subject.

Staphylococcus Aureus

Is Gram positive bacteria widespread in Nature although it is mainly found on the skin, skin glands and mucous membranes of mammals and birds but can cause infection under certain circumstances [8].

Sauers is more pathogenic than the other common members of genus.

Sauers has many potential virulence factors:

- Surface preteens
- Leukocidin
- Kinases Hyaluronidase
- Capsule, protein A
- Carotenoids, Catalase, Coagulase
- Hemolysins, Leukotoxin, Leukocidin

S. aureus acquires genetic resistance against multiple classes of antibiotics through a variety of mechanism an existing gene undergoes mutation or changes in genetic configuration through the insertion of insertion sequence.

Methicillin Resistant Staphylococcus Aureus (MRSA):

Infection was reported first in 1950 however the rate of infections with MRSA has increased dramatically in recent decades reaching up to 50%. thirty percent of infected patients with MRSA will die within 30 days [9].

Hospital acquires MRSA (HA_MRSA) is an important Nosocomial pathogen and is usually associated with predisposing risk factors. Community acquired MRSA (CA_MRSA) has emerged worldwide particularly among Children [10].

The MRSA rate is higher in hospitalized patients (HA_MRSA), however recent reports indicate the growing frequency of community acquired MRSA [11].

MRSA has Acquired the ability to survive in the presence of beta lactam antibiotics, including penicillin, methicillin and cephalosporin.

The resistant mechanism is the production of a penicillin binding protein PBP encoded by the mecA gene [12].

The Mec A gene is located on a mobile genetic element called Staphylococcal cassette chromosome mechanism (sccmec) [12,13].

Detection of MRSA;
All S. aureus isolates were screened for MRSA, using the oxacillin salt screening method [14].

Cefoxitin and oxacillin disk agar diffusion method [15].

Vancomycin Resistant Staphylococcus Aureus (VRSA)
The name vancomycin was derived from vanquish because of its ability to vanquish resistant Staphylococcus it was first used clinically after the food and drug administration (FDA) approval in 1955 to treat penicillin resistant strains of S. aureus [16].

Vancomycin became an antibiotic of choice for treatment of MRSA infections in hospital settings in the late 1980 [17-19].

Resistant to vancomycin was discovered in enterococci in the 1980s and this finding elicited significant concern with regard to the future use of vancomycin as an effective treatment for MRSA [20].

Vancomycin intermediate resistant S. aureus (VISA) was first reported in Japan in 1997 [21]. VISA has been mostly described in Asia and Europel/America where prevalence in MRSA samples is reportedly at 3.42% and 2.75% respectively [22].

Laboratory Diagnosis of Staphylococcus Aureus

- **Catalase Test**
  Is test used to detection S. aureus produce abundant catalase which can interact with hydrogen peroxide to produce oxygen,
  S. aureus catalase positive produce Air bubble.
  S. aureus catalase negatives No produce Air bubble.
- **Coagulase Test**
  this test is used to differentiate Staphylococcus aureus which produces the enzyme Coagulase, from other species.
  Positive Coagulase _clumping within 10 secs.
  Negative Coagulase _no clumping within 10 secs.
- **Latex Agglutination**
  Proteins on the surface of S. aureus (e.g. protein A, clumping factor group specific antigens, capsular polysaccharides) are recognized by specific latex spheres, resulting in Aggregation.
- **Mannitol Salt Agar**
  S. aureus strains grow well in high salt and are able to ferment mannitol to produce Acid which can be detected using ph indicator, produced small yellow colonies.
- **Polymyxin B and Novobiocin Susceptibility**
  Polymyxin B is a cationic detergent antibiotic specific for grem Negative bacilli, S. aureus are resistant to Polymyxin B.
  Novobiocin is an amino coumarin antibiotics which can be used to differentiate S. aureus are sensitive to Novobiocin.

DNase and Heat Stable Nuclease
Identification of S. aureus bases on detection of specific DNase and heat stable nucleases common to S. aureus strains.

Automated Methods
Automated systems employ a bettery of tests to achieve S. aureus Identifications.

Commercial system includes

- VITEK/VITEK2 (biomerienx)
- Phoenix (BD biosciences)
- Microscen (Dade Behring)

Molecular Methods
S. aureus contains a number of genes that allow them to be distinguished from other Staphylococcal species.
methods include PCR (simple, multiplex and real time).
DNA sequencing.
Hybridization based techniques.
Species _specific genes commonly used include nuclease (nuc) coagulase (coa) protein A(spa) fem A and fem B, Sa442, and S. aureus specific 16S rRNA.

Methicillin Sensitive Testing
There are many options for testing Methicillin Susceptibility of S. aureus including disc diffusion, MlC measurements (in broth or by E test), chromomeric Agar, Latex agglutination, Automated methods, Rapid Screening methods and Molecular approaches [23].

Treatment of Staphylococcus Aureus:
The sensitivity of Staphylococcus aureus to antimicrobial medicines is different,90%of strain isolated from patients or carriers are resistant to penicillin due to beta lactamase (penicillinase) production or to changes in the Nature of penicillin binding proteins (PBP).

Beta lactam clavulanic Acid (such as co_amoxiclav) was used to treat infection caused by Staphylococcus aureus B lactamase producing strains.

Methicillin sensitive B Lactamase producing strains can be treated with Oxacillin, Cloxacillin and Nafcillin, however for the Treatment of Methicillin Resistant strains, Vancomycin teicoplanin and Mupirocin are used [24].

Trimethoprin/Sulfamethoxazole may be substituted by vancomycin infections caused by Staphylococci Sensitive or Resistant to Methicillin respond to trimethoprim-Sulfamethoxazole.
Trimethroprim _Sulfamethoxazole may be used in patients Who are Sensitive to Vancomycin, in Combination with Rifampin, trimethoprim is used to eradicate resistant Staphylococcus aureus Spread by Nasal Carriers in Epidemic of Nosocomial infections [25].

Fusidic Acid is also active against Methicillin Resistant Staphylococcus. Vancomycin is Considered a selective drug for the treatment of Methicillin Resistant Staphylococcus.

Infection Prevention and Control
S. aureus is associated with healthcare related infection.
Its diagnosis is based on performing microbial detection and Identification tests within bacterial colonies.
A Staphylococcus aureus infection can be prevented and controlled by:

- practicing good hand hygiene.
- hygienic conditions for medical procedures and appropriate use of antimicrobial drugs.
- proper environmental control methods including the regular monitoring of Air water and surfaces.
- Stringency Cleaning and disinfection of Equipment and Environments.
- in clinical Environments isolating patients when appropriate.

- Close monitoring of at-risk patients and populations.

Justification and Rational

The purpose of the present study was to assess the compliance of university students in National Ribat University with active MRSA and VRSA surveillance guidelines, assess adherence of university students to hand hygiene, and investigate its impact on rates of nosocomial Methicillin Resistant *Staphylococcus aureus* and Vancomycin Resistant Staphylococcus aureus carriage.

In order to allow an epidemiological analysis and to direct prevention and treatment policies for these infections, as well as to encourage the inclusion of university students in these actions.

Objectives

General Objective

To determine the rates of, and risk factors for, Methicillin Resistant *Staphylococcus aureus* and Vancomycin Resistant *Staphylococcus aureus* carriage and acquisition among university students in National Ribat University.

Specific objectives

1. To identify the most common carriage strains were isolated from university students in National Ribat University.
2. To estimate the extent of Staphylococcal resistant strains carriage among different students and investigate the relationship of several characteristics of this group to the carrier state.

Literature Review

- Thawatchai kitten et al found in 2014 the incidences of nasal colonization with coagulase negative Staphylococcus (CNS) and S. aureus among 200 healthy young Thai adults were 11% (20 of 200) and 15% (30of 200) respectively of the 30 S. aureus Isolates 28 were MSSA and 2 were MRSA [26].
- Eshetie et al found in 2016 the pooled prevalence of Methicillin Resistant Staphylococcus aureus was 32.5% moreover, Methicillin Resistant Staphylococcus aureus strains were found to be highly resistant to penicillin, ampicillin, erythromycin and amoxicillin, with a pooled resistance ratio of 99.1,98.1,97.2 and 97.1%respectively on the other hand Comparably low levels of resistance ratio were noted to Vancomycin 5.3% [27].
- Benard Okamoto et al found in 2016, 21%(66/314) of which 1.5(1/66) was MRSA Giving an overall MRSA Nasal carriage prevalence of 0.3(1/314).
- Staphylococcus aureus carriage among per clinical and clinical students were 19.9% (33/166) and 22.3(33/148) respectively [28].
- Federick K. Wangai et al in 2019 found antimicrobial susceptibility data from a total of 187 Staphylococcus aureus isolates revealed an overall MRSA prevalence of 53.4%isolates remained highly susceptible to Iinezolid, tigecycline, teicoplanin and Vancomycin [29].
- Barbara kot et al in 2017 found a large number of MRSA isolates showed resistance to levofloxacin (83.9%), cirprofloxacin (83%), erythromycin (77.7%) and Clindamycin)72.372.
- A lower number of MRSA isolates showed resistance to tetracycline (10.7%) amikacin (14.2%) gentamicin and trimethoprim with sulfamethoxazole (8% (None of the MRSA isolates were resistant to linezolid and teicoplanin, among MRSA isolates 92.9% were Multidrug resistant MDR [30].

Materials and Methods

Study Area

This study was carried out in National Ribat University.

Study Subject

- Any healthy university students (laboratory technician, accountants, and nurses) not suffering from any upper respiratory tract infection and who have not received any antibiotics for the past 30 days was included in this study.
- Any non-university students and diseased students or other personnel's with chronic diseases were excluded from this study.

Study Type

Cross-sectional community base study type was conducted in selected university students in National Ribat University colleges.

Study Design

Observational cross sectional study design

Study Duration

This study was conducted during the period from March to June 2022.

Sample Size

371 nasal swabs were been collected from a target group of this study.

Methodology

Data Collection

371 nasal swab samples were been collected from a university students in National Ribat University, as a primary data were been transported in Amies transport medium. Secondary data were been collected by using a direct interviewing questionnaire and were included: personal information (name, age, sex, residence).

Sampling Technique

Collection of Nasal Swab Samples

Nasal swab samples were collected randomly from 371 university students in National Ribat University according to the important quality assurance of collecting the specimens. The containers were labeled carefully and correctly including medical students name, number, age, date and time of collection.

By using a sterile cotton wool swab moistened with sterile peptone water gently swab the inside surface of the nose, must be taking care not to contaminate the swab, replace it in its sterile container, then label, and within 2 hours deliver

Sample Transporting

All samples were been transported to the laboratory in Stuart transport medium (OXOID, England); they were then transported to our microbiology lab and processed within 4 hours. S. aureus isolates were identified by previously described methods (92). Antibiotic susceptibility was assessed by the disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines (93). The antibiotics tested were: cefoxytin, erythromycin, clindamycin, gentamycine, vancomycin and rifampin.

Sample Processing

Swabs were collected from the anterior nares of the patients and streaked on Blood agar, Chocolate blood agar and For the identification of the MRSA among the isolates of Staphylococcus aureus, the Hi-Media (India) made "HI Chrome Me Re Sa HiVeg

Agar Base (MV 1674) was used. Plates were incubated overnight at 37Co, colonies with typical morphology were confirmed as S. aureus using coagulase test, DNase production on DNase agar, and Mannitol fermentation test.

Diagnostic Methods
Laboratory Examination:
Culture the Specimen:
- On Sterile blood agar media inoculate swabs and incubated in aerobic incubator at 37Co/overnight.

Identification of Colony:
Colonial Morphology
On the blood agar medium plates was observed and wrote the colonial morphology which was small, 1-2 mm indiameter, white, Beta haemolytic colonies.

Indirect Gram Stain:
Requirements
- Slides
- Wire loop
- Crystal violet
- Lugol Iodine
- Alcohol
- Safranin

Methodology
- A drop of normal saline was placed on middle of the clean, dry slide.
- By using of sterile wire loop. Flame sterilizes the loop, with it, a small portion of colony (just touch the colony) was picked up and emulsified in the saline drop, then the smear was spread evenly covering an area of about 15-20 mm in diameter.
- After that the smear was left in safe place to air dry, fixed by passed it over the flame of bunzen burner for 3-4 time.
- Covered the fixed smear with crystal violet stain for 1minute, washed with clean water, covered by gram (lugol,s) iodine stain for 1 minute.
- Washed with clean water decolorized rapidly by alcohol (few seconds) washed immediately with clean water, covered with safranin for 2minute, washed the smear was placed on draining rack to air dry.
- Examined under light microscope in systemic way to cover the entire smear preparation by using 100X objective (oil immersion).

Catalase Test
Requirement
- Hydrogen peroxide, 3% H2O2 (10 volume solution)
- Wooden stick

Methodology
2–3 ml of the hydrogen peroxide solution was poured into a test tube Hydrogen peroxide, 3% H2O2 (10 volume solution) then several colonies of the test organism were removed by using a sterile wooden stick or a glass rod (not a nichrome wire loop), and immersed in the hydrogen peroxide solution. Then Looked for immediate bubbling.

Coagulase Test
Requirement
- Sterile un diluted rabbit or human plasma
- Slide
- Pasteur pipette

Methodology
- Drop of normal saline were placed on each side of the slide, by using wire loop, small portion of colony of the test organism was emulsified in two drops of normal saline to made thick suspension, loopful of plasma was added to one suspension. mix gently, looking for clumping of organism within 10 seconds.
- Other suspension as negative control

DNase Test
Requirement
- Sterile DNAase agar; consist of (Tryptose, deoxyribonucleic acid, sodium chloride,agar). Hydrochloric 1 mol/1 (1 N).

Methodology
- By Using a sterile loop or swab, and the test and control organisms were inoculated as spot in the medium (center), Incubated the plate at 35–37Co overnight.
- Then covered the surface of the plate with 1 mol/l hydrochloric acid solution. after Tip off the excess acid.
- Looked for clearing around the colonies within 5 minutes of adding the acid.

Mannitol salt agar medium
Requirement
- Sterile mannitol salt agar medium

Methodology
- Small portion of colony was removed and inoculated by using sterile wire loop, the well area A was made, then the wire loop was sterile by the flame, then the area B, C, and the zigzagging were inoculated by streaking.
- Those plates were incubated aerobically at37CO for 24 hours.

Sensitivity Testing Technique
Disc diffusion method (Kirby Bauer method)

Requirement
- Sensitivity testing agar (Mueller and Hinton agar medium)
- Methicillin disc
- Normal saline
- Swab
- Mcfarland (turbidity) standard
- Foreceps

Methodology
Several colony of test organism was emulsified in sterile normal saline, that suspension was compared with the turbidity standard, loopful of the test organism was taken by sterile wire loop, then inoculated on Mueller and Hinton agar medium by using sterile cotton wool swab by seeding, the inocula was allowed to dry for few minutes, Methicillin disc was placed on the medium by using sterile foreceps, then incubated aerobically at 35-37CO/over night.

Methicillin resistance was determined using the Kirby-Bauer disk diffusion test with a 30 µg cefoxitin disk. An inhibition zone diameter of = 22 mm was considered diagnostic for methicillin-susceptible *Staphylococcus aureus*. Susceptibility testing for mupirocin was performed for MRSA eradication purposes using a 200-µg mupirocin disk on a Mueller-Hinton agar plate. Any zone was considered susceptible.

Data Analysis
Different variables were presented and analysis of significant variance (ANOVA, Chi-squire, and T-test), were applied for

comparison between different results.
The P-value was been considered significant at a value less than (0.05).

Statistical analysis was done by using Statistical Package for Social Science (SPSS) Program (version.22) under windows (IBM) computer system.

Ethical Consideration
The protocol was ethical cleared by ethical approval comity of National ribat university Faculty of medical laboratory sciences. An official letter from faculty was submitted to faculty of higher education for approval before data collection. The purpose of the study was clearly described to the study participants. Written informed consent was obtained from the eligible participants before enrollment. Clinical and laboratory data collected were kept confidential through anonymity. For those with MRSA colonization, treatment was recommended.

Results
A total of 371 students were enrolled in the study with the response rate of 99.2%. Of these, 84.9% (315/371) were males. Over 80% (299/371) of the participants were 25 years old or younger. With respect to category of the students, the medical laboratory students were 44.7% (166/371), nurses students were 33.7% (125/371) and accountants were 21.6% (80/371)

(Table-1): Distribution of S. aureus according to Sex, Age and Type of Study Students on University from March to June, 2022.

Variables	S. aureus colonization (n = 371)		Total, n (%)
	Yes, n (%)	No, n (%)	
Sex			
Male	69 (21.9%)	246 (78.1%)	315 (100%)
Female	13 (23.2%)	43 (76.8%)	56 (100%)
Age (in years)			
≤25	62 (20.7%)	237 (79.3%)	299 (100%)
≥25	20 (27.7%)	52 (72.3%)	72 (100%)
Category of the students			
Clinical year I	28 (16.9%)	138 (83.1%)	166 (100%)
Clinical year II	23 (18.4%)	102 (81.6%)	125 (100%)
Medical Interns	31 (38.8%)	49 (61.2%)	80 (100%)
Total	82 (22.1%)	289 (77.9%)	371 (100.0)

Discussion
Worldwide, occurrence of healthcare-associated infections (HAI) is one of the main public health problems, with severe human and economic repercussions. According to the Centers for Disease Control and Prevention (CDC), MRSA infections have outperformed HIV as the leading cause of morbidity and mortality in the United State Studies have revealed high prevalence of MRSA in Data in the literature have highlighted occurrences of MRSA infection in healthy populations that live in agglomerations or experience such conditions but which have little or no contact with health-care services, as is the case of undergraduate students within the field of University.

Identification of high frequencies of MRSA in students in university is a matter for concern. It indicates that there is a need for effective infection prevention and control policies, in relation to hygiene and surveillance.

Possibly for this reason, the nasal according to the scenarios within which they occur. This may be due to measures that are taken to control infection and may be dependent on effective implementation. Likewise; the MRSA rate also varies in different locations.

Conclusion
Another point to be considered is that the presence of other factors may also be related to the port ability of S. aureus, such as chronic sinusitis, smoking, male gender, individuals older than 23 years old, other upper respiratory tract infections and the presence of chronic diseases. In general, the use of antibiotic therapy in the last 3 months had divergences regarding its influence on the carriage of S. aureus.

Therefore, it is clear the need for structuring educational actions, in students' institutions, aimed at hand washing practices, monitoring of university student's infections and awareness of staff and students about these issues.

In the long-term care facility in which our study took place, MRSA was endemic, and the infection rate was low. In such settings, the cost effectiveness of aggressive management of MRSA (widespread screening for MRSA and eradication with antimicrobial agents) needs to be assessed.

Recommendations
- Surveillance is required to determine the degree and extent of the community reservoir and determine the changing pattern of resistance of staphylococcus aureus.
- More studies should be done with increase size sample.

Appendix
Culture Media Preparation
Blood Agar Preparation
To make about 35 blood agar plates:
Nutritious agar . . . . . 500 ml
Sterile defibrinated blood . . . . . 25 ml

Dehydrated agar products from which to prepare blood agar include: Blood agar base (Oxoid product CM 0055), Columbia agar, or Tryptone soya agar (available from most suppliers of culture media).

For most pathogens, haemolysis-free defibrinated horse, sheep, goat, or rabbit blood can be used.
1. Prepare the agar medium as instructed by the manufacturer. Sterilize by autoclaving at 121OC for 15 minutes. Transfer to a 50OC water bath.
2. When the agar has cooled to 50OC, add aseptically the sterile blood and mix gently but well. Avoid forming air bubbles.

Important: The blood must be allowed to warm to room temperature before being added to the molten agar.
3. Dispense aseptically in 15 ml amounts in sterile petri dishes.
4. Date the medium and give it a batch number.
5. Store the plates at 2–8 _C, preferably in sealed plastic bags to prevent loss of moisture.

DNase Agar
This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.
Contents: Tryptose, deoxyribonucleic acid, sodium chloride, agar.
The medium is usually used at a concentration of 3.9g in every 100 ml distilled water (concentration may vary depending on manufacturer).
1. Prepare and sterilize as instructed by the manufacturer.
2. When the medium has cooled to 50–55OC, mix well and dispense in sterile petri dishes. Date the medium and give it a batch number.
3. Store the plates at 2–8OC in sealed plastic bags to prevent loss of moisture.

Mannitol Salt Agar
This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, Lab-Lemco powder, mannitol, sodium chloride, phenol red, agar.
The medium is usually used at a concentration of 11.1g in every 100 ml distilled water (concentration may vary depending on manufacturer).
1. Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at 121OC for 15 minutes.
2. When the medium has cooled to 50–55OC, mix well, and dispense it aseptically in sterile petri dishes. Date the medium and give it a batch number.
3. Store the plates at 2–8 _C preferably in plastic bags to prevent loss of moisture.
PH of medium: This should be within the range pH 7.3–7.7 at room temperature.

Mueller Hinton Agar
Prepare and sterilize the medium as instructed by the manufacturer. The pH of the medium should be 7.2–7.4. Pour into 90mm diameter sterile petri dishes to a depth of 4 mm (about 25ml per plate). Care must be taken to pour the plates on a level surface so that the depth of the medium is uniform.

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