Mediterranean Journals
Integrative Journal of Medical Sciences
2021, Volume 8, ID 440
DOI: 10.15342/ijms.2021.440

RESEARCH ARTICLE

Identification of Nasal Carriage of *Staphylococcus aureus* among Nursing Students during Curricular Clinical Internships: An Observational Study

Maria João Coelho [a b], Ricardo Magalhães [a b], Inês Lopes Cardoso [a b], Cristina Pina [a b], Maria Pia Ferraz [a b]
[a] Health Sciences Faculty, University Fernando Pessoa, Porto, Portugal
[b] FP-ENAS – UFP Energy, Environment and Health Research Unit, University Fernando Pessoa, Porto, Portugal

ABSTRACT
Staphylococcus aureus is considered one of the most frequently isolated bacteria in the community and in the hospital environment, being associated with several infections. Healthcare professionals represent a group vulnerable to *Staphylococcus aureus* and MRSA colonization, therefore being potential disseminators of these microorganisms during their care activities. The aim of this study was to evaluate the dynamics of *S. aureus* and MRSA nasal colonization among nursing students over the four years of university attendance, including pre-clinical exposure and at different moments during clinical rotations. Samples were collected from students from the 1st, 2nd, 3rd and 4th year. The study identified 55.9% MSSA positive samples and 31.4% MRSA positive samples from the total studied population. Simultaneous carriage of MRSA and MSSA was observed in students from all years of the nursing degree, but a highest MSSA colonization (61.5%) was linked to a lower MRSA colonization (30.8%). MRSA colonization seems to be dependent on the type of clinical internship, since the group attending internship in emergency rooms and surgery wards presented a significant increase in the amount of MRSA samples. Nursing students should be educated on the risks involved in carrying *S. aureus* and MRSA and informed about infection control measures.

KEYWORDS: MSSA; MRSA; Nasal Colonization; Nursing Students.

Correspondence: Maria João Coelho. Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal. Email: mcoelho@ufp.edu.pt

INTRODUCTION

Staphylococcus aureus is considered one of the most frequently isolated Gram-positive bacteria in the community and in the hospital environment and is associated to several infections ranging from mild skin infections to life-threatening invasive infections, such as septicemia. It is a commensal microorganism that exists in the nostrils and skin of about 37% of the population[1-4]. Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection. In healthy subjects, over time, three patterns of carriage can be distinguished: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry *S. aureus*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* identified for the first time in the United Kingdom in 1961, which is resistant to β-lactam antibiotics [4,5]. Initially, these bacteria were considered a concern for hospitalized patients until in the last two decades strains of MRSA have emerged in the community. These strains were found to be associated with aggressive virulence and strong resistance to antibiotics, making it a public health concern [3].In fact, MRSA is often also resistant to multiple other antibiotic classes, as *S. aureus* has the ability to acquire resistance to any antibiotic, which has major implications for current as well as future treatment options for this pathogen [3].

According to the European Center for Disease Prevention and Control (ECDC), the percentage of MRSA in the European Union has declined significantly between 2012 and 2019. Despite this reduction, these bacteria are still a public health priority in Europe as seven of the 30 countries evaluated have MRSA rates above 25% [6]. According to this agency, the percentage of MRSA in Portugal is among the highest in Europe (34.8% in 2019).

Healthcare workers (HCWs), especially nursing professionals, are considered a population susceptible to

colonization by multi-resistant microorganisms in their work environment. Work activities performed by nursing professionals involve close physical contact with patients. This, associated with the lack of adherence to standard precautions, may favour the colonization and dissemination of microorganisms.

National and international studies characterize healthcare professionals as a group vulnerable to the colonization by *Staphylococcus aureus*, in addition to potential disseminators of these microorganisms in their care activities.

According to a review on MRSA nasal carriage among HCWs, the reported carriage rate was 4.6% [7]. Nurses have a two-fold increased risk of being colonized with MRSA comparing with medical doctors and a three-fold higher risk than other HCWs, despite having a better compliance with hand hygiene and infection control protocols. Therefore, the higher colonization rate may be due to closer and more frequent contact between nurses and patients. HCWs, particularly nurses, may play an important role in MRSA transmission. Although several studies evaluated the prevalence of *S. aureus* and MRSA among medical students, showing carriage rates of 14–45% and 0–5.4%, respectively [8], few studies focused on nursing students.

Due to the relevance of the theme, combined with the lack of national studies on the prevalence of MRSA, specifically with nursing students, the present study was considered relevant, as its aim was to evaluate the dynamics of *S. aureus* and MRSA nasal colonization among nursing students over the four years of university attendance, including pre-clinical exposure and at different moments during clinical rotations.

This study aims to identify patterns of nasal colonization in nursing students while they do their clinical internships. The specific objectives are:

- to measure nasal colonization by MSSA and MRSA after each clinical internship
- to identify the possibility of a difference in the pattern of colonization dependent on a particular internship.

METHODS

Study design: A cross sectional study was implemented. The researchers collected data after the participants go through their clinical internships along their academical year. The participation on the study was proposed to students from all years of the nursing degree. The students were able to participate by their own free will, with no impact on their academic outcome. They were also able to withdraw from the study at anytime.

Setting and Participants: Students from the 1st, 2nd, 3rd and 4th year were eligible to the study. 1st year students were used as control group (n = 20) due to no participation on any clinical internship and were sampled on the first recruiting session. 2nd, 3rd and 4th year student (n = 44) were the focus group and samples were collected after their periods of clinical internships).

Procedures: The study was proposed to students in the beginning of the academic year. A questionnaire was filled to collect socio-demographic data (sex, age, year of degree…) and information about the clinical internship the student had just participated in. The questionnaire was repeated on every sampling session. On the first session a written informed consent was also signed. One hundred and fifty samples were collected with sterile soft nylon fiber swabs (Copan Liquid Amies Elution Swab (ESwab) Brescia, Italy).

After the sampling procedure, samples were transported and processed, being cultured in a biplate chromogenic medium (chromID MRSA/chormID*S. aureus*, bioMérieux, France), incubated at 37ºC for 48h as recommended [9,10] to identify methicillin sensitive *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) strains.

MRSA/MSSA strains were confirmed by PCR on the *mecA* gene. For the identification of *mecA* gene, DNA (5 µL) was amplified in a reaction mixture containing 10 µL of 5x PCR buffer, 3 µL of $MgCl_2$ 25mM, 2 µL of dNTP mixture 10mM, 5 µL of each primer 10 µM and 0.25 µL Taq polymerase in a total volume of 50 µL [11,12]. Primers used for identification of *mecA* gene were 5'-GGGATCATAGCGTCATTATTC-3' and 5'-AACGATTGTGACACGATAGCC-3' [12]. PCR was performed as followed: 10 minutes at 94ºC; 30 cycles consisting of 30 seconds at 94ºC, 1 minute at 55ºC and 1 minute at 72ºC; final extension for 10 minutes at 72ºC. PCR products were analyzed by electrophoresis on a 1% agarose gel [12].

RESULTS

A total of 64 students from all the classes in the 2018/2019 academic year, were enrolled in sampling. The students were recruited at the beginning of the academic year and samples were collected after each clinical rotation group. The exception was the first year students, used as control group, whose samples were collected at the end of the academic year. Students' compliance is described on the flow diagram in Figure 1.

A total of 102 samples was collected, being *S. aureus* present in 69of collected samples as shown in Table 1. As can be seen in Figure 2,MSSAwas identified in 55.9% and MRSA in 31.4% of total samples. The control group (students from the first year) showed colonization by MSSA in 65% of the cases and only 10% presented MRSA. Moreover, MSSA was found in 50% of second year students, while MRSA was detected in 21.4%. In addition, MSSA were identified on 52.1% and 60% of the samples collected from third- and fourth-year students respectively, and MRSA was identified on 43.8% and 30% of the samples for both years.

Results presented in Figure 3 show the relative amount of MSSA and MRSA samples by academic year after each clinical rotation, particularly in third year. The percentage of samples presenting MSSA and MRSA are 45% and 60% respectively in the period 3Y1, 61.5% and 30.8% in 3Y2 period and 53.3% and 33.3% in the 3Y3 period.

Individuals with MRSA colonization or carriage have an increased risk of subsequent infection and are an important source of person-to-person transmission. Health-care facilities enable the epidemic spread of MRSA in hospitals since they host persons who are predisposed to infection and are environments with high antibiotic selection pressure and frequent contact between individuals.

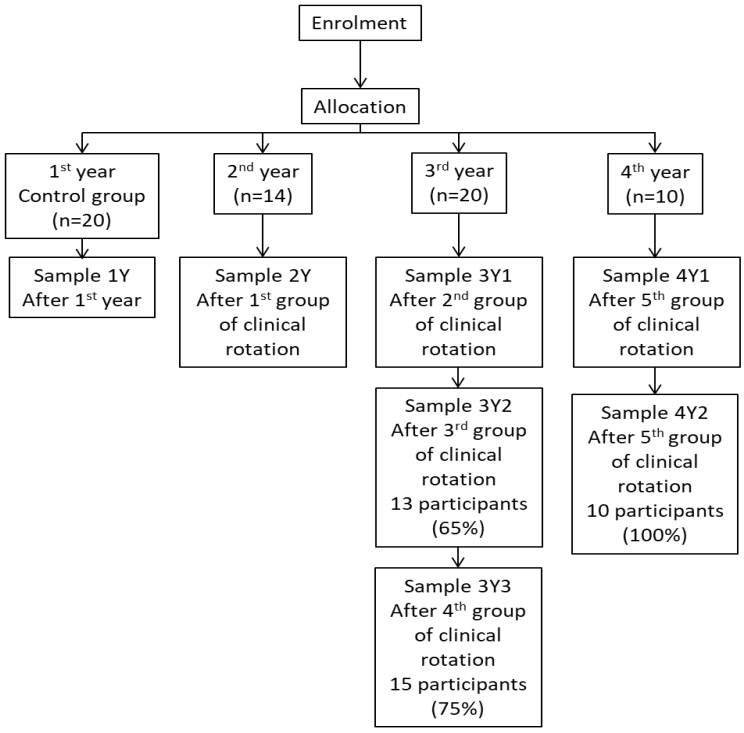

Fig 1. Flow Diagram of the Participating Students and Moments of Sampling.

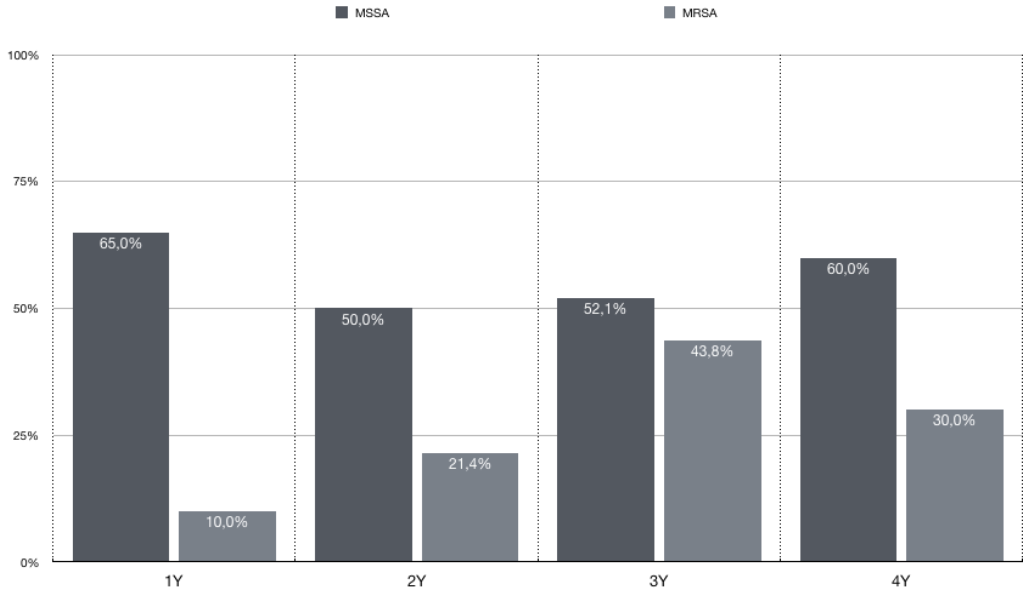

Fig 2. Relative amount of MSSA and MRSA positive samples compared to the total amount of samples in each academic year.

Table 1. Sample Distribution for the Presence of *Staphylococcus aureus* (MSSA) Or Methicillin-Resistant *Staphylococcus aureus* (MRSA).

				Total samples	n= 102
Variable	Sampled group	No S. aureus	S. aureus	MSSA	MRSA
	n	n (%)	n (%)	n (%)	n (%)
1Y	20	6 (30)	14 (70)	13 (65)	2 (10)
2Y	14	5 (35,7)	9 (64,3)	7 (50)	3 (21,4)
3Y1	20	5 (25)	15 (75)	9 (45)	12 (60)
3Y2	13	3 (23,1)	10 (76,9)	8 (61,5)	4 (30,8)
3Y3	15	7 (46,7)	8 (53,3)	8 (53,3)	5 (33,3)
4Y1	10	7 (70)	3 (30)	2 (20)	2 (20)
4Y2	10	0 (0)	10 (100)	10 (100)	4 (40)

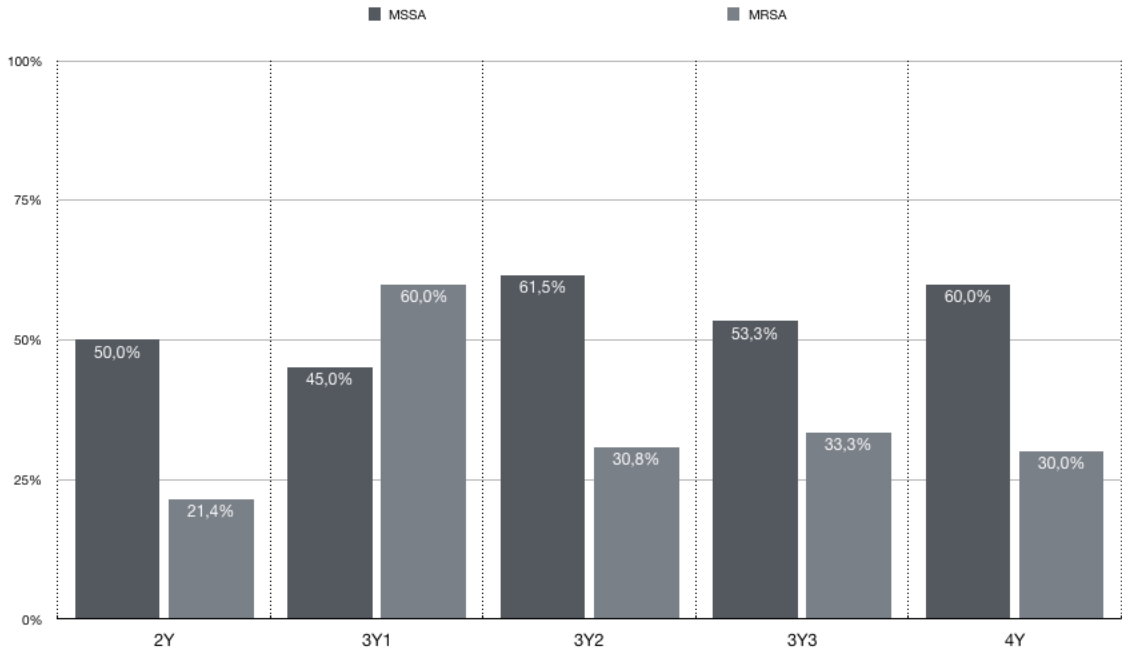

Fig3. Relative amount of MSSA and MRSA positive samples compared to the total amount of samples after clinical rotation in each academic year

DISCUSSION

Individuals with MRSA colonization or carriage have an increased risk of subsequent infection and are an important source of person-to-person transmission. Health-care facilities enable the epidemic spread of MRSA in hospitals since they host persons who are predisposed to infection and are environments with high antibiotic selection pressure and frequent contact between individuals.

Although the vehicles of dissemination of MRSA between hospital and community are not firmly established and may differ in different countries, patients and healthcare workers may represent one of the main means of dispersal. Since HCWs, such as nurses, work at the intersection between hospitals, healthcare facilities and nursing homes on one hand and interact with the community (schools, athletic facilities, prisons, universities, etc.) on the other, they may serve as reservoirs, vectors, or victims of MRSA, *S. aureus*, and/or other multiple drug resistant organisms' cross-transmission. A study showed that in a country with a high rate of nosocomial MRSA, like Portugal, the population structure of MRSA in the community might mirror that found in the hospital sett [13].

In this study, *S. aureus* colonization rate at the first year (70%) was higher than the one estimated in the general population (37%). Moreover, among the 20 control students (at the first year), 13 (65%) carried methicillin-susceptible *S. aureus* (MSSA), and 2 (10%) were colonized with MRSA.

Results seem to indicate that there are no significant differences in the percentage of MSSA colonization during the four years of the course, being the highest value in the first year (65%) and the lower (45%) after the first internship on second year. However, MRSA colonization seems to be dependent on the type of clinical internship. The 3Y1 group presents a significant increase in the relative amount of MRSA samples. The presence of MRSA varies from 21.4% on the second year to 60% on the 3Y1 group, indicating colonization by MRSA during this clinical internship, presumably because of the increased exposure to the hospital environment, namely in emergency rooms and surgery wards. After this internship, relative amount of MRSA colonization remains practically stable.

Also, higher rates of MRSA carriage were found in the present study compared to other reports on medical or nursing students that found rates of nasal colonization up to 11% of the students harbouring MRSA[8,14]. Possible explanations for these discrepancies may be the number of sampling timesand the size of the sample. Portugal is a country with a very high MRSA prevalence, one of the highest in Europe [6], which may increase the possibility of MRSA acquisition in the healthcare environment. This seems to be corroborated by the fact that MRSA colonization increased after healthcare exposure during a specific internship.

Simultaneous carriage of MRSA and MSSA was observed in students from all years of the nursing degree. This observation was previously reported either as a rare occurrence, assuming competition for colonization space and nutrients, or in contrast, as a relatively common occurrence.When we examined results in subjects carrying both MSSA and MRSA, we observed that a highest MSSA colonization (61.5%) was linked to a low MRSA colonization (30.8%). Our data seemto support the idea that colonization with MSSA has a protective effect against colonization with MRSA, as reported by Orlin et al. [8]. The fact that MRSA was not detected in some samples could be due to the transient nature of MRSA carriage. Although the simultaneous presence of strains is not yet clinically explained, there is a possibility that co-colonization could influence the pathogenicity and virulence of *S. aureus* strains [15].

LIMITATONS AND RECOMENDATIONS

This study presents some limitations. The number of participating students was relatively low, the sampling was not done simultaneously in all students and the nose was the only tested spot. Moreover, only one medical school was involved in the study. The next steps in this study would be centred in identifying, on the several clinical rotations, of the stress points involved in the colonization of the students by MSSA and MRSA.

CONCLUSION

Education on infection control measures in nursing schools is of major importance as well as implementation of adequate and effective infection control programs to reduce the extremely high prevalence of MRSA in Portuguese hospitals that remains over 45%.

MRSA, along with other healthcare associated infections, has emerged as a growing world-wide problem in the past decades. Prevention along with intelligent use of the laboratory (culture of wounds, antibiotic susceptibility testing, etc.) can protect individuals from this threat. Healthcare officials, community leaders, and public health policymakers should be aware of the potential transmission risk and outbreak scenario that could develop in the rich environment of HCW populations and their daily work-related tasks.

In our study, colonization by MSSA and MRSA was detected in students from all years of the nursing degree. MSSA were detected in larger numbers in initial years, maintaining its regular presence on students throughout the years of clinical training. As for MRSA, an increase was identified in the second and third years of contact with the clinical rotations, on the fourth year a reduction in the identification of MRSA strain occurred.

MRSA carriage occurred in most students after healthcare exposure. Nursing students are exposed to pathogens and will constitute potential transmission vectors of nosocomial strains to the community.

ACKNOWLEDGMENTS

None.

AUTHORS' CONTRIBUTIONS

The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors. Indeed, all the authors have actively participated in the redaction, the revision of the manuscript, and provided approval for this final revised version.

COMPETING INTERESTS

The authors declare no competing interests with this case.

FUNDING SOURCES

This work was supported by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P. in the project UID/Multi/04546/2019.

REFERENCES

[1] David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev.2010;23(3):616-87. DOI: 10.1128/CMR.00081-09

[2] ECDC (European Centre for Disease Prevention and Control), Antimicrobial resistance surveillance in Europe 2014. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).ECDC, Stockholm. 2015. DOI : 10.2900/93403

[3] Lee AS, De Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, Harbarth S. Methicillin-resistant Staphylococcus aureus. Nat Rev Dis Prim.2018;4:18033. DOI: 10.1038/nrdp.2018.33.

[4] Okamo B, Moremi N, Seni J, Mirambo MM, Kidenya BR, Mshana SE. Prevalence and antimicrobial susceptibility profiles of Staphylococcus aureus nasal carriage among pre-clinical and clinical medical students in a Tanzanian University. BMC Res Notes.2016;9:47. DOI: 10.1186/s13104-016-1858-0

[5] Ottomeyer M, Graham CD, Legg AD, Cooper ES, Law CD, Molani M, Matevossian K, Marlin J, Williams C, Newman R, Wasserman JA, Segars LW, Taylor TAH. Prevalence of nasal colonization by Methicillin-Resistant Staphylococcus aureus in persons using a homeless shelter in Kansas City. Front Public Health.2016;4:234. DOI: 10.3389/fpubh.2016.00234.

[6] ECDC (European Centre for Disease Prevention and Control), Antimicrobial resistance surveillance in Europe 2019. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). 2019. Available: https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2019.pdf.

[7] Albrich WC, Harbarth S. Health-care workers: source, vector, or victim of MRSA? Lancet Infect Dis. 2008; 8(5): 289-301. DOI: 10.1016/S1473-3099(08)70097-5

[8] Orlin I, Rokney A, Onn A, Glikman D, Peretz A. Hospital clones of methicillin-resistant Staphylococcus aureus are carried by medical students even before healthcare exposure. Antimicrob Resist Infect Control. 2017;6:15. DOI: 10.1186/s13756-017-0175-2.

[9] Mukovnikova M, Yusuf E, Cossey V, Schuermans A, Saegeman V. Evaluation of a chromogenic biplate medium (ChromID MRSA/ChromID S. aureus) for the simultaneous detection of methicillin-resistant and methicillin-susceptible Staphylococcus aureus in preoperative screening samples from the anterior nares. J Clin Microbiol.2014;52(2):678-80. DOI: 10.1128/JCM.03311-13

[10] Wassenberg MWM, Kluytmans JAJW, Box ATA, Bosboom RW, Buiting AGM, Van Elzakker EPM, Melchers WJG, Van Rijen MML, Thijsen SFT, Troelstra A, Vandenbroucke-Grauls CMJE, Visser CE, Voss A, Wolffs PFG, Wulf MWH, Van Zwet AA, De Wit GA, Bonten MJM. Rapid screening of methicillin-resistant Staphylococcus aureus using PCR and chromogenic agar: A prospective study to evaluate costs and effects.Clin Microbiol Infect.2010;16(12):1754-61. DOI: 10.1111/j.1469-0691.2010.03210.x

[11] BeckWD, Berger-BächiB, KayserFH. Additional DNA in methicillin-resistant Staphylococcus aureus and molecular

cloning of mec-specific DNA. J Bacteriol. 1986; 165(2): 373-8.
DOI: 10.1128/jb.165.2.373-378.1986

[12] KoukosG, PapadopoulosC, TsalikisL, SakellariD, ArsenakisM, KonstantinidisA.Prevalence of antibiotic resistance genes in subjects with successful and failing dental implants. A pilot study. Open Dent J.2015;8:257-63.
DOI: 10.2174/1874210601408010257

[13] Espadinha D, Faria NA, Miragaia M,Lito LM, Melo-Cristino J., Lencastre H.Extensive dissemination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. PLoS One. 2013; 8(4): e59960.
DOI: 10.1371/journal.pone.0059960

[14] Conceição T, LencastreH, Aires-de-SousaM. Carriage of Staphylococcus aureus among Portuguese nursing students: A longitudinal cohort study over four years of education. PLoS One. 2017;12(11):e0188855.
DOI; 10.1371/journal.pone.0188855

[15] Mongkolrattanothai K, Gray BM, Mankin P, Stanfill AB, Pearl RH, Wallace LJ, Vegunta RK. Simultaneous carriage of multiple genotypes of Staphylococcus aureus in children. J Med Microbiol.2018;60(Pt 3):317-22.
DOI: 10.1099/jmm.0.025841-0