

ANTIBIOTIC RESISTANCE PHENOTYPES OF *S. AUREUS* ISOLATES FROM NOSE AND PHARYNX OF HEALTHY YOUNG ADULTS

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Abstract

The aim of this work was to identify the antibiotic resistance phenotypes of *S. aureus* isolates from nose and pharynx in healthy adults represented by students at the Faculty of Dentistry, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania. The antimicrobial susceptibility investigation was performed by the Kirby-Bauer method and D-test on a collection of *S. aureus* strains isolated in 2017 from students in the second year of study. Discs with: penicillin G, cefoxitin, erythromycin, clindamycin, gentamicin, tetracycline, norfloxacin, trimethoprim-sulphamethoxazole and linezolid were used. In addition, the minimum inhibitory concentration of oxacillin was determined by E-test for all *S. aureus* strains with a growth inhibition area of less than 22mm in diameter around the cefoxitin disc. The 5 strains of methicillin-resistant *S. aureus* (MRSA) and more than half of the methicillin-sensitive *S. aureus* (MSSA) isolates showed inducible MLSB resistance phenotype and many of them were multidrug-resistant. The study underlines, that young adults in preclinical study years should be informed about the risk of the cross-infection with such bacteria.

Rezumat

Scopul lucrării a fost identificarea fenotipurilor de rezistență ale tulpinilor *S. aureus* izolate din nasul și faringele unor adulți tineri sănătoși, reprezentati de studenți de la Facultatea de Medicină Dentară, Universitatea de Medicină și Farmacie „Carol Davila” din București, România. Sensibilitatea la antibiotice a fost investigată prin metoda Kirby-Bauer și testul D pe o colecție de tulpini de *S. aureus* izolate de la studenți stomatologi, în anul 2017. Au fost utilizate discuri cu: penicilină G, cefoxitină, eritromicină, clindamicină, gentamicină, tetraciclină, norfloxacină, trimetoprim-sulfametoxazol și linezolid. Pe lângă aceasta, a fost determinată concentrația minimă inhibitoare a oxacilinei, prin testul E, pentru toate tulpinile de *S. aureus* cu zona de inhibiție a creșterii cu diametrul mai mic de 22 mm în jurul discului de cefoxitină. Cele 5 tulpini de *S. aureus* metilino-rezistente (MRSA) și mai mult de jumătate din numărul izolatelor de *S. aureus* metilino-sensibile (MSSA) au prezentat fenotip MLSB inductibil și multe dintre acestea au fost multirezistente la antibiotice. Studiul subliniază faptul că tinerii trebuie să fie informați despre riscul infecțiilor încrucișate cu astfel de bacterii.

Keywords: MRSA, MSSA, antibiotic resistance

Introduction

Staphylococcus aureus is very often involved in various types of infections in hospitals and community and especially the methicillin-resistant *S. aureus* (MRSA) causes a lot of serious health and socio-economic problems [1, 4, 7, 8, 12, 13, 15-17]. Healthy nasal carriers of *S. aureus* are estimated to account for approximately 30% of the world's population [10], but some publications suggest that the frequency of nasal carriage of *S. aureus* is 20 - 70%. In hospitals and dental offices there is a risk of cross-infection through the possibility of transmitting these micro-organisms from the patient to the health staff and environment, as well as from doctor to patient and environment, at risk of contamination of other people. In the community, the transmission of MRSA and methicillin-sensitive *S. aureus* (MSSA) from one

person to another may be favoured by living in very crowded areas, without following the proper hygiene rules. MRSA are resistant to all beta-lactam antibiotics except fifth-generation cephalosporins [20].

Currently, few data are available on *S. aureus* carriage in the case of healthy young Romanian adults. Thus, the present study was focused on the detection of antibiotic resistance phenotypes of the *S. aureus* isolates from a collection of the Microbiology Department of the Faculty of Dentistry, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania (UMFCD), which contained strains of *S. aureus* isolated from second-year students in 2017.

Materials and Methods

The investigation of antibiotic sensitivity was performed on a retrospective collection of 63 *S. aureus* isolates

stored at -70°C , in the laboratory of the Department of Microbiology of the Faculty of Dentistry, UMFC, Romania. These isolates came from 56 of the 254 healthy dental students (73 men and 181 women between the ages of 19 and 29, of whom 68 were active smokers) from the second year of study at the Faculty of Dentistry, UMFC, Romania, screened for nasal and pharyngeal carriage of *S. aureus*, in 2017. The students have not used antibiotics in the last 6 months. Of the 56 carriers of *S. aureus*, only 4 women and 3 men were both nasal and pharyngeal carriers, but the isolates showed different biochemical profiles. In the present study, the 41 nasal and 22 pharyngeal isolates of *S. aureus* belonging to this collection of strains were tested for their susceptibility against some commonly used antibiotics and linezolid. For this, the Kirby-Bauer technique was used, supplemented with the double-disc test (D-test), in order to allow the detection of the macrolide - lincosamide - streptogramin B (MLSB) resistance phenotype. The strains were initially sub-cultured on blood agar with incubation at 35°C for 24 h, and afterwards on Müller-Hinton agar and incubated at 35°C for 24 h. The prepared inoculum from each strain was then adjusted to a turbidity of 0.5 McFarland and dispersed by swab on 2 Müller-Hinton agar plates (Cantacuzino Institute, Romania). The following antibiotic discs were used: penicillin G - 1 U (P-1) and penicillin G - 10 U (P-10), cefoxitin - 30 μg (FOX-30), erythromycin - 15 μg (E-15), clindamycin - 2 μg (DA-2), gentamicin - 10 μg (CN-10), sulfamethoxazole - trimethoprim - 25 μg (SXT-25), tetracycline - 30 μg (TE-30), norfloxacin - 10 μg (NOR-10) and linezolid - 10 μg (LZD-10) (Oxoid, U.K.).

The antibiotic discs were applied at a distance of at least 25 mm from each other, with the exception of erythromycin and clindamycin, placed at a distance of 14 mm between their edges, according to D-test requirements [11, 20]. *S. aureus* ATCC 29213 was used as quality control strain when testing the above mentioned antibiotics, except for penicillin G-10 U, when *S. aureus* ATCC 25923 was used as quality control strain.

The diameters of the growth inhibition zone were measured after 20 h of incubation at 35°C with a caliper on the back of the agar plate in reflected light, except for penicillin G and linezolid when read in transmitted light. The interpretation of sensitivity was based on the recommendation of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [20], with the exception of penicillin G-10 U, when the recommendation of the Clinical and Laboratory Standards Institute (CLSI) was followed [11].

The minimum inhibitory concentration (MIC) of oxacillin was detected by the E-test for *S. aureus* strains that were considered to be MRSA by producing

a growth inhibition zone with a diameter of less than 22 mm around the cefoxitin disc. For this, an oxacillin strip (BioMérieux, France) was applied to the surface of a Müller-Hinton agar plate inoculated with the bacterial suspension of 0.5 McFarland turbidity, as soon as the surface was no longer wet. After incubation overnight at 35°C , the MIC value was read and interpreted based on the recommendation of the manufacturer and CLSI [11].

Statistical analysis

The statistical analysis aimed to determine the frequency of all antibiotic resistance phenotypes and also to find any statistically significant correlation between MRSA carriage and student sex and between MRSA carriage and smoking, using Fisher's exact test at a significance level of 0.05.

Results and Discussion

The frequency of MRSA isolates was 7.9% and these strains were isolated from the pharynx from 3 female smokers and 2 female non-smokers. The MIC of oxacillin for each of the 5 strains was 4 $\mu\text{g}/\text{mL}$, consistent with the inclusion of the isolates in the MRSA phenotype, based on result of the cefoxitin disc diffusion test. The other 58 strains showed zone diameters greater than 22 mm for cefoxitin and were considered to be MSSA. All MSSA isolates were resistant to penicillin G and belonged to the penicillin G-resistant and methicillin-susceptible (Peni-R Meti-S) phenotype. Of these, 15 strains were resistant to tetracycline and 12 strains to erythromycin and clindamycin, while 16 strains were resistant to tetracycline, erythromycin and clindamycin and 3 nasal strains were resistant to: gentamicin, tetracycline, erythromycin and clindamycin.

All *S. aureus* strains showed inhibition zones greater than 19 mm in diameter around the clindamycin disc. However, the D-test was positive for all erythromycin-resistant strains, indicating flattening of inhibition zone around the clindamycin disc proximal to the erythromycin disc (inhibition zone shaped like the letter D). Thus, all 31 erythromycin-resistant MSSA strains were shown to induce clindamycin resistance and were included in the inducible macrolide - lincosamide - streptogramin B (MLSB) resistance phenotype. All MRSA strains expressed the inducible MLSB phenotype, but only one isolate was also resistant to gentamicin and tetracycline and was considered a multidrug-resistant (MDR) MRSA strain.

Figure 1 illustrates the percentage distribution of strains by antibiotic susceptibility, with the observation that the interpretation of methicillin/oxacillin susceptibility was based on the result of the cefoxitin disc diffusion test (Figure 1). The resistance phenotypes found among the *S. aureus* strains isolated from this group of students are listed in Table I.

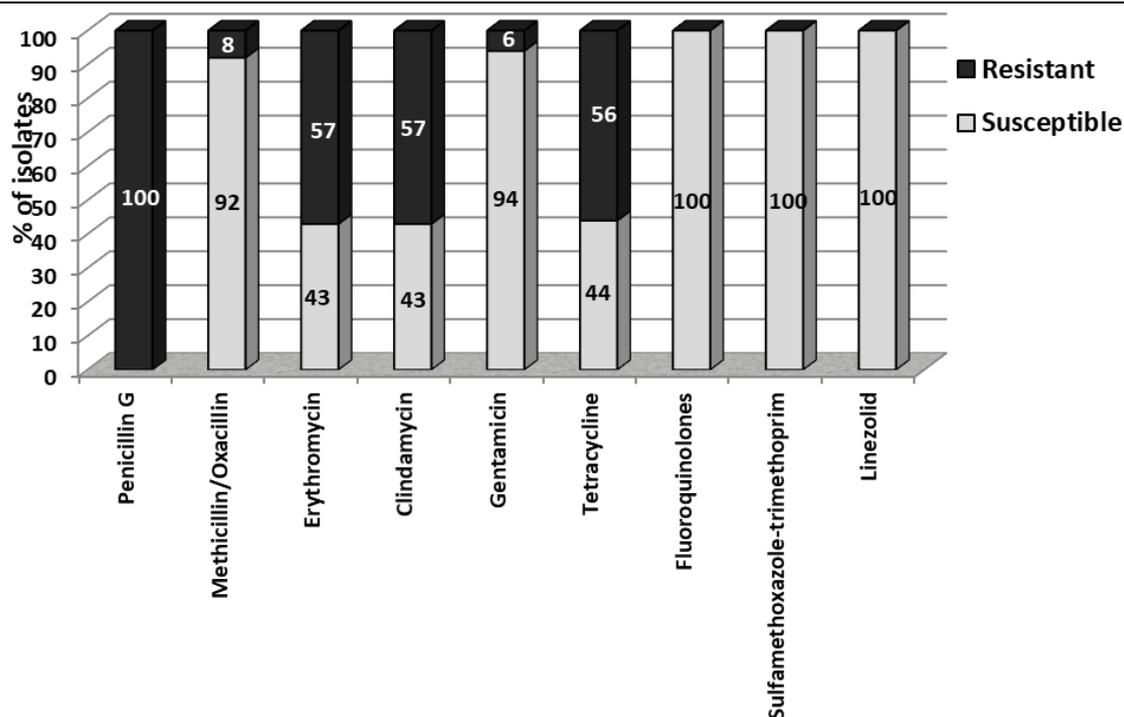


Figure 1.
Distribution (%) of the 63 *S. aureus* isolates by antibiotic susceptibility

Table I
Antibiotic resistance phenotypes detected among the 63 *S. aureus* isolates

Resistance phenotypes	No. of isolates
MRSA ¹ + inducible MLSB ² -R ³	4
MRSA + inducible MLSB-R + Tetracycline-R + Gentamicin-R	1
Peni ⁴ -R Meti ⁵ -S ⁶	12
Peni-R Meti-S + Tetracycline-R	15
Peni-R Meti-S + inducible MLSB-R	12
Peni-R Meti-S + inducible MLSB-R + Tetracycline-R	16
Peni-R Meti-S + inducible MLSB-R + Tetracycline-R + Gentamicin-R	3

¹MRSA = methicillin-resistant *S. aureus*, ²MLSB = macrolide - lincosamide - streptogramin B, ³R = resistant, ⁴Peni = penicillin G, ⁵Meti = methicillin, ⁶S = susceptible.

The p-value was 0.3255 when investigating the association between MRSA carriers and the sex of the subjects by Fisher's exact test and 0.1209 when investigating the association between MRSA carriers and smoking. No statistically significant association was found, the calculated *p* value being greater than 0.05 for both pairs of variables.

The frequency of MRSA carriage (~ 2%) was similar to that found in the student population of some medical universities in the USA [6] and Ireland [9] and close to the frequency (2.4%) reported by Prates *et al.* [18]. In contrast, other researchers reported higher values of MRSA nasal carriage among dental students, such as 12.5% [14] or 21% [19]. Most MRSA carriers were found among students in the final years of study [5, 19]. Regarding the predominance of MRSA carriers according to the student's gender, there are studies that indicated a higher frequency of female carriers, while other studies indicated a higher frequency of male carriers.

It is already known that MRSA are resistant to all beta-lactam antibiotics, except for fifth-generation cephalosporins, such as ceftaroline [17]. In the present work, 7.93% of the *S. aureus* isolates were included in the MRSA phenotype, based on the result of the cefoxitin disc diffusion test and a MIC value of oxacillin higher than 2 µg/mL.

Over 57% of the *S. aureus* isolates were resistant to erythromycin and implicitly to: azithromycin, clarithromycin and roxithromycin. If D-test had not been applied, all 36 erythromycin-resistant isolates would have been falsely declared to be susceptible to clindamycin. The D-test indicated that the macrolide-resistant strains were invariably clindamycin-resistant and showed an inducible MLSB phenotype. A resistance rate similar to the erythromycin and clindamycin resistance rate was also found in tetracycline (55.5%). In contrast, the frequency of gentamicin-resistant strains was much lower (6.3%), but all those strains were MDR.

Like trimethoprim-sulfamethoxazole and linezolid, norfloxacin (used to screen for fluoroquinolone sensitivity) was fully active, indicating susceptibility to the fluoroquinolones group of antibiotics. In contrast, a recent study on antibiotic sensitivity of pharyngeal and nasal strains of *S. aureus* isolated from 186 healthy Ecuadorian medicine students reported a frequency of 3.2% of fluoroquinolone-resistant strains and a frequency of 0.5% of trimethoprim-sulfamethoxazole-resistant strains [3]. The erythromycin-resistance rate (57.7%) was similar to that found in the present study (57.1%), while clindamycin and tetracycline resistance rates were 39.2% and 19.4%, respectively. The frequency of oxacillin-resistant strains was 10 times higher (79.6%) and the penicillin G-resistance rate was 93.5% [3].

Another similar research work, performed at the Medical School in Amman, in 2018 - 2019, reported a prevalence of 8.5% for nasal carriage of *S. aureus* and 1.9% for nasal MRSA carriage among the pre-clinical students, while the prevalence of nasal carriage among students in last 3 years of study was 4.2% for *S. aureus* and 2.8% for MRSA [2].

To our knowledge, this is the first Romanian study focused on the detection of antibiotic resistance phenotypes of *S. aureus* strains isolated from the nose and pharynx from dental students. Both screening for MRSA and MSSA carriage in larger groups of dental students and investigation of antibiotic resistance patterns of *S. aureus* isolates are worth continuing.

Conclusions

S. aureus strains isolated from this group of healthy young adults belonged to different resistance phenotypes, and the frequency of MDR strains was high. The results clearly showed the importance of applying the D-test whenever there is interest in knowing whether the *S. aureus* isolates are susceptible to clindamycin. It is important for dental students to be informed before working with patients about the risk of transmitting such bacteria during dental practice.

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Conflict of interest

The authors declare no conflict of interest.

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