

Low nasal carriage of drug-resistant bacteria among medical students in Vienna

Niedrige nasale Besiedlung mit resistenten Bakterien bei Medizinstudenten in Wien

Abstract

Background: Multi-drug resistant bacteria are increasing and remain a major public health challenge worldwide. In order to understand the potential role of medical students as a reservoir for circulating pathogenic bacteria and their transmission, we analysed the nasal colonisation among 86 clinically exposed medical students of the Medical University of Vienna, which is integrated into General Hospital of Vienna.

Methods: Nasal swabs obtained from 79 students were eligible for further analysis. Nasal swabs were analysed for Gram-positive and Gram-negative bacteria with special emphasis on methicillin-resistant *Staphylococcus aureus*.

Results: 25.3% of participants were positive for *Staphylococcus aureus* colonization; none of the isolates showed methicillin-resistance or expression of Pantoin-Valentine-leukocidin. However, 2.5% were positive for methicillin-resistant *Staphylococcus epidermidis*. No participant showed *Streptococcus pneumoniae* colonisation. Furthermore, 10.1% of the samples displayed growth of Gram-negative bacteria, yet none showed any relevant drug-resistance.

Conclusion: In conclusion, our investigation did not reveal any clinically relevant multi-drug resistant bacterial colonisation among clinically exposed medical students in Vienna. This might be explained by well-established hygienic precautions or comparably low circulation of resistant bacteria.

Keywords: MRSA, MSSA, resistant bacteria, nasal colonization, medical students

Zusammenfassung

Hintergrund: Multiresistente Bakterien stellen eine große Herausforderung für das öffentliche Gesundheitswesen weltweit dar. Um die potenzielle Rolle von Medizinstudenten als ein Reservoir für zirkulierende pathogene Bakterien und deren Verbreitung besser zu verstehen, analysierten wir die nasale Besiedlung bei 86 klinisch tätigen Medizinstudenten der Medizinischen Universität Wien, die in das Allgemeine Krankenhaus Wien integriert ist.

Methoden: Nasenabstriche von 79 Studenten konnten in die Studie eingeschlossen werden. Die Nasenabstriche wurden auf Gram-positive und Gram-negative Bakterien mit dem Schwerpunkt Methicillin-resistenter *Staphylococcus aureus* untersucht.

Ergebnisse: 25.3% der Teilnehmenden zeigten eine *Staphylococcus aureus* Kolonisation; keines dieser Isolate zeigte jedoch eine Methicillin-Resistenz oder die Expression des Pantoin-Valentine-Leukocidin. 2.5% der Teilnehmenden waren positiv auf methicillin-resistenten *Staphylococcus epidermidis*. *Streptococcus pneumoniae* Kolonisation zeigte sich bei keinem der Teilnehmenden. Des Weiteren fanden sich Gram-negative Bakterien in 10.1% der Proben, allerdings ohne relevante Antibiotika-Resistenz.

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Schlussfolgerung: Insgesamt konnten wir keine maßgebliche nasale Kolonisation mit Antibiotika-resistenten Bakterien bei klinisch exponierten Medizinstudenten feststellen. Dies könnte durch gut etablierte Hygienemaßnahmen oder eine vergleichsweise geringe Menge von zirkulierenden pathogenen Bakterien erklärt werden.

Background

Nosocomial infections are a challenge to all health care systems worldwide. Numbers of infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are consistently high and remain a major risk to patients, especially when hospitalized [1]. Furthermore, a growing prevalence of infections caused by multi-drug resistant (MDR) Gram-negative bacteria is reported [2].

Testing for nasal colonization with clinical relevant bacteria is an uncomplicated approach for estimating the extent of bacterial resistance-pattern in hospital settings. Therefore, analysis of nose colonizing bacteria serves not only for monitoring circulating pathogenic bacteria and their transmission pathways, but may also allow estimation of the compliance of infection control precautions among health care professionals.

Therefore, the aim of this study was to characterize the effects of clinical exposure and evaluate the possible role of medical students in the transmission of pathogenic bacteria at a 2,200 bed tertiary care university teaching hospital. In total, 86 medical students voluntarily presented themselves to perform a nasal swab after a written announce of the study. As the medical school is integrated into the Vienna General Hospital, students are daily exposed to the hospital environment. Furthermore, the sample collection was performed directly after the summer period of compulsory internships, thus guaranteeing additional exposure. A special focus was put on epidemiologically significant bacteria such as MRSA, methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Streptococcus pneumoniae* and Gram-negative MDR-bacteria (e.g. extended spectrum beta-lactamase producing *Enterobacteriaceae*). Furthermore, we determined the expression of Panton-Valentine Leucocidin (PVL) in order to characterize the pathogenicity of yielded *S. aureus* isolates.

Material and methods

The study protocol was approved by the ethic committee of the Medical University of Vienna (Ethic committee no.: 2010/713). A cohort of 86 medical students participated in the study. Nasal swabs were used to collect samples for microbiological analysis. Of 86 participants, 79 fulfilled the inclusion criteria, which consisted in i) no severe respiratory infection ii) no hospitalization iii) no antibiotic treatment in the last six months. These criteria were established in order to ensure nasal carriage associated to health care environment. Nasal swabs were taken from both nostrils and transferred on blood agar (BA), chocolate agar (CHOC) and MacConkey Agar (MCK) plates.

Incubation for 24 hours at 37 °C followed; CHOC plates under CO₂ environment.

Verification of *Staphylococci* was performed by i) characteristic phenotypically growth on BA plate ii) Gram stain iii) positive katalase reaction. Colonies meeting these criteria were incubated on Mannitol Salt Agar (MSA) plates. Isolates displaying yellow growth in MSA plates were deemed as *Staphylococcus aureus* (*S. aureus*) and subsequently verified by Analytical Profile Index (API) and PCR. The PCR for identification of *S. aureus* and PVL was performed according to Kocsis et al. 2009 [3].

Identified isolates of *S. aureus* were screened for oxacillin-resistance by 24 h incubation on Oxacillin Screen Agar (Mueller Hinton Agar, Oxacillin 6 µg/ml, 4% NaCl). Additionally, oxacillin minimum inhibition concentrations (MICs) of *Staphylococcus aureus* isolates were determined by microtiter dilution method according to CLSI guidelines [4].

Staphylococci isolates not displaying yellow growth on MSA plates were deemed as coagulase-negative *Staphylococci* (CoNS). Screening for oxacillin-resistance was performed as above. Further identification by API was only executed in case of positive oxacillin-resistance screening.

S. pneumoniae was identified by alpha-haemolysis on CHOC plates and sensitivity to optochin. Further identification was done by API.

Bacterial colonies growing on MCK plates, indicating Gram-negative bacteria, were subsequently analysed by Gram-stain, oxidase reaction and API.

Resistance-testing for all isolates was performed on Mueller Hinton II Agar using disk diffusion susceptibility testing following EUCAST criteria

Results

An overview of bacterial nasal colonization is shown in Table 1. All 20 *S. aureus* isolates showed no growth on Oxacillin-Screen agar and displayed a MIC range of 0.25–0.5 mg/L with a MIC₅₀ of 0.25 mg/L and a MIC₉₀ of 0.5 mg/L (Table 2). Furthermore, all isolates were susceptible to fosfomicin, linezolid, mupirocin, rifampin and vancomycin. No isolate expressed PVL.

Among CoNS, two isolates identified as *Staphylococcus epidermidis* showed growth on Oxacillin-Screen agar and displayed MICs of 8 and 4 mg/L, respectively.

Overall, eight Gram-negative bacteria were identified. These were sensitive to amoxicillin/clavulanic acid, cefuroxime, cefepime, ciprofloxacin, fosfomicin and imipenem.

Table 1: Overview of Isolated Bacteria

	Number	Percent
Participants	79	100
Gram-positive Bacteria overall:	79	100
MSSA ¹	20	25.31
MRSA ²	0	0
MRSE ³	2	2.53
other unspecified CoNS ⁴	77	97.46
<i>Streptococcus pneumoniae</i>	0	0
Gram-negative Bacteria overall:	8	10.12
<i>Citrobacter koseri</i>	3	3.79
<i>Klebsiella oxytoca</i>	3	3.79
<i>Morganella morganii</i>	1	1.26
<i>Proteus mirabilis</i>	1	1.26

1: MSSA: methicillin-sensitive *Staphylococcus aureus*

2: MRSA: methicillin-resistant *Staphylococcus aureus*

3: MRSE: methicillin-resistant *Staphylococcus aureus*

4: CoNS: coagulase-negative Staphylococci

Table 2: Susceptibility against Oxacillin among the 20 MSSA Isolates

	MIC (mg/L)		
	Range	MIC ₅₀	MIC ₉₀
Oxacillin	0.25–0.5	0.25	0.5

MSSA: methicillin-sensitive *Staphylococcus aureus*

Discussion

The results of this control sample indicate a very low prevalence of multidrug resistant bacteria among medical students at the Medical University of Vienna. Moreover, the *S. aureus* isolates did not express PVL, thus indicating low pathogenicity. Colonisation with *S. aureus* did not significantly correlate with clinical exposure, sex or smoking habits, the latter finding contrary to previous studies [5]. Pertaining to methicillin-sensitive *S. aureus*, our findings are consistent with two other studies among medical students in Europe [6], [7]. However, the low prevalence of MRSA found among Viennese medical students diverged from findings in Gdansk, Poland, which found a prevalence of 4.5% among clinically exposed students. Nevertheless, PVL expression was not detected as well [7]. The other study, performed in Budapest, Hungary, came to similar results concerning MRSA with 0.67% [6]. Interestingly, two studies performed in the United States came to different findings. Healthy non-medical students at Texas State University showed similar *S. aureus* colonisations, whereas the MRSA prevalence was remarkably high with 7.4% [8]. Another study among healthcare professionals detected 43.8% *S. aureus* colonisation and 6.6% MRSA prevalence among the study population [9]. A study conducted in Malaysia among non-medical individuals found a prevalence of 23.4% concerning *S. aureus* and 0.28% MRSA [10]. Overall, this data indicates strong geographical differences among nasal colonization by MRSA, with low prevalence rates in Central Europe. However, the pattern

corresponds to the local epidemiologic prevalence of MRSA.

Surprisingly, 10.1% of our study population were positive on Gram-negative bacterial colonisation. However, the bacteria found did not show resistance against tested antibiotics, indicating low pathogenic potential.

Conclusion

Considering our data we conclude that clinical exposure does not contribute to nasal carriage of multidrug-resistant bacteria among medical students in Vienna. This might be explained by well-established hygienic precautions or comparably low circulation of resistant bacteria or the small sample size of investigated individuals, which might be the limiting factor of this study.

Notes

Authorship

The two first authors Gualdoni GA and Lingscheid T contributed equally to the study.

Competing interests

The authors declare that they have no competing interests.

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