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To screen or not to screen medical students for carriage of multidrug-resistant pathogens?☆

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SUMMARY

Background: The carriage of multidrug-resistant (MDR) pathogens in medical students has not been studied extensively, despite the fact that they are in contact with patients and exposed to a hospital environment.

Aim: To investigate the intestinal and nasal carriage of MDR pathogens among medical students and its association with their lifestyle and demographic data.

Methods: In 2021, first- and final-year medical students were invited to the study. Two rectal swabs were used for detection of extended-spectrum β-lactamase (ESBL)-producing, colistin-, tigecycline- or carbapenem-resistant Gram-negative bacteria and vancomycin-resistant enterococci. Nasal swab was used for *Staphylococcus aureus* culture. *S. aureus* isolates were characterized by *spa* typing; Gram-negative resistant isolates and meticillin-resistant *S. aureus* (MRSA) were subjected to whole-genome short and/or long sequencing.

Findings: From 178 students, 80 (44.9%) showed nasal carriage of *S. aureus*; two isolates were MRSA. In rectal swabs, seven ESBL-producing strains were detected. Sixteen students were colonized by colistin-resistant bacteria, three isolates carried the *mcr-1* gene (1.7%). The *mcr-9* (10.7%, 19/178) and *mcr-10* (2.2%, 4/178) genes were detected by quantitative polymerase chain reaction, but only two colistin-susceptible *mcr-10*-positive isolates were cultured. The *S. aureus* nasal carriage was negatively associated with antibiotic and probiotic consumption. *S. aureus* and colistin-resistant bacteria were detected more frequently among students in contact with livestock.

Conclusion: Medical students can be colonized by (multi)drug-resistant bacteria with no difference between first- and final-year students. The participation of students in self-screening increases their awareness of possible colonization by resistant strains and their potential transmission due to poor hand hygiene.

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Introduction

Antimicrobial resistance poses one of the greatest threats to global health. The increasing occurrence of multidrug-resistant (MDR) pathogens poses an economic burden to the healthcare system and increases in-hospital mortality [1].

Active microbiological surveillance for multidrug-resistant pathogen carriage is crucial for hospital infection prevention and control. The intestinal or nasal carriage of resistant strains among hospitalized patients or healthcare workers has been studied and it was shown that both groups participated in the transmission of MDR strains, mainly due to insufficient hand hygiene compliance and a lack of awareness of MDR organisms and mechanisms of their transmission [2—6]. The carriage of MDR in medical students has not been studied extensively despite the fact that they are also in contact with patients and are also exposed to a hospital environment [7].

The majority of studies involving medical students focused mainly on the nasal carriage of meticillin-susceptible *S. aureus* (MSSA) and/or MRSA [7—9]. Data on the intestinal carriage of MDR pathogens in medical student cohorts are mainly from Asia and/or are often limited to extended-spectrum β-lactamase (ESBL) carriage [10,11]. Therefore, to supplement limited knowledge, the aim of our study was to investigate the faecal and nasal carriage of MDR pathogens among first- and final-year medical students and its association with demographic data and the students' lifestyle. In addition, we characterized the acquired MDR isolates by whole-genome sequencing.

Methods

Sample collection

Between March and June 2021, first- and final-year medical students of the 2nd Faculty of Medicine at Charles University, Prague, Czech Republic were invited to participate in the study. After being given detailed instructions, each student self-collected two rectal and one nasal swab with a sterile tampon placed in transport Amies media (Copan, Brescia, Italy) and immediately transferred to the laboratory. Simultaneously, students completed a questionnaire to evaluate the association between the carriage and demographics and lifestyle including age, gender, overweight, nationality, type of household (urban or rural), antibiotic consumption within the month preceding sampling, frequency of use of antibiotics and other drug consumption, employment of a family member in the health sector, smoking, travelling within six months preceding sampling, owning pets, and type of diet. At the end of the study, the anonymized results were presented to students.

The study has been approved by the Ethics Committee of the University Hospital Motol (reference number EK-1075/20) and all the involved subjects signed informed consent.

Bacterial culture and antimicrobial susceptibility testing

Nasal swabs

Nasal swabs were enriched in the thioglycollate broth (Oxoid, ThermoFisher, Waltham, MA, USA) at 37 °C overnight. The enrichment was plated on to a mannitol salt agar plate (Oxoid) and a mannitol salt agar supplemented with 2 mg/L of oxacillin (Sigma—Aldrich; St Louis, Missouri, USA) for detection of *S. aureus* and MRSA, respectively [12]. Suspected colonies were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using Biotyper v 3.1 (Bruker Daltonics, Hilden, Germany). Susceptibility testing of *S. aureus* isolates to trimethoprim/sulfamethoxazole, erythromycin, clindamycin, cefoxitin, tetracycline, gentamicin, ofloxacin, rifampicin, linezolid, fusidic acid, and mupirocin (Oxoid) was determined by the disc diffusion method. The results were evaluated according to the breakpoint values defined by EUCAST or the Clinical Laboratory Standards Institute (CLSI) for ofloxacin only. Inducible clindamycin resistance was tested by D-test [13,14].

All MRSA and MSSA isolates were characterized by *spa* typing according to the protocol of the European Network of Laboratories for sequence-based typing of microbial pathogens (http://www.seqnet.org) [15]. Ridom StaphType software (Ridom, Münster, Germany) was used for the *spa* type assignment. MRSA isolates were subjected to the whole-genome sequencing described below.

Rectal swabs

One rectal swab was enriched in Enterobacteriaceae enrichment (EE) broth-Mossel (ThermoFisher) overnight at 37 °C. Enrichment cultures were plated onto the following media using a sterile dacron swab (Dulab s.r.o., Dubné, Czechia). For the detection of colistin-resistant Gram-negative bacteria, the Brilliance™ UTI Clarity™ Agar (ThermoFisher) supplemented with 3.5 mg/L of colistin (Sigma—Aldrich) was used as described previously [16,17].

In addition, 1 mL of enrichment broths was tested by quantitative polymerase chain reaction (qPCR) after DNA extraction for the presence of genes *mcr-1—10* (Supplementary Table S1). The specificity of PCR amplicons was confirmed by Sanger sequencing. As *mcr-9* and *-10* genes are also detected among colistin-susceptible isolates, PCR-positive enrichments for the presence of *mcr-9* and/or *mcr-10* genes were plated on to Brilliance UTI Clarity Agar without colistin and processed as described above [18]. Suspected colonies were identified by MALDI-TOF MS and antibiotic susceptibility testing to colistin was performed by broth microdilution (Mikrolatest®; Erba Lachema s.r.o., Brno, Czechia) in isolates grown on selective media supplemented with colistin and/or showing the presence of *mcr-9* and/or *mcr*-10 by qPCR.

For ESBL and carbapenem-resistant Gram-negative bacteria, CHROMagar™ Orientation medium supplemented with 0.4 mg/L of KPC supplement (CHROMagar, Paris, France) and CHROMagar Orientation medium supplemented with 0.57 mg/L of ESBL supplement (CHROMagar) were used. The ESBL phenotype was confirmed by the double-disc synergy test (DDST) according to the EUCAST recommendation [19].

For detection of tigecycline resistance, 10 μL of EE broth suspension was inoculated into thioglycollate broth supplemented with 1 mg/L of tigecycline (Sigma—Aldrich), the breakpoint for tigecycline resistance in *E. coli* and *C. koseri* according to EUCAST [13]. All tubes showing bacterial turbidity were plated on to non-selective agar and a disc diffusion test was performed in growing isolates to test tigecycline resistance. If the disc diffusion test predicted tigecycline resistance, broth microdilution (Mikrolatest, Erba Lachema s.r.o., Brno, Czechia) was performed for its verification. Among tigecycline-resistant isolates, plasmid-bounded resistance mediated by the

tet(X) and *tet*(X1) genes was tested by qPCR amplification using primers described previously with the new design for forward primer *tet*(X1) because of a mismatch in the original primer sequence (Supplementary Table S1).

In addition, the antibiotic susceptibility testing to clinically commonly used antibiotics against Gram-negative bacteria was performed in isolates resistant to tigecycline, colistin, and/or ESBL producers using the microdilution method (SENSILAtest G-I, G-II; Mikrolatest) with the EUCAST breakpoints or CLSI breakpoints for the antibiotics not defined by EUCAST [13,14]. For cefoperazone/sulbactam, the breakpoint ≥64 mg/L determined previously was used [20].

An additional rectal swab was used for VRE detection. After the overnight incubation in VRE Broth Base supplemented with 2 mg/L of meropenem (Oxoid) at 37 °C, suspensions were plated on to CHROMagar VRE base plates supplemented with 60 mg/L of VRE supplement (CHROMagar) using a sterile dacron swab (Dulab). All isolates phenotypically matching *Enterococcus* spp. were verified using MALDI-TOF MS and tested for vancomycin resistance by disc diffusion.

Isolates were defined as multidrug resistant if they were resistant to at least one agent in three or more classes of antibiotics [21].

Whole genome sequencing

All detected resistant isolates excluding MSSA were characterized by whole genome sequencing. DNA from one bacterial colony was extracted using MasterPure Complete DNA & RNA Purification Kit (Biosearch Technologies, Hoddesdon, UK), quantified using Qubit™ dsDNA HS Assay Kit (ThermoFisher) and the purity was measured using NanoDrop (ThermoFisher).

For the short reads sequencing, the DNA sequencing library was prepared by Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturers' instructions, pooled libraries were sequenced on Illumina sequencer (HiSeq X Ten, Macrogen, Amsterdam, Netherlands). Genomes of all isolates were assembled using SPAdes v3.15.5 [22].

To investigate the genetic localization of *mcr* genes, the long reads sequencing was performed using Ligation Sequencing Kit, #SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) and a MinION #FLO-MIN106 flow cell (Oxford Nanopore Technologies). FAST5 read files were base-called and converted to FASTQ using Guppy v3.0.3+7e7b7d0 (Oxford Nanopore Technologies). The hybrid assembly of long and short reads was done using Unicycler v0.4.7 [23].

All raw sequence data in this study were submitted in the NCBI Sequence Read Archive (SRA) under accession number PRJNA882601. According to NCBI Taxonomy database rules, *E. hermannii* was listed as *Atlantibacter hermannii*.

Bioinformatic analysis

FastQ, fasta and hybrid assemblies (for *mcr*-positive strains) data were analysed using the following tools available at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk). Sequence types (ST) and core genome ST (cgST) were defined using MLST 2.0 and cgMLSTFinder 1.2; acquired antimicrobial resistance genes and virulence factors were identified using ResFinder 4.1 and VirulenceFinder 2.0, respectively. PlasmidFinder 2.1 was used for plasmid classification. KmerFinder 3.2 was used for distinguishing *Enterobacter* spp. strains [24–29]. In *E. coli* isolates, serotype and FimH/FumC type were determined by SerotypeFinder 2.0 and CHTyper 1.0 [27,30]. SCCmecFinder 1.2 was used for the determination of SCCmec type in MRSA isolates [31].

For the detection of chromosomal mutations leading to colistin or tigecycline resistance, single nucleotide polymorphism (SNP) analysis was performed using Snippy [32]. The following strains were re-annotated with RASTtk and used as reference strains: wild-type *E. coli* K-12 MG1655 (NC_000913), *Escherichia hermannii* ATCC 33651 (NZ_CP042941), *K. pneumoniae* MGH 78578 (CP000647) and *mgrB* gene from *K. pneumoniae* KP51 (MF431845), *Klebsiella michiganensis* KCTC 1686 (CP003218), *Pseudomonas aeruginosa ATCC 27853* (CP011857), *Enterobacter cancerogenus* FDAARGOS 1428 (NZ_CP077290), *Enterobacter cloacae* subsp. *dissolvens* SDM (NC_018079), *Enterobacter roggenkampii* FDAARGOS (NZ_CP077407) and *Enterobacter hormaechei* subsp. *hoffmannii* DSM 14563 (NZ_CP017186) [33]. For *E. hermannii*, *K. michiganensis* and *Enterobacter* spp., phenotype including antibiotic profile or heteroresistant subpopulation is unknown.

Statistical analysis

Statistical analysis was performed in R v. 2021.09.1. The correlation between variables was determined using Fisher's exact test or χ^2-test as appropriate. Within statistically significant results ($P < 0.05$), an odds ratio was determined. Body mass index was calculated to predict underweight or overweight.

Results

Study participants

A total of 389 medical students were invited to participate in the study, of whom 221 were in their first year of study and 168 were in their final (sixth) year of study. A total of 178 of them agreed; 118 (66.3%) were first-year students and 60 (33.7%) participants were final-year students. Females represented 53.4% (63/118) and 60.0% (36/60) ($P = 0.40$) among the first-year and final-year students, respectively. The age ranged from 19 to 24 years with a mean age of 21.3 years (SD ± 1.9) and from 25 to 30 years with a mean age of 25.8 (SD ± 1.0) among first-year and final-year students, respectively. The other characteristics acquired from the questionnaires are summarized in Supplementary Table S2.

Nasal carriage of Staphylococcus aureus *and MRSA*

The prevalence of nasal carriage of *S. aureus* and MRSA among students was 44.9% (80/178) and 1.1% (2/178), respectively. There was no statistically significant difference between first- (47.5%, 56/118) and final-year (40.0%, 24/60) students ($P = 0.34$). Men were significantly more often colonized ($P = 0.049$) with an almost two-fold odds increase (1.82; 95% CI: 1.00 to 3.32).

The negative correlation with nasal carriage of *S. aureus* was observed among students taking antibiotics within one month preceding sampling ($P = 0.02$). Of 11 students with recent antibiotic treatment, only one carried *S. aureus* (9.1%) (nine-fold decrease in the odds of colonization: 0.11; 95% CI:

0.01 to 0.89). Similarly, only two students (2/14, 14.3%) using probiotics and/or prebiotics were colonized by *S. aureus* ($P = 0.02$, a five-fold decrease in the odds: 0.18; 95% CI: 0.04 to 0.85). By contrast, 11 out of 14 students who reported contact with livestock (poultry, pigs, rabbits, bulls and/or horses) were carriers of *S. aureus* ($P = 0.01$, a five-fold increase in the odds: 5.05; 95% CI: 1.36 to 18.78). The complete evaluations of associations for the nasal carriage of *S. aureus* and *P*-values are presented in Supplementary Table S2.

Staphylococcus aureus isolates were resistant to erythromycin (21.3%, 17/80), cefoxitin (2.5%, 2/80), tetracycline (2.5%, 2/80), gentamicin (2.5%, 2/80), fusidic acid (3.8%, 3/80), clindamycin (1.3%, 1/80) and mupirocin (1.3%, 1/80). The clindamycin resistance was inducible in 16 isolates (20.0%, 16/80). All isolates tested remained susceptible to co-trimoxazole, ofloxacin, rifampicin and linezolid. Five (6.3%) isolates were defined as MDR (both MRSA and three isolates of MSSA) (Supplementary Table S3).

The 54 different *spa* types were assigned to 80 *S. aureus* isolates (Supplementary Table S3). The most frequent *spa* type was t084 with six first-year and two final-year students colonized (8/80, 10.0%). Among first-year students, t1451 was very common (5/80, 6.3%) but none of the final-year students was colonized by this *spa* type.

MRSA isolates characterization

The two MRSA carriers were first- (G26B) and final-year (G171B) students, respectively. The first-year student was a Slovak male with no contact with animals. The final-year student was a Czech female owning a dog and a cat. Both were non-smokers, had no relatives working in a healthcare facility, no travel history within six months preceding sampling, without a specific diet, and had not been hospitalized or treated by antibiotics within one-month preceding sampling.

Both MRSA isolates belonged to community-acquired MRSA (CA-MRSA) lineage, i.e. ST80 the European CA-MRSA (G26B, t044, SCC*mec* type IVc) and ST59 the Asian CA-MRSA (G171B, t3527, SCC*mec* type Vb). Both isolates carried Panton–Valentine leucocidin (PVL). The isolate G26B was resistant to cefoxitin, tetracycline and fusidic acid and carried corresponding resistance genes *mecA*, *tet*(K), and *fusB*. Two plasmids, rep7c and rep20, were detected in this isolate. The rep20 plasmid carried the *fusB* and *blaZ* genes as they were located in the same contig. The isolate G171B was resistant to cefoxitin, erythromycin, and clindamycin, and carried *mecA* and *ermB* genes explaining its resistant phenotype. No plasmid was identified in this isolate. The characterization of isolates using WGS data is summarized in Supplementary Table S4.

Rectal carriage of ESBL-producing bacteria

Twelve isolates from 12 students (12/178, 6.7%) were cultured on ESBL chromogenic agar. All isolates were resistant to ampicillin, cefazolin and cefuroxime. The resistance to aztreonam, piperacillin, cefotaxime, ceftazidime, cefoperazone was detected in 11 out of 12 isolates. A summary of minimum inhibitory concentration (MIC) values is listed in Supplementary Table S5. The ESBL phenotype was confirmed in seven isolates (six *E. coli* and one *K. pneumoniae*) by DDST, with a prevalence of 3.9% (7/178). In five isolates (two *Citrobacter* spp. and three *Enterobacter* spp.), ESBL production was not confirmed by DDST. The isolates were frequently detected among males (eight-fold increase in odds: 8.05; 95% CI: 0.95 to 68.37; $P = 0.045$) (Supplementary Table S6) with no difference between first- and final-year students ($P = 1.00$).

All 12 isolates growing on ESBL chromogenic agar were sequenced and analysed for carriage of ESBL genes. ESBL genes were detected among seven confirmed ESBL producers: $bla_{\text{CTX-M-14}}$ ($N = 1$), $bla_{\text{CTX-M-15}}$ ($N = 4$), $bla_{\text{TEM-1B}}$ ($N = 3$), $bla_{\text{CTX-M-55}}$ ($N = 2$), $bla_{\text{OXA-1}}$ ($N = 2$), $bla_{\text{SHV-187}}$ ($N = 1$), as some of them carried more than one ESBL gene. Interestingly, two *E. coli* isolates of the same ST773 were detected in two first-year students. *Citrobacter* spp. and *Enterobacter* spp. carried only *bla* genes belonging to the class-C β-lactamase family naturally occurring in these bacteria ($bla_{CMY\text{-}104}$, $bla_{ACT\text{-}7}$, $bla_{ACT\text{-}15}$, $bla_{CMY\text{-}109}$, and $bla_{MIR\text{-}3}$). The results of the characterization of isolates are summarized in Supplementary Table S7.

Rectal carriage of colistin-resistant bacteria

Furthermore, 17 colistin-resistant isolates among 16 students were identified with a prevalence of 9.0% with no difference between first- and final-year students ($P = 0.58$). Contact with livestock was only associated with colistin-resistant isolate colonization identified with a five-fold increase in the odds ratio (5.07; 95% CI: 1.38 to 18.59) (Supplementary Table S6). Six isolates were resistant only to colistin with MIC ranging between 4 and 16 mg/L. The remaining isolates were resistant to other antimicrobials as well (Supplementary Table S8).

The *mcr-1* gene-mediated plasmid-borne colistin resistance was found among three *E. coli* isolates (3/17, 17.6%) of STs 744, 69 and 10 with a prevalence of 1.7% (3/178). IncX4 and IncI2 plasmids were found to be the vectors of the *mcr-1* gene in two and one isolates, respectively (Supplementary Table S9). Two *mcr-1*-carrying IncX4 plasmids showed 100% similarity and they did not carry any other genes of resistance (Supplementary Figure S1). Two of the *mcr-1*-carrying strains were isolated from Czech women living in the city, one first-year and one final-year student. The third isolate was cultured from a Slovak man in the first year of study. They did not report relatives in healthcare settings, smoking, specific diet, or contact with livestock.

In the remaining 14 colistin-resistant isolates, no *mcr* genes were detected. In all colistin-resistant isolates (including *mcr-1*-harbouring strains), SNP analysis was performed to find mutations in genes associated with polymyxin resistance phenotype. Several missense and frameshift mutations were found in genes encoding PmrABC, PmrG, PmrK, PhoPQ, MgrB, and ArnC proteins (Supplementary Table S10). In the PmrD protein, several mutations were found; however, all of them were identified as naturally occurring alternative protein isoforms according to the Uniprot database (www.uniprot.org/uniprotkb/P37590/entry). In addition to the *mcr-1* gene, two of three *mcr-1*-positive isolates carried two identical amino acid substitutions: S29G in PmrA and C27Y in PmrC. However, amino acid substitution in PmrA (S29G) was a natural isoform of this protein (www.uniprot.org/uniprotkb/P30843/entry). *E. hermannii* carried only one SNP within the whole genome leading to amino acid substitution in MgrB protein (Q22L). The results of SNP analysis are listed in Supplementary Tables S11–27. New putative polymorphisms

Table I
Summary of cultured bacterial isolates and the carriage prevalence among medical students

Detected isolates	First-year students ($N = 118$)	Final-year students ($N = 60$)	Total ($N = 178$)	Risk factors
Staphylococcus aureus	56 (47.5%)	24 (40.0%)	80 (44.9%)	Males (+); antibiotics one month preceding sampling (−); probiotics/prebiotics consumption (−); contact with livestock (+)
MSSA	55 (46.6%)	23 (38.3%)	78 (43.8%)	
MRSA	1 (0.8%)	1 (1.7%)	2 (1.1%)	
ESBL	5 (4.2%)	2 (3.3%)	7 (3.9%)	Males (+)
Escherichia coli	4 (3.4%)	2 (3.3%)	6 (3.4%)	
Klebsiella pneumoniae	1 (0.8%)	0	1 (0.6%)	
Colistin-resistant	13 (10.2%)[a]	4 (6.7%)	17 (9.0%)[a]	Contact with livestock (+)
Klebsiella spp.	1 (0.8%)	2 (3.3%)	3 (1.6%)	
E. coli[b]	7 (5.9%)	2 (3.3%)	9 (5.1%)	
Enterobacter spp.	4 (3.4%)	0	4 (2.2%)	
Pseudomonas aeruginosa	1 (0.8%)	0	1 (0.6%)	
Tigecycline-resistant	2 (1.7%)	1 (1.7%)	3 (1.7%)	Relatives in healthcare settings (+) ($P = 0.06$)[c]
E. coli	1 (0.8%)	0	1 (0.6%)	
Enterobacter hormaechei	1 (0.8%)	0	1 (0.6%)	
K. pneumoniae	0	1 (1.7%)	1 (0.6%)	

−, negative correlation; +, positive correlation; MSSA/MRSA, meticillin-susceptible/resistant *Staphylococcus aureus*; ESBL, extended-spectrum β-lactamase.
[a] One student was colonized by two isolates of *Enterobacter* spp.
[b] Three *mcr-1*-positive strains (two from first-year student and one from final-year student).
[c] Trend towards significance.

which have not previously been published as associated with colistin resistance are listed in Supplementary Table S28.

Screening for the presence of *mcr* genes in enrichments showed positivity for *mcr-1* in two samples from which two *mcr-1*-carrying isolates were acquired, and none of the *mcr* genes *2–8* was detected. The prevalence of the *mcr-9* gene carriage by qPCR was 10.7% (19/178) but none of these isolates was cultured on selective agar without colistin. The *mcr-10* gene was detected among five students (2.8%) and only two *Enterobacter roggenkampii* were cultured on selective agar without colistin. In *mcr-10*-carrying isolates, the *mcr-10* gene was located on two variable IncFIB (pECLA) plasmids (Supplementary Figure S2). Both isolates were susceptible to colistin. Although one isolate (G151A) was susceptible also to other antimicrobials tested, the other *mcr-10*-positive isolate (G171A) was resistant to first to third generations of cephalosporins, aztreonam and piperacillin/tazobactam (Supplementary Table S8). The WGS characteristics of *mcr-10*-harbouring isolates are listed in Supplementary Table S29.

Rectal carriage of tigecycline-resistant bacteria

Three tigecycline-resistant isolates were detected in three students (1.7%, 3/178). One isolate of *K. pneumoniae* (4 mg/L) was detected in a final-year Czech woman and isolates of *E. coli* (2 mg/L) and *Enterobacter hormaechei* (8 mg/L) were detected in first-year students (one woman and one man) ($P = 0.26$) (Supplementary Table S6). All three tigecycline-resistant isolates were also resistant to tetracycline, ampicillin and ampicillin/sulbactam. Tigecycline-resistant *K. pneumoniae* and *E. hormaechei* showed MDR phenotype, as both were resistant also to the first- and second-generation cephalosporins and chloramphenicol (Supplementary Table S30). No statistically significant associations were found but all three students recorded relatives in healthcare settings ($P = 0.06$).

In the tigecycline-resistant *E. coli* isolate, the *tet*(A) gene was present without mutations. In the other two isolates, no *tet* genes were detected. SNP analysis comparing tigecycline-resistant isolates with the reference showed several non-synonymous substitutions in the proteins associated with the tigecycline-resistant phenotype and multidrug efflux systems but some of the mutations were found also among tigecycline-susceptible isolates acquired in this study (Supplementary Tables S31–S34).

Rectal carriage of vancomycin and carbapenem-resistant bacteria

No isolate was cultured on selective media for VRE and carbapenem resistance detection.

The summary of antimicrobial-resistant isolate carriage among first- and final-year medical students and its association with demographic data and lifestyle are listed in Table I. Altogether, 17 MDR isolates from 15 students (8.4%, 15/178) were detected with no significant difference between first- and final-year students ($P = 0.78$). The most frequent were *E. coli* (7/17), *S. aureus* (5/17), *K. pneumoniae* (3/17), and *Enterobacter* spp. (2/17). The remaining 97 isolates were resistant to fewer than three classes of tested antimicrobials.

Discussion

Hospital screening for antimicrobial resistance is often limited to patients and healthcare workers but medical students are in general not included in the screening. In the present study, we evaluated the carriage of clinically important drug-resistant bacteria among medical students including the nasal carriage of *S. aureus* or MRSA and intestinal carriage of VRE, carbapenem-, colistin-, tigecycline-resistant and ESBL-producing bacteria and its association with demographic data and lifestyle.

A high prevalence of MSSA (43.8%) with a low prevalence of MRSA (1.1%) was found in our study, which is in line with data from Portugal where nasal carriage of 37.1% and 0.2% of MSSA and MRSA, respectively, was detected in 475 students of two biomedical faculties. By contrast, another Portuguese study that screened nursing students in Portugal showed that a considerable portion of students was colonized by MRSA during their education (10.6%) [34]. Lower prevalence rates were found in Irish second-year medical students where 28.8% (128/444) and 2.0% (9/444) were nasal carriers of MSSA and MRSA, respectively, and 955 Polish students without clinical practice (MSSA 25.5% and MRSA 0.1%) [35,36]. In another Polish study, MRSA strains were detected only among the students with clinical practice (1.8%, 3/165) when compared to the students without practice in the hospital (0/156) [37]. Similarly, two studies from Spain found a difference in MRSA nasal carriage between healthcare students who had not had clinical practice (0%) and who already worked in a hospital (1.3 and 4.3%) [38,39]. In this study, no significant difference in MRSA nor *S. aureus* nasal carriage between first- and final-year students was observed, which is in line with another Czech study that also showed no significant difference in *S. aureus* nasal carriage among 307 first- (32%) and fifth-year (30%) medical students, but no MRSA was cultured [40]. In *S. aureus* isolates, the most prevalent *spa* type found was t084, which was previously detected in healthy young males with intermittent *S. aureus* carriage [41]. The MRSA spa t044 detected in this study was found in one case in the Czech surveillance study including 441 MRSA isolates from asymptomatic carriers of inpatients and outpatients, and MRSA *spa* type t3527 was not detected [42].

Analysis of demographic and lifestyle data showed that contact with livestock but not with companion animals (cats and dogs) was found to be positively associated with *S. aureus* nasal carriage. It was previously reported that farm animals (especially poultry and pigs) could serve as a reservoir of *S. aureus* for humans, but companion animals were not frequent carriers of *S. aureus* [43]. By contrast, antibiotic, probiotic, or prebiotic consumption showed a negative correlation with *S. aureus* nasal colonization. Regarding the effect of probiotics, some probiotic bacteria, i.e. *Bacillus* spp., were found to inhibit *S. aureus* nasal colonization by interference with the quorum sensing signalling system [44].

Data on the intestinal carriage of drug-resistant strains by medical students are scarce. Most of the available studies are from China and Nepal and are often limited to ESBL carriage [10,11]. In Europe, a Portuguese study detected 16 (14.4%) out of 111 faecal samples of healthcare students positive for ESBL-producing Enterobacterales, which is higher than in our study (3.9% (7/178)). Interestingly, the authors found $bla_{CTX\text{-}M\text{-}1}$ as the most frequent ESBL gene (76%), which is commonly found in livestock isolates, and only 18% of Portuguese ESBL producers carried $bla_{CTX\text{-}M\text{-}15}$, which predominates in most European studies including our study (57%) [45].

Our study detected more ESBL producers among first-year students (5/7) similar to the above-mentioned Portuguese study which also found zero prevalence of colistin- and carbapenem-resistant strains using SuperPolymyxin and SuperCarba media, respectively, by contrast with a remarkably high prevalence of colistin resistance (9.0%) in our study [45]. Moreover, three students (1.7%) carried *mcr-1*-positive *E. coli*. Similar to *S. aureus* nasal carriage, a significantly higher prevalence of ESBL producers was found among males than among females. The higher carriage rate of opportunistic pathogens in males such as *S. aureus* or *Neisseria meningitidis* has been reported previously [46,47]. This may be associated with lifestyle-related factors that vary between males and females including hand hygiene habits or participation in contact sports [48]. Sex hormones can be another attribute, as contraceptives have been linked with a higher risk of *S. aureus* nasal carriage [49]. However, no association between contraceptives and the carriage of any pathogen was found in our study.

Travelling abroad (especially to Asia) was suggested as a risk factor for colonization with colistin- and carbapenem-resistant strains in the study of Dao *et al.*, where 2.6% of students became carriers of carbapenemase-producing Entero-bacterales after an internship abroad and 6.8% of students acquired the *mcr-1* gene-carrying bacteria [50]. In our study, travelling was not found to be a significant factor, but because of the coronavirus pandemic, only a limited number of students had travelled abroad (21.3%) and none of them had travelled to Asia. On the other hand, contact with livestock significantly increased the odds to acquire colistin-resistant strains. This is not unexpected as livestock is probably the main source of colistin-resistant strains to people because of the extensive usage of colistin in the veterinary sector [51].

In this study, the *mcr-1*, *-9* and *-10* genes were detected, which is in concordance with other studies as the *mcr-1* and *mcr-9* are the most disseminated *mcr* genes in the world [52]. Unfortunately, only two *mcr-10*-positive *E. roggenkampii* were cultivated as these isolates are often susceptible to colistin and other antimicrobials making their isolation problematic [51]. Other *mcr* genes from *2* to *8* are isolated mainly from the environment, food, and animals, and their detection among people is also scarce in other studies [16,17,53,54].

In *mcr*-negative isolates, amino acid substitutions related to colistin resistance were found. There are several studies describing polymorphism in *E. coli* and *K. pneumoniae* isolates, mainly in PmrABCD, PmrK, MgrB, and PhoPQ proteins (Supplementary Table S28). Also, for other strains including *K. oxytoca*, *E. hermannii*, *Enterobacter* spp. and *Pseudomonas aeruginosa*, some putative amino acid changes leading to colistin resistance were suggested, as knowledge about the mechanism of colistin resistance in these isolates is insufficient (Supplementary Table S28). Interestingly, in colistin-resistant *E. hermannii*, only one SNP in the whole genome was identified, leading to amino acid substitution in MgrB regulator (Q22L) which was previously related to colistin resistance in *K. pneumoniae* [55].

Tigecycline resistance was detected in three isolates. However, these samples represent more incidental interceptions rather than a reflection of prevalence because of the

unavailability of selective media for screening. Tigecycline belongs to the bacteriostatic agents with various breakpoints for different strains (EUCAST v11), making screening of tigecycline resistance problematic [56].

Unfortunately, the mechanism of resistance remained unclear in cultured tigecycline-resistant isolates. Some types of nucleotide mutations in transcriptional regulators such as *ramR* or *acrR* can lead to overexpression of the efflux system [57]. However, amino acid substitutions detected in AcrR in this study were found also in tigecycline-susceptible strains. Some other mutations in MATE, MFS and RND family efflux pumps were detected only in tigecycline-resistant isolates when compared to tigecycline-susceptible isolates in this study. However, there is still insufficient knowledge about the significance of these mutations.

Surprisingly, the prevalence of colonization with MDR strains did not differ significantly between first- and final-year students. The level of student involvement in patient care increases over the course of their medical studies and includes months of compulsory individual clinical practice at different hospital wards, especially in their final year of study. Nevertheless, the finding did not support our original hypothesis that the final-year students were at considerable risk of acquisition of hospital-associated MDR strains upon six years of their attendance at the university hospital. It is apparent that the result of such observations depends on the local epidemiological situation in the hospital and the level of student involvement in patient care, and therefore may differ between medical student groups from different universities and over time.

Although the colonization rates in this study were generally low, some clinically relevant pathogens including colistin-resistant bacteria (*mcr-1*-positive), ESBL producers, or MRSA were detected. The students in the study were young healthy carriers, so their colonization by MDR strains poses a risk of transmission of these pathogens while working with patients and moving around the hospital environment rather than developing the disease associated with MDR strain carriage [58,59].

Limitations of our single-centre study included a relatively small sample size, which does not allow generalization of associations found between lifestyle factors and carriage of resistant pathogens; and the fact that only CHROMagar KPC and ESBL were used to detect carbapenem-resistant Gram-negative bacteria and ESBL producers. This posed a risk of not detecting cephalosporin-susceptible OXA-48 producers with only decreased susceptibility to carbapenems [60].

In conclusion, medical students may be colonized by drug-resistant bacteria with no difference between first- and final-year students. The participation of students in self-screening increases their awareness of possible colonization by resistant strains and their potential transmission due to poor hand hygiene.

Author contributions

E.S.: investigation, writing original draft; P.D.: supervision, writing (review and editing); O.N.: supervision; M.B.: investigation, J.T.: study design, investigation, writing (review and editing), supervision; M.K.: study design, coordination of the study, investigation, writing (review and editing), supervision.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.06.028.

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