Acquisition of multidrug-resistant bacteria and colistin resistance genes in French medical students on internships abroad

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Abstract

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Background: In France, no previous studies had addressed the acquisition of multidrug resistant (MDR) bacteria and colistin resistance genes by medical students when undertaking internships abroad.

Methods: Nasopharyngeal, rectal, and vaginal swabs samples were collected from 382 French medical students before and after travel to investigate the acquisition of MDR bacteria. The bacterial diversity in the samples was assessed by culture on selective media. We also genetically characterised the isolates of MDR bacteria including Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), methicillin-resistant *Staphylococcus aureus* (MRSA), and Carbapenemase-producing *Enterobacteriacae* (CPE) using the real-time polymerase chain reaction method. The samples were collected from 293 students and were investigated for *mcr* colistin-resistance genes using RT-PCR directly on the samples, followed by conventional PCR and sequencing.

Results: A proportion of 29.3% of the participants had acquired ESBL-E and 2.6% had acquired CPE. The most common species and ESBL-E encoding gene were *Escherichia coli* (98.4%) and $bla_{CTX\text{-}M\text{-}A}$ (95.3%), respectively. A proportion of 6.8% of the participants had acquired *mcr*-1 genes, followed by *mcr*-3 (0.3%) and *mcr*-8 (0.3%). We found that taking part in humanitarian missions to orphanages, being in contact with children during travel, the primary destination of travel being Vietnam and north India, using antibiotics during travel, and studying in 2017 were associated with the acquisition of ESBL-E. When the primary destination of travel was Vietnam and the year of study was 2018, this was associated with acquisition of colistin resistance genes.

Conclusion: Medical students are at a potential risk of acquiring ESBL-E, CPE and colistin resistance genes. A number of risk factors have been identified, which may be used to develop targeted preventive measures.

Key words: medical students; travellers; MDR bacteria; CPE; *mcr-1*; *mcr-8*

Introduction

International travellers are not only exposed to travel-associated diseases [1] but also to the acquisition of multidrug resistant (MDR) bacteria [2,3], with extended spectrum β lactamase producing *Enterobacteriaceae* (ESBL-E) acquisition by travellers being the most frequent [4, 5]. The main risk factors associated with the acquisition of ESBL-E have been identified as travelling in Asia, the occurrence of diarrhoea during the stay, and the use of antibiotics [5-8]. The few studies that have evaluated the duration of MDR bacteria carriage, showed that there was a rapid decrease in the carriage rate with a possible persistence up to six months in between 8.6% and 25.0% of cases [7-11]. Of the Gram negative MDR bacteria, the current emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) has become a major concern [12-14]. Although the vast majority of MDR bacteria acquired during international travel are Gram-negative bacteria, the acquisition of Gram-positive bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA), during travel has also been documented [15, 16]. On the other hand, the acquisition of colistin-resistant bacteria is an emerging threat in international travellers [3, 17-19]. Recently, the transmission of colistin-resistant genes when international travellers return to developed countries from China, Vietnam and India has been reported [19-21].

Resistance to the polymyxin antibiotic colistin and to antibiotics in the carbapenem class is of particular concern, because these antibiotics are last line treatments for MDR bacterial infections [22].

A large number of medical students from high-income countries often participate in a clinical elective abroad, which is also known as an international medical elective. The most popular destinations are low- and middle-income countries. International medical electives are generally clinical immersion experiences in a hospital, but younger medical students may also take part in humanitarian missions to improve their medical knowledge through clinical experience, helping those in conditions of extreme poverty and exploring new cultures [23].

We recently reported on the occurrence of diarrhoea, respiratory and genital infection symptoms, and the acquisition of respiratory, gastro-intestinal and vaginal pathogens in French medical students who undertook an internship abroad during the summer months [24]. In France, no previous studies have addressed the acquisition of MDR bacteria and colistin resistance genes by medical students in relation to internships abroad. This study aimed to investigate the acquisition of MDR bacteria and colistin resistance genes and associated risk factors in cohorts of medical students from Marseille undertaking internships abroad during the summer. We also genetically characterised the isolates of MDR bacteria including ESBL-E genes, MRSA genes and CPE.

Materials and methods

Study design

A monocentric cohort survey was conducted over three years (2017–2019) among medical students from the Faculty of Medicine in Marseille, France who planned to take part in an international internship during the summer. Recruitment was performed on a voluntary basis, during their vaccination and pre-travel consultation at the Institut Méditerranée Infection, which is located on the Marseille University medical campus. Participants were required to sign a

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written informed consent form and to complete an inclusion questionnaire covering demographic data, intended travel dates and destination, and history of chronic illness. Necessary vaccination and antimalarial chemoprophylaxis for specific destinations were provided. Comprehensive advice on preventing diarrhoea when travelling (hand hygiene and safe food and water consumption) and vector-borne diseases (use of mosquito nets and repellent), and warning regarding rabies risks were given to all participants. Participants were requested to self-collect throat, nasal, rectal and vaginal samples during the week preceding departure (pre-travel samples) and during the week following their return to France (post-travel samples). For participants found to be carrying MDR bacteria on their return, follow-up samples were taken and analysed six months after return. A flow diagram of the study is presented in Figure 1.

Samples were collected using commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK) and placed in Sigma Transwab®. Samples were returned by participants to our institute for processing. A return questionnaire documenting the exact place where the internship took place, the type of activities performed, tourism and travel to other countries over the period of the internship, accommodation conditions, contacts with children and animals, symptoms and treatment during the stay, was given to students.

Influenza-like illness (ILI) was defined as the association of cough, sore throat, and subjective fever [25]. Diarrhoea was defined by at least three loose or liquid stools per day.

Microbiological methods

Bacterial isolates and species identification

Suspension of samples in Sigma Transwab® medium were streaked on selective media to screen for MRSA, Gram-negative bacteria including, notably, ESBL-E and CPE, *Acinetobacter*

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baumannii complex, Cephalosporin-resistant *Pseudomonas aeruginosa* and Glycopeptide-resistant *Enterococcus spp* (GRE), and were further tested using E-tests (Biomérieux, Marcy l'Etoile, France), the double disk diffusion test and ß-Carbatest. Species identification was performed using MALDITOF mass-spectrometry (Microflex LT, Bruker Daltonik, Bremen, Germany). (Supplementary Table S1).

Antibacterial susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. The results were interpreted according to EUCAST guidelines 2017 [26]. ESBL-E and CPE isolates were tested against 13 antibiotics (amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefepime, ceftriaxone, ertapenem, imipenem, fosfomycin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, doxycycline, and amikacine). MRSA isolates were tested against 16 antibiotics (benzylpenicillin, cefoxitin, oxacillin, rifampicin, clindamycin, erythromycin, pristinamycin, gentamicin, vancomycin, teicoplanin, ciprofloxacin, doxycycline, fosfomycin, fusidic acid, linezolid, and sulfamethoxazole-trimethoprim).

Molecular characterisation of antibiotic resistance genes

DNA extraction from isolates of MDR bacteria was performed using EZ1 DNA extraction kits (Qiagen, Courtaboeuf, France) with the EZ1 Advanced XL biorobot according to the manufacturer's instructions.

Real-time PCR was performed to detect bla_{SHV}, bla_{TEM}, $bla_{\text{CTX-M-A}}$ and $bla_{CTX\text{-}M\text{-}B}$ genes of ESBL –E [27], bla_{Oxa48}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{KPC} genes of CPE [28], *mecA, mecC* genes of MRSA [29, 30]. Positive results were defined as those with a cycle threshold (CT) value ≤ 26.

Screening for colistin resistance genes directly from samples

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131 ***DNA extraction and pool***

132 DNA and RNA were extracted from the all samples using the EZ1 Advanced XL (Qiagen,
133 Hilden, Germany) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's
134 recommendations. DNA pooling was performed as previously described [31].

135 ***Real-time PCR***

136 All quantitative real-time PCR (qPCR) reactions were performed using a C1000 Touch™
137 Thermal Cycle (Bio-Rad, USA) with the ready-to-use reaction mix ROX qPCR Master
138 according to the manufacturer's recommendations. Negative control (PCR mix) and positive
139 control templates were included in each qPCR experimental run. Results were considered
140 positive when the cycle threshold value of real-time PCR was ≤35. Individual retesting of each
141 specimen was carried out from the positive pools. qPCR amplification was used to confirm the
142 presence of colistin resistance genes using primers as described in Supplementary Table S2: *mcr*-
143 1, *mcr*-2 (including the detection of *mcr*-6) [37], *mcr*-3, *mcr*-4, *mcr*-5, and *mcr*-8.

144 ***Conventional PCR and sequencing***

145 To better characterise these genes, positive qPCR samples were simultaneously tested using
146 standard PCR. The purified PCR products were sequenced using specific primers and the BigDye
147 Terminator® version 1.1 cycle sequencing ready reaction mix (Applied Biosystems, Foster City,
148 CA, USA). All primers used in this study have previously been described (Supplementary Table
149 S2). Sequencing was performed on Applied Biosystems 3130 platform (ABI PRISM, PE Applied
150 Biosystems, USA). The sequences obtained were edited and assembled using Chromas Pro 1.77
151 (Technelysium Pty Ltd, Australia) and were then aligned with reference genes from the
152 ARGANNOT site (Gupta SK, 2014) and Genbank site. These sequences are available in

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GenBank at accession numbers from **MT475739** to **MT475760** (for mcr-1), **MT475761** (for mcr-3) and **MT475762** (for mcr-8).

The acquisition of MDR bacteria or colistin resistance genes was defined by the detection of MDR bacteria or colistin resistance genes after travel in individuals who were negative before travel. A flow diagram of the methods used to assess the acquisition of MDR bacteria is shown in Supplementary Figure 1.

Statistical analysis

Differences in the proportions were tested using Pearson's chi-square or Fisher's exact tests when appropriate. Univariate analysis was used to examine unadjusted associations between multiple factors and the prevalence of ESBL-E acquisition or colistin resistance gene acquisition, presented by percentages and risk ratios (aRR) with a 95% confidence interval (95%CI). Variables with p values <0.2 in the univariate analysis were included in the multivariate analysis. Log-binomial regression was used to estimate factors' adjusted risk ratios regarding the acquisition of ESBL-E and colistin resistance genes. Statistical analysis was conducted using STATA software version 14.2. A p value ≤0.05 was considered as statistically significant.

Ethics

The protocol was approved by our Institutional Review Board (2019- 006). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants gave their written informed consent. Analysis was conducted retrospectively.

Results

Characteristics of study participants

A total of 382 students were included in the study. The gender ratio (male/female) was 0.36. The median age was 21 years (ranging from 18 to 29 years) (Table 1). Twelve (3.1%) students suffered from chronic respiratory diseases and five (1.3%) used long-term corticosteroids. Most participants (78.8%) were students in their second year of medical school and were taking part in non-medical humanitarian missions including social and cultural activities with orphan children, school renovations, and supplying medical equipment and providing health education to children. Other students were in their fourth year of medical studies and were taking part in clinical work in surgical or medical departments in hospitals (Supplementary Table S3). The primary travel destinations were in South East Asia (31.7%), Africa (27.7%), South Asia (19.9%), and South America (17.5%). The top-five primary destination countries were Vietnam (25.7%), India (19.9%), Peru (13.4%), Madagascar (11.3%) and Tanzania (7.9%) (Supplementary Table S4). 83.3% of the participants had contacts with local children and 53.7% had contact with animals. Accommodation conditions were judged clean by 41.4% and basic by 39% of students, respectively. The mean travel duration was 42 days ± 11 days (ranging from 19 to 78 days). During their time abroad, 95.3% also travelled as tourists in the country of primary destination and 75 (19.6%) visited additional countries. The top five additional destination countries were Cambodia (5.5%), Laos (3.9%), Thailand (3.9%), Bolivia (3.1%), and Indonesia (1.3%).

One-third of students reported respiratory symptoms and two-thirds reported gastrointestinal symptoms during their stay, leading to a low rate of antibiotic consumption. The majority no longer had symptoms on return. Among the female students, ten (3.6%) reported vaginitis symptoms including leucorrhoea, during travel. Two of them took antibiotics for this purpose

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and three (1.1%) were still symptomatic upon their return to France. In addition, 19 (5.0%) students reported symptoms of skin infection and seven (1.8%) took oral antibiotics for these symptoms. Finally, 15 (3.9%) students reported symptoms of other infections during their stays, and ten (2.6%) received antibiotics for these symptoms (Table 1). Overall, 13.6% of students took antibiotics for the purpose of treating an infection during travel and 17.8% took doxycycline as a chemoprophylaxis against malaria.

Microbiological culture results

Before travel, 19 (4.5%) students carried MDR bacteria, compared to 119 (31.2%) after travel. A total of 119 (31.2%) participants acquired at least one MDR bacteria, mostly isolated from rectal (29.6% acquisition rate), and vaginal samples (4.5% acquisition rate), with the highest acquisition rate being for ESBL-E (29.3%) and CPE (2.6%) (Table 2, Figure 1).

We did not detect any acquisition of MRSA, Carbapenem-resistant *A. baumannii*, Cephalosporin-resistant *P. aeruginosa* or GRE.

Acquisition of ESBL-E carriage and characterisation of bacterial isolates

ESBL-E acquisition was found in 112 students including 48/121 (39.7%) who had travelled to South-East Asia, 31/76 (40.7%) who had travelled to South Asia, 26/106 (24.5%) who had travelled to Africa, and 7/67 (10.5%) who had travelled to South America. At the country level in South-East Asia, the acquisition rate was 75% in students who had travelled to Cambodia, and 39.8% in Vietnam. In South Asia, the acquisition rate was 40.8% in India. In Africa, the acquisition rate was 63.8% in Togo, 30.2% in Madagascar, 15% in Senegal and 10% in Tanzania. Finally, in South America, the acquisition rate was 13.7% in Peru (Figure 2). From the 112 participants who acquired an ESBL-E during travel, 127 isolates with an ESBL phenotype

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were recovered and analysed. *E. coli* was the species most commonly found, with 125 (98.4%) isolates from 112 carriers. Two isolates (1.6%) of *K. pneumoniae* were recovered from two (1.8%) carriers (Figure 1).

From these 127 isolates, 221 ESBL-encoding genes could be amplified. $Bla_{CTX\text{-}M\text{-}A}$ was found in 121/127 (95.3%) isolates from 108/112 (96.4%) students who acquired an ESBL-E. Bla_{TEM} was found in 91/127 (71.7%) isolates from 83/112 (74.1%) students. Bla_{SHV} was found in 9/127 (7.1%) isolates from 9/112 (8.0%) students (Table 3). Regarding geographical distribution, the highest $bla_{CTX\text{-}M\text{-}A}$, bla_{TEM} and bla_{SHV} acquisition rates were in South Asia, followed by South East Asia, Africa, and South America (Table 3).

Factors associated with the acquisition of ESBL- E carriage (Table 1)

In a univariate analysis, being younger, taking part in a humanitarian mission in an orphanage, primarily travelling to South-East or South Asia and to Vietnam, contacts with children and animals, use of antibiotics during travel (notably against respiratory symptoms) and travelling in 2017 were significantly associated with higher ESBL-E acquisition rates. Internships in hospitals and notably in the surgical department was associated with a lower risk of ESBL-E acquisition. Travelling to South America in general, and Peru and Tanzania specifically, was associated with a lower risk of ESBL-E acquisition. In multivariate analysis, taking part in a humanitarian mission in an orphanage, being in contact with children, travelling primarily to Vietnam and north India, antibiotic use during travel, and travelling in 2017 were independently associated with the acquisition of ESBL-E.

Acquisition of CPE carriage and characterisation of bacterial isolates

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Overall, 11 isolates from ten carriers (ten isolates from rectal swab samples and one isolate from a vaginal swab sample, all obtained from post-travel samples) exhibited a CPE phenotype and were screened for resistance genes (Figure 1). *Bla*$_{Oxa48}$ and *bla*$_{NDM}$ were found in six (54.5%) and five (45.5%) isolates from six (60%) and five (50%) carriers, respectively. Nine/ten carriers had travelled to India for humanitarian missions in two distinct facilities (Supplementary Table S5). Antibiotic susceptibility testing results showed eleven distinct CPE resistance patterns. One of the rectal swab samples (patient 146) led to the growth of CPE isolates with two different resistance patterns from two distinct species (*E. coli* and *E. cloacae*), as shown in Supplementary Figure 2. All isolates were positive with ß-Carbatest. No isolates were found to be resistant to fosfomycin. The MICs of imipenem and ertapenem ranged from 2 to 32 mg/l and from 1 to 512 mg/l, respectively.

MRSA carriage and characterisation of bacterial isolates

We detected two MRSA isolates from two participants in pre-travel samples only and both were positive for the *mecA* gene by qPCR. No cases of MRSA were detected in any post-travel samples (Figure 1).

MDR-bacteria carriage at follow-up

Of the 119 participants that tested positive for MDR bacteria after travel, 88 samples from four anatomical sites from 79 participants (66.4%) were re-tested six months later and the others participants were lost to follow-up (Figure 1). 19/79 (24.1%) tested positive for at least one MDR bacteria and all were detected in rectal samples. In 17 students (21.5%), the same ESBL-E bacterial species (*E. coli*) was observed both upon return and at six months after return. None of these participants reported a clinical infection or antibiotic use during the six-month follow-up

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period. Antibiotic susceptibility testing and gene resistance PCR results comparing these 17 distinct ESBL-E isolates upon return and six months later are presented in Supplementary Figure 3. Strictly similar patterns in samples obtained upon return and six months later were observed in only two isolates (p040 and p197). In addition, one isolate only differed regarding sensitivity to piperacillin-tazobactam that was absent on return and intermediate six months later (p074). In all the other 14 paired isolates, marked differences in both antibiotic susceptibility and resistance gene patterns were observed. In one student, where ESBL *E. coli* was detected on return, MRSA but no ESBL-E was detected six months later. In one case where *E. coli* resistant to carbapenem was isolated on return, ESBL *E. coli* (sensitive to carbapenem) was detected six months later.

Detection of colistin resistance genes

In total, 78 pools (28 pools of respiratory samples, 28 pools of rectal samples and 22 pools of vaginal samples) from 1,552 pre- and post-travel samples were screened over two years (2018–2019) (554 respiratory samples, 565 rectal samples and 433 vaginal samples). Of the 78 DNA pools tested by qPCR screening, 28 pools (35.9%) were positive for at least one colistin resistance gene, including 33.3% (26/78) for the mcr-1 gene, 1.3% (1/78) for the mcr-3 gene, and 1.3% (1/78) for the *mcr*-8 gene. None of the DNA pools tested positive for the *mcr*-2 (including the *mcr*-6 group), *mcr*-4 and *mcr*-5 genes. By individual retesting, 26 (8.9%) students were positive for at least one colistin resistance gene (two students were positive in both rectal and vaginal samples). None of the nasopharyngeal swab tested positive for any genes.

A total of 24 genes in 22 students could be sequenced out of 28 genes of 28 samples. The sequences obtained were successfully assembled and aligned with reference genes (22 for the *mcr-1* gene, one for the *mcr-3* gene and one for the *mcr-8* gene).

We found a prevalence of 6.8% (20/293) detection of the *mcr*-1 gene. All were detected in post–travel samples. Interestingly, two female students were positive for mcr-1 in both their rectal and vaginal samples in 2019. For the genotypic identification of *mcr*-1, we succeeded in amplifying 22 sequences (of 26 samples, 84.6%). Only one male student (0.3%) was positive for *mcr*-3 by both qPCR and sequencing in a rectal post–travel swab sampled in 2018. Only one female student (0.3%) was positive for *mcr*-8 by both qPCR and sequencing in a rectal post–travel swab sampled in 2019.

Characteristics of students carrying colistin resistance genes

The acquisition of resistance genes occurred mostly in 2018 (81.8%). Of the 22 students carrying colistin resistance genes, 77.3% were female. The mean age was 20.5 years (ranging from 19 to 23). All were in their second year of medical studies and 18/22 (81.8%) had taken part in a non-medical mission consisting of renovating a school and 4/22 (18.2%) took part in supplying medical equipment and providing health advice. The primary destination countries were Vietnam (77.3%) and Peru (22.7%). The acquisition rate was 40% (14/35) in students who had travelled to Vietnam, and 15.4% (4/25) in those who had travelled to Peru, in 2018. Most students (86.4%) had contact with local children and 45.5% had contact with animals. A total of 12/22 (54.6%) students reported at least one respiratory symptom during their stay. Sore throat was the most prevalent symptom (40.9% of all students found to be carriers), followed by rhinitis (36.4%) and a cough (27.3%). A total of 17 (77.3%) reported gastrointestinal symptoms. Abdominal pain and diarrhoea were the most frequent symptoms with prevalence of 59.1% and 50.0%, respectively. Also, of the 18 female students, none reported vaginal symptoms, during their stay. A proportion of 4.6% (1/22) were prescribed malaria chemoprophylaxis with doxycycline. 13.6%, 4.6% and

4.6% received antibiotics for respiratory symptoms, gastrointestinal symptoms, and for vaginal and other symptoms, respectively (data not shown).

Of the 22 students who acquired at least one colistin resistance gene, nine (40.9%) students had acquired at least one MDR bacteria (8 ESBL-E type *E. coli* and 1 CPE type *E. cloacae*). We found no significant correlation between the acquisition of a colistin resistance gene and the acquisition of MDR bacteria (Supplementary Table S6).

Factors associated with the acquisition of colistin resistance genes (Table 1)

The results of univariate analysis presented in Table 1. In a multivariate analysis, resistance gene acquisition remained significantly higher in 2018. When the primary destination of travel was Vietnam, this was independently associated with the acquisition of colistin resistance genes.

Discussion

Our study revealed a 29.3% acquisition rate of ESBL-E (mainly *E. coli*, but also *K. pneumoniae*) in French medical students taking part in an internship abroad. Our results are in line with those obtained in a cohort of Swedish healthcare students on a clinical assignment abroad with a 35% colonisation rate with a new ESBL-E strain during travel [38]. Also, in a study conducted in 205 young, healthy German travellers, a 30.4% ESBL-E carriage rate was found upon return to Germany [9].

The high prevalence of $bla_{CTX\text{-}M\text{-}A}$ gene (95.3%) in ESBL-E acquired by students reflects the worldwide dominance of this ESBL-E type [39], including in Vietnam and India, where we identified a higher risk of acquisition [40, 41]. Similar data were obtained among travellers from

Sweden, with 73% of acquired ESBL-E resulting positive for *bla*$_{CTX-M}$ gene detection [42]. Also, in a cohort of Canadian travellers returning from South Asia, 88% of acquired ESBL *E. coli* resulted positive for *bla*$_{CTX-M}$ gene amplification [43].

A key finding in our study is that 83.3% of the participants had had contact with local children and that contact with children and activities in an orphanage were associated with increased odds of ESBL-E acquisition. This finding is in agreement with the result of a multi-centre cohort study by Arcilla *et al.* [7]. In that study, the authors indicated that one of the strongest predictors of ESBL-E acquisition was contact with orphan children. As human-to-human transmission of ESBL-E has been recorded [44], our observation suggests an acquisition of ESBL-E when in contact with the children in the orphanage through faecal contamination in connection with probably unhygienic conditions in these facilities. However, the data collected are not sufficient to substantiate this hypothesis.

Our results showed that the main factor associated with ESBL–E acquisition was the region visited. In our study, students with a primary destination in Vietnam or north India were 2-2.4 times more at risk of ESBL-E acquisition, reflecting results of other studies [7, 8, 42, 45, 46]. In the medical literature, traveling to South Asia [7, 8] and in particular to India [9, 43, 47] is associated with a significant risk of acquiring ESBL-E, while travelling to Africa, the Middle East, and South and Central America were associated with lower risk. The acquisition of ESBL-E after a trip to Europe in general appears to be rare and could be explained by most studies not including travellers to Southern Europe. However, one study identified travel to Greece as a risk factor [48].

In our study, overall antibiotic use during travel (excluding prophylactic use of doxycycline against malaria) was associated with a higher risk of acquisition of ESBL-E. This finding is in

line with the results of other studies [7, 29, 43, 47] but contrasts with the results of others [8, 9, 42]. Arcilla *et al*. showed that quinolone use during travel was the strongest predictor of ESBL-E acquisition [7]. In our study, the use of beta-lactam during travel led to a 1.5 times higher risk of ESBL-E acquisition but this was not statistically significant. However, in the present study, beta-lactam and quinolone use was very low overall, probably because medical students are well aware of the rational use of antibiotics during travel.

In this study, univariate analyses indicated that students reporting gastrointestinal symptoms and diarrhoea during travel were 1.3 times more at risk of ESBL-E acquisition, but this was not statistically significant. Diarrhoea has been identified as a major risk factor for ESBL-E acquisition in other studies [7, 38, 47, 49]. In our study, the onset of gastro-intestinal symptoms including diarrhoea occurred early during travel, while sampling was performed on return, several weeks later. It could be that the potential acquisition of ESBL-E in connection with diarrhoea was partially cleared at the time of sampling. A cohort study among 132 German and Dutch travellers who provided daily stool samples before, during, and after travel showed that the number of travellers with a temporary colonisation during the journey exceeded the number of travellers still colonised after return [18].

We observed a 21.5% persistence rate of ESBL-E at six months in our study, which corroborates the result of a study that showed 16/63 (25%) travellers were still carrying ESBL-E six months after return travel [11]. By contrast, several recent studies showed lower rates of persistent carriage at six months post-return, ranging 4.7% to 16.7% [4, 7-9]. To date, a limited number of studies on the acquisition of MDR Enterobacteriaceae in travellers have included follow-up sampling at regular time intervals in the months following return. Such studies have shown that the median carriage duration was less than a month after return [7].

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We found a CPE acquisition rate of 2.6% (10/382) with 9/10 cases returning from India. The acquisition rate of CPE in our study was five times higher than that described in a recent study reporting that 0.5% French travellers returning from India were colonised with CPE [12]. In our study, all students who acquired CPE had taken part in a non-medical humanitarian mission consisting of social and cultural activities with orphan children and supplying medical equipment and providing health advice to children. This result suggests a relationship between the acquisition of CPE and contact with local children in India. As the number of students who acquired CPE was low, it was not intended to analyse predictive factors. Of the 11 isolates producing carbapenemase, we detected four *bla*$_{Oxa48}$ *E.coli*, two *bla*$_{Oxa48}$ *E. cloacae* and five *bla*$_{NDM}$ *E.coli*. In a group of 2001 Dutch travellers, CPE were acquired by five travellers returning from Asia (0.25%), but outside the Indian subcontinent, including *E. cloacae bla*$_{IMI-2}$, *E. coli bla*$_{OXA-244}$, *Klebsiella pneumoniae bla*$_{Oxa48}$, *E. coli bla*$_{NDM-1/2}$, and *E. coli bla*$_{NDM-7}$ [10]. In total, of 31 Polish patients returning from South and South-East Asia, CPE acquisition was detected in three patients (9.7%) returning from India with six isolates – five *bla*$_{NDM-1}$ *E. coli* and one *bla*$_{NDM-1}$ *K. pneumoniae* [14].

We observed a 7.5% acquisition rate of colistin-resistance genes following travel. Our result are in line with those obtained in a study conducted on 412 US international travellers with 5% acquiring mcr-mediated colistin-resistant genes after travel [17]. A prevalence of 6.8 % students acquired the *mcr*-1 gene. Our result is in the range of that obtained in a study conducted on 122 Dutch international travellers with a post-travel prevalence of *mcr*-1 (4.9%) in their faecal microbiota [50]. We found no significant association between the acquisition of colistin resistance genes and the acquisition of MDR bacteria in our study. It suggests that the sources of contamination were different. Indeed, environmental bacteria, especially those from water

sources, appears to be the main reservoir and source of *mcr*-like genes [51]. This should be further explored in a future study on colistin-resistant bacteria including environmental sampling in addition to student sampling.

In addition, we report here for the first time the circulation of the *mcr*-8 gene in a rectal sample of a student who had returned from Vietnam. The *mcr*-8 gene has rarely been reported. This gene was identified for the first time in *K. pneumoniae* strains isolated from animals and humans in 2016 in China [36]. The *mcr*-8 gene has been described in China in *Stenotrophomonas* spp., *Raoultella ornithinolytica* and *K. quasipneumoniae* from animals and environmental origins [52-54]. To date, *mcr*-8 genes have been found in samples from human specimens (faeces, urine, and blood) in *K. pneumoniae* isolates from Laos, Algeria, Bangladesh, and Morocco [34, 55-57]. To our knowledge, the circulation of *mcr*-8 gene in Vietnam has not been previously described. Our result suggests that *mcr*-8 gene is in circulation in Vietnam.

We observed that the primary travel destination being Vietnam was associated with a higher risk of acquisition of colistin resistance genes. Yamaguchi *et al.*, revealed a high percentage (36.8%) of colistin-resistant *E. coli* carrying chromosomal *mcr*-1 in the faecal microbiota of a community of Vietnam residents [58]. In this study, we revealed that 40% of students who participated in a school renovation project in Vietnam became carriers of *mcr* genes. Even though the details of transmission mechanisms are not clear, our results indicate that students who stayed for a few weeks in Vietnam came colonised with colistin-resistant bacteria with a prevalence similar to that of Vietnamese residents.

Finally, we observed significant annual variations regarding the acquisition rates of ESBL-E and colistin resistance genes. However, our study was only carried out over two-three years. It is necessary to carry out studies over a longer period of time in the future to clarify this result.

Our study had some limitations including, notably, its monocentric design in a very specific population of travellers, which impairs generalisation of findings. The distinction between the persistence of ESBL-E carriage at six months post-return and recolonisation with a new strain was based on antibiotic resistance patterns and detection of resistance genes. Only genome sequencing could formally make it possible to distinguish between persistent carriage and recolonisation. This is the first study addressing the direct detection of colistin resistance genes in nasopharyngeal, rectal, and vaginal samples among medical students returning from travel abroad. We did not identify the bacteria that housed the antibiotic resistance genes.

In conclusion, the acquisition of MDR bacteria and colistin resistance genes during travel abroad by medical students is very frequent. A number of risk factors have been identified, allowing identifying students at increased risk of ESBL -E acquisition on which targeted preventive measures could be based. Because of a theoretical risk of community and hospital spread, healthcare staff including medical students should apply reinforced standard precautions (hand disinfection with an alcohol based solution) after return from areas with a high prevalence of MDR bacterial carriage. In addition, direct PCR-based screening is a sensitive method to detect mcr genes in international travellers. Medical students returning from electives abroad may contribute to the dissemination of mcr genes.

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Table 1: Characteristics of 382 study participants and risk factors for the acquisition of ESBL-producing *Enterobacteriaceae* (ESBL-E) and colistin resistance genes

		Risk factors for the acquisition of ESBL				**Risk factors for the acquisition of colistin resistance genes**			
Variables	**Total**	**Univariate analysis**			**Multivariate analysis**	**Univariate analysis**			**Multivariate analysis**
	n (%)	No n (%)	Yes n (%)	RR (95%CI)	aRR (95%CI)	No n (%)	Yes n (%)	RR (95%CI)	aRR (95%CI)
Year of study									
2017	89 (23.3)	44 (49.4)	45 (50.6)	4.25**** (2.34-7.71)	1.98**** (1.34-2.93)	NA	NA	NA	NA
2018	134 (35.1)	108 (80.6)	26 (19.4)	Rfr	Rfr	116 (86.6)	18 (13.4)	6.01*** (1.98 -18.24)	5.33 *** (2.01-14.11)
2019	159	118	41	1.44*	1.28*	155	4	Rfr	Rfr

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	(41.6)	(74.2)	(25.8)	(0.83-2.52)	(0.93-1.75)	(97.5)	(2.5)	
Gender								
Female	281 (73.6)	198 (73.3)	83 (74.1)	1.04 (0.63-1.72)		207 (92.4)	17 (7.6)	1.05 (0.37-2.96)
Male	101 (26.4)	72 (26.7)	29 (25.9)	rfr		64 (92.8)	5 (7.3)	Rfr
Age (years)								
Mean	20.8							
Interquartile	20-22	20.9 ± 1.2	20.5 ±1.2	t= 2.83***	-	20.7 ± 1.1	20.5 ± 0.9	t= 0.68
Range	18 - 29							
Mission during travel								
Social and cultural activities in an orphanage	90 (23.6)	71 (24.3)	41 (45.6)	1.87**** (1.38-2.54)	2.01**** (1.38-2.92)	NA	NA	NA

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School renovation	159 (41.4)	69 (30.9)	43 (27.0)	0.87 (0.63-1.21)		4 (2.6)	18 (13.1)	5.74** (1.89-17.44)	-
Supply of medical equipment and health advice	52 (13.6)	94 (28.5)	18 (34.6)	1.22 (0.81-1.83)		18 (7.3)	4 (8.5)	1.18 (0.38-3.65)	
Internship in hospitals	81 (21.2)	102 (33.9)	10 (12.4)	0.36**** (0.2-0.66)	-	NA	NA	NA	
Internship in surgery department	28 (7.3)	110 (31.1)	2 (7.1)	0.23*** (0.06-0.88)	-	NA	NA	NA	
Primary destination of travel									
South-East Asia	121 (31.7)	64 (25.1)	48 (39.7)	1.62** (1.19-2.20)	-	5 (2.4)	17 (21.0)	11.0**** (3.90 -30.98)	-

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Vietnam	98 (25.7)	73 (25.7)	39 (39.8)	1.55*** (1.13-2.12)	2.15**** (1.53-3.02)	5 (2.3)	17 (23.9)	13.67**** (4.83-38.68)	7.58**** (3.21-17.92)
South Asia	76 (19.9)	81 (26.5)	31 (40.8)	1.54*** (1.11-2.14)	-	NA	NA	NA	
South India	46 (12.0)	94 (28.0)	18 (39.1)	1.40* (0.94-2.08)	-	NA	NA	NA	
North India	30 (7.9)	99 (28.1)	13 (43.3)	1.54* (0.99-2.40)	2.41*** (1.41-4.14)	NA	NA	NA	
South America	67 (17.5)	105 (33.3)	7 (10.5)	0.31** (0.15-0.64)	-	17 (7.4)	5 (7.8)	1.05 (0.37-2.98)	
Peru	51 (13.4)	105 (31.7)	7 (13.7)	0.43** (0.21-0.88)	-	17 (6.9)	5 (10.4)	1.56 (0.55-4.45)	
Africa	106	86	26	0.79*	-	22	0	NA	

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	(27.8)	(31.2)	(24.5)	(0.54-1.15)		(10.6)		
Madagascar	43 (11.3)	99 (29.2)	13 (30.2)	1.04 (0.64-1.68)		NA	NA	NA
Tanzania	30 (7.9)	109 (31.0)	3 (10.0)	0.32** (0.11-0.96)	-	NA	NA	NA
Senegal	20 (5.2)	109 (30.1)	3 (15.0)	0.50* (0.17-1.43)	-	NA	NA	NA
Conditions during travel								
Contact with children	318 (83.3)	6 (9.4)	106 (33.3)	3.56*** (1.63-7.74)	1.78*** (1.18-2.69)	3 (5.1)	19 (8.1)	1.65 (0.47-5.78)
Contact with animals	205 (53.7)	37 (20.9)	75 (36.6)	1.75** (1.25-2.46)	-	12 (7.1)	10 (8.0)	1.13 (0.47-2.71)
Type of accommodation, as perceived by students								

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Very clean	75 (19.6)	5 (66.7)	25 (33.3)	Rfr	-	36 (83.7)	7 (16.3)	Rfr	
Clean	158 (41.4)	119 (75.3)	39 (24.7)	0.66* (0.36-1.20)	-	121 (93.8)	8 (6.2)	0.34* (0.12-1.01)	-
Basic	149 (39.0)	101 (67.8)	48 (32.2)	0.95 (0.53-1.72)	-	114 (94.2)	7 (5.8)	0.32 ** (0.10-0.96)	
Duration of travel (days) Mean ± SD	42 ± 11	42.2 ±11.7	41.1± 11	t=0.87		40.9± 11.2	42.4±1 0.7	t= -0.61	
Symptoms of infection during travel									
At least one respiratory symptom	131 (34.3)	75 (29.9)	37 (28.2)	0.95 (0.68-1.32)		10 (5.3)	12 (11.4)	2.30* (0.96-5.51)	-
Cough	71 (18.6)	91 (29.3)	21 (29.6)	1.02 (0.55-1.84)		16 (6.8)	6 (10.2)	1.54 (0.58-4.13)	

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Dyspnoea	22 (5.8)	104 (28.9)	8 (36.4)	1.01 (0.68-1.51)		18 (6.6)	4 (21.1)	3.79** (1.14-12.62)	-
Sore throat	77 (20.2)	91 (29.8)	21 (27.3)	0.91 (0.61-1.37)		13 (5.7)	9 (14.1)	2.72** (1.11-6.69)	-
Voice failure	17 (4.5)	NA	NA	NA		20 (7.2)	2 (13.3)	1.98 (0.42-9.41)	
Rhinitis	104 (27.2)	79 (28.4)	33 (31.7)	1.12 (0.80-1.57)		14 (6.5)	8 (10.1)	1.61 (0.65-4.00)	
Fever	39 (10.2)	96 (28.0)	16 (41.0)	1.47 * (0.97-2.21)	-	21 (7.9)	1 (3.6)	0.43 (0.06-3.33)	
Influenza-like illness	23 (6.0)	104 (29.0)	8 (34.8)	1.2 (0.67-2.15)		18 (6.7)	4 (17.4)	2.95* (0.91-9.59)	-
Persistence of respiratory	31	103	9	0.99		18	4	2.95*	-

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symptoms on return	(8.1)	(29.3)	(29.0)	(0.56-1.76)		(6.7)	(17.4)	(0.91-9.59)	
At least one gastro-intestinal symptom	253 (66.2)	30 (23.3)	82 (32.4)	1.39* (0.97-1.20)	-	5 (4.7)	17 (9.1)	2.05* (0.73-5.73)	-
Diarrhoea	196 (51.3)	47 (25.3)	65 (33.2)	1.31* (0.96-1.80)	-	11 (7.2)	11 (7.8)	1.08 (0.56-2.59)	
Nausea	111 (29.1)	72 (26.6)	40 (36.0)	1.36* (0.98-1.86)	-	17 (7.9)	5 (6.5)	0.81 (0.29-2.28)	
Vomiting	80 (20.9)	83 (27.5)	29 (36.3)	1.32* (0.94-1.86)	-	19 (8.0)	3 (5.6)	0.68 (0.19-2.39)	
Abdominal pain	183 (47.9)	51 (25.6)	61 (33.3)	1.30* (0.95-1.78)	-	9 (5.7)	13 (9.6)	1.74 (0.72-4.20)	
Persistence of gastro-intestinal symptoms on return	32 (8.4)	100 (28.6)	12 (37.5)	1.31 (0.81-2.11)		NA	NA	NA	

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Vaginitis symptoms	10 (3.6)	NA	NA	NA	NA	NA	NA	
Skin infection symptoms	19 (5.0)	NA	NA	NA	NA	NA	NA	
Other symptoms of infection[1]	15 (3.9)	NA	NA	NA	23 (8.2)	1 (7.1)	0.95 (0.12-7.58)	
Drug use								
Antibiotic (ATB) use in the week before departure from France	21 (5.5)	104 (28.8)	8 (38.1)	1.32 (0.74-2.34)	19 (6.8)	3 (20.0)	3.41* (0.89-13.12)	-
Malaria chemoprophylaxis during travel	72 (18.8)	91 (29.4)	21 (29.2)	0.99 (0.67-1.48)	21 (8.7)	1 (1.9)	0.21 (0.03-1.56)	
Doxycycline	68 (17.8)	92 (29.3)	20 (29.4)	0.99 (0.67-1.51)	21 (8.7)	1 (1.9)	0.21 (0.03-1.56)	

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Atovaquone-Proguanil	4 (1.0)	NA	NA	NA		NA	NA	NA	
Overall oral ATB use during travel (excluding malaria chemoprophylaxis)[2]	52 (13.6)	87 (26.4)	25 (48.1)	1.82 (1.3-2.55)**	1.77 (1.15-2.72)***	18 (7.1)	6 (15.8)	2.47* (0.91-6.68)	-
ATB treatment against diarrhea	15 (3.9)	NA	NA	NA		NA	NA	NA	
ATB treatment against respiratory tract infection	21 (5.5)	101 (28.0)	11 (52.4)	1.87 (1.21-2.91)**	-	19 (6.9)	3 (18.8)	3.13* (0.82-11.96)	-
ATB treatment of skin infection	25	107	5	1.23		NA	NA	NA	

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symptoms, vaginitis symptoms and other symptoms of infection	(6.5)	(29.1)	(20.0)	(0.60-2.53)				
Beta-lactam during travel	21 (5.5)	103 (28.5)	9 (42.9)	1.50 (0.89-2.53)*	-	NA	NA	NA
Quinolone during travel	7 (1.8)	NA	NA	NA		NA	NA	NA
Macrolid	6 (1.6)	NA	NA	NA		NA	NA	NA
Fosfomycin	6 (1.6)	NA	NA	NA		NA	NA	NA
Cycline	4 (1.1)	NA	NA	NA		NA	NA	NA
Aminosid	3 (0.8)	NA	NA	NA		NA	NA	NA
Sulfamid	3 (0.8)	NA	NA	NA		NA	NA	NA

Metronidazole	2 (0.5)	NA	NA	NA	NA	NA	NA
Fungicide	1 (0.3)	NA	NA	NA	NA	NA	NA
Antiviral	1 (0.3)	NA	NA	NA	NA	NA	NA

NA: not applicable

[1]Urinary tract infection (9), cervical lymphadenopathy (2), otitis (3) and conjunctivitis (1)

[2]Some individuals used more than one ATB

*p-value ≤0.2; **p-value ≤0.05; ***p-value ≤0.01; ****p-value ≤0.001.

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Table 2: Carriage of multi-drug resistant bacteria

	Before-travel		After-travel		Acquisition[1]		P value[2]
	n	%	n	%	n	%	
Carriage of MDR bacteria							
Yes	19[a]	5.0	119[b]	31.2	119	31.2	< 0.000
Type of resistance							
Extended-spectrum β-lactamase producing *Enterobacteriaceae*	17	4.5	112	29.3	112	29.3	< 0.000
Methicillin resistant *S. aureus*	2	0.5	0	0	0	0	NA
Carbapenemase-producing Enterobacteriaceae	0	0	10	2.6	10	2.6	NA
Anatomical site							
Nasal	0	0	1	0.3	1	0.3	NA
Pharyngeal	2	0.5	1	0.3	1	0.3	NA
Rectal	14	3.7	119[c]	31.2	113	29.6	<0.000
Vaginal[d]	3[e]	1.4	11[f]	4.9	10	4.5	0.02

a, e, f: one student was positive for two samples, b: 16 students were positive for two samples, two students were positive for three samples; c: five students were positive for two samples; d: n = 224 (female students in 2018-2019)

[1]Negative before travel and positive after travel; [2]p-value, McNemar's Test.

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Table 3: Bacterial species and resistance genes in 127 isolates of extended-spectrum β-lactamase producing *Enterobacteriaceae* acquired by students following travel according to regions to which they travelled.

Genes	Number of isolates with ESBL-encoding genes per region and acquisition rates in students (%)				Total	Species (n)
	South East Asia n=54	South Asia n=36	Africa n=29	South America n=8	N=127	
bla$_{CTX-M-A}$	49 (40.5%)	35 (46.1%)	29 (27.4%)	8 (11.9%)	121	*Escherichia coli* (119) *Klebsiella pneumoniae* (2)
bla$_{CTX-M-B}$	0	0	0	0	0	0
bla$_{TEM}$	36 (29.8%)	24 (31.6%)	25 (23.6%)	6 (8.9%)	91	*E. coli* (90) *K. pneumoniae* (1)
bla$_{SHV}$	4 (3.3%)	4 (5.3%)	0	1 (1.5%)	9	*E. coli* (8) *K. pneumoniae* (1)
Total	89	63	54	15	221	*E. coli* (125) *K. pneumoniae* (2)

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Figure 1: Number of participants with positive detection of resistant bacteria pre-travel, post-travel and at six months following return. Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), Carbapenemase-producing *Enterobacteriacae* (CPE). *Escherichia coli* (*E. coli*), *Enterobacter cloacae* (*E. cloacae*), *Klebsiella pneumoniae (K. pneumoniae), Hafnia alvei (H. alvei*

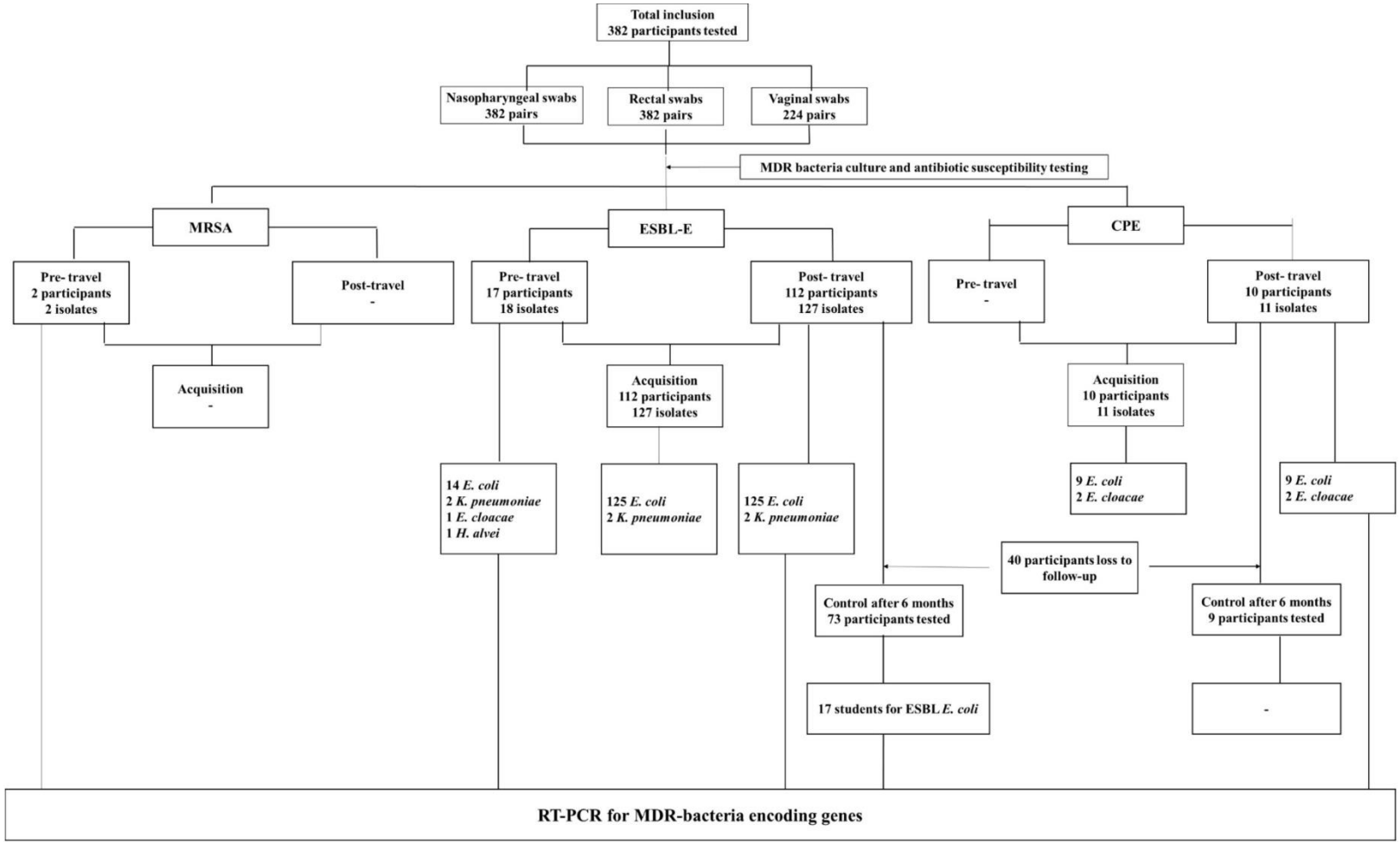

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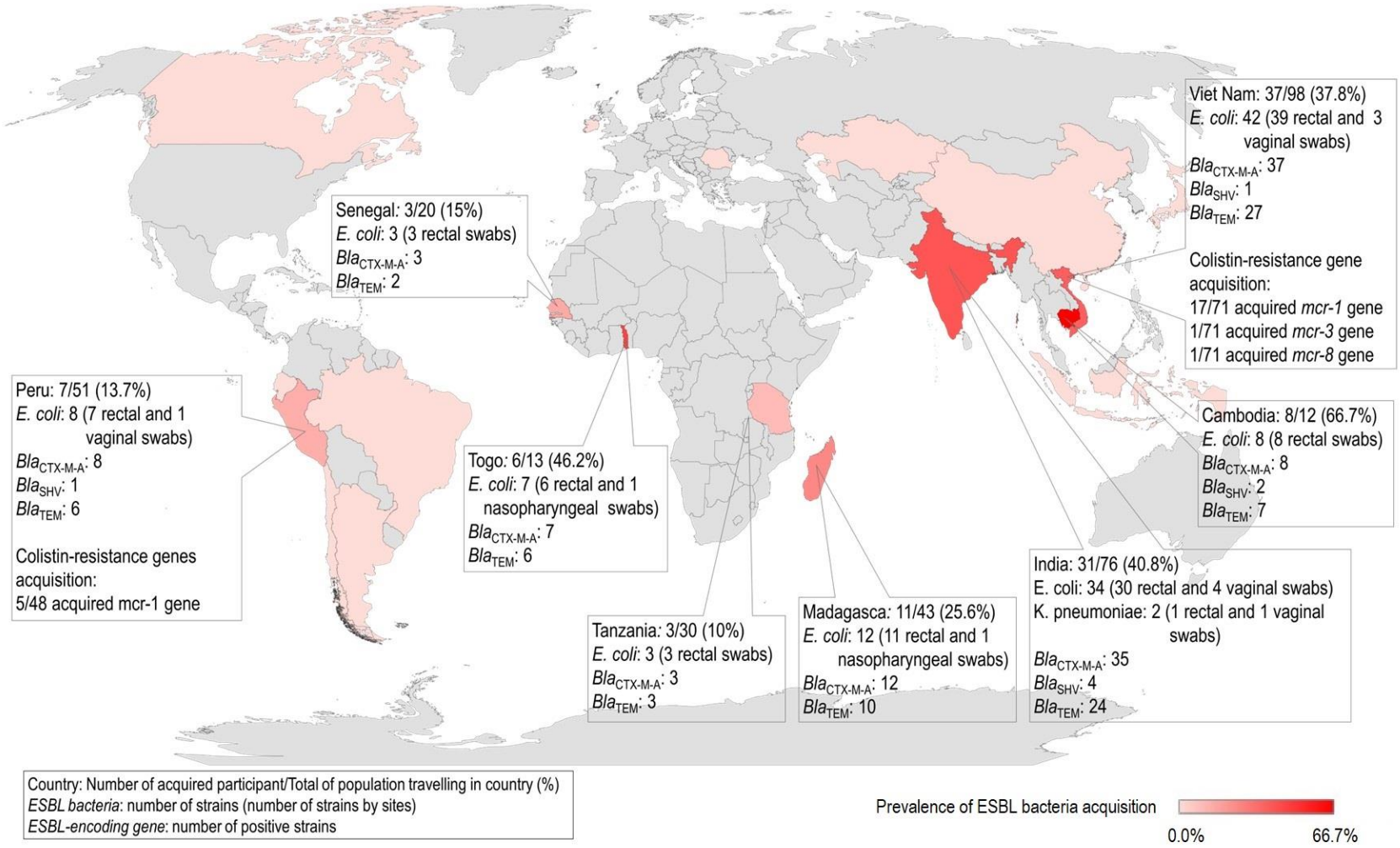
Figure 2: Geographical distribution of 127 isolates of extended-spectrum β-lactamase producing *Enterobacteriaceae,* bacterial species by anatomic site and resistance genes, and geographical distribution of colistin resistance genes acquired by students following travel.



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Supplementary Table S1: Set up of microbiological screening for antimicrobial-resistant bacteria

Medium	Manufacturer/Reference	Objective	Culture conditions	Identification	Further testing
MRSA agar	bioMérieux, Marcy l'Étoile, France	Meticillin-resistant *Staphylococcus aureus*	35±1°C, 18–24 h, ambient air	MALDI-TOF mass-spectrometry	E-test Methicillin
McConkey agar	bioMérieux, Marcy l'Étoile, France	ESBL-producing *Enterobacteriaceae*	35±1°C, 18–24 h, ambient air	MALDI-TOF mass-spectrometry	Double disk diffusion test (I2A, Montpellier, France)
McConkey agar	bioMérieux, Marcy l'Étoile, France	*Acinetobacter baumannii*	35±1°C, 18–24 h, ambient air	MALDI-TOF mass-spectrometry	E-test Imipenem E-test Ceftriaxone
SMART agar	bioMérieux, Marcy l'Étoile, France	Carbapenemase-producing *Enterobacteriaceae*/Gra	35±1°C, 18–24 h, ambient air	MALDI-TOF mass-spectrometry	β-Carbatest (Biorad, Hercules, California) E-test Imipenem

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		m-negative rods			E-test Ertapenem
VRE agar	bioMérieux, Marcy l'Étoile, France	Glycopeptide-resistant *Enterococcus spp*	35±1°C, 24–28 h, ambient air	MALDI-TOF mass-spectrometry	E-test Vancomycin
McConkey agar	bioMérieux, Marcy l'Étoile, France	Cephalosporin-resistant *Pseudomonas aeruginosa*	35±1°C, 24–28 h, ambient air	MALDI-TOF mass-spectrometry	E-test Ceftazidime

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Supplementary Table S2: Sequences of primers and probes used for real-time PCRs and conventional PCRs

Gene	Name	Primers (5'- 3') and probes	Amplicon size (bp)	Reference
Real-time PCRs				
mcr-1	Forward	GCAGCATACTTCTGTGTGGTAC	145	[32]
	Reward	ACAAAGCCGAGATTGTCCGCG		
	Probe	FAM–GACCGCGACCGCCAATCTTACC-TAMRA		
mcr-2	Forward	CTGTGCCGTGTATGTTCAGC	151	[33]
	Reward	TTATCCATCACGCCTTTTGAG		
	Probe	VIC-TGACCGCTTGGGTGTGGGTA-TAMRA		
mcr-3	Forward	TGAATCACTGGGAGCATTAGGGC	144	[33]
	Reward	TGCTGCAAACACGCCATATCAAC		
	Probe	FAM-TGCACCGGATGATCAGACCCGT-TAMRA		
mcr-4	Forward	GCCAACCAATGCTCATACCCAAAA	112	[33]
	Reward	CCGCCCCATTCGTGAAAACATAC		
	Probe	FAM-GCCACGGCGGTGTCTCTACCC-TAMR		
mcr-5	Forward	TATCCCGCAAGCTACCGACGC	126	[33]
	Reward	ACGGGCAAGCACATGATCGGT		
	Probe	FAM-TGCGACACCACCGATCTGGCCA-TAMRA		
mcr-8	Forward	TCCGGGATGCGTGACGTTGC	158	[34]
	Reward	TGCTGCGCGAATGAAGACGA		
	Probe	FAM-TCATGGAGAATCGCTGGGGGAAAGC-TAMRA		
Conventional PCRs				
mcr-1	Forward	GCAGCATACTTCTGTGTGGTAC	554	[32]
	Reward	TATGCACGCGAAAGAAACTGGC		
mcr-3	Forward	AAATAAAAATTGTTCCGCTTATG	929	[35]
	Reward	AATGGAGATCCCCGTTTTT		

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mcr-8	Forward	CCCAAGCTTTTGATTGTCCCTGTCGCCAT	2631	[36]
	Reward	CACCGATAAGAGGAACCAGTGAATTCCGG		

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Supplementary Table S3: Missions during electives.

	N=382	%
Humanitarian missions	301	78.8
Social and cultural activities in an orphanage	90	23.6
School renovation	159	41.4
Supply of medical equipment and health advice to children	52	13.6
Internship in hospitals[1]	81	21.2
Surgery	28	7.3
Infectious diseases	17	4.5
Cardiology	16	4.1
Urology	13	3.4
Gynaecology	10	2.6
Paediatrics	10	2.6
Dermatology	6	1.6
Gastro-enterology	6	1.6
Pneumology	6	1.6
Emergency	5	1.3
Radiology	4	1.0

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Oncology	3	0.9
Internal medicine	2	0.5
Orthopaedics	2	0.5
Psychiatry	1	0.5

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Supplementary Table S4: Missions during travel according to primary destination country (where the internship took place).

	Mission				
	Social and cultural activities in an orphanage n (%)	School renovation n (%)	Supply of medical equipment and health advice to children n (%)	Treating patients in hospitals n (%)	Total n (%)
South East Asia					121 (31.7)
Vietnam	0	76 (19.9)	9 (2.4)	13 (3.4)	98 (25.7)
Cambodia	8 (2.1)	0	0	4 (1.0)	12 (3.1)
Indonesia	0	0	0	11 (2.9)	11 (2.9)
South Asia					76 (19.9)
South India	47 (12.3)	0	0	0	47 (12.3)
North India	0	0	28 (7.3)	1 (0.3)	29 (7.6)
Other Asia					6 (1.6)
Japan	0	0	0	3 (0.8)	3 (0.8)
Taiwan	0	0	0	1(0.3)	1 (0.3)
China	0	0	0	1(0.3)	1 (0.3)

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Kazakhstan	0	0	0	1(0.3)	1 (0.3)
Africa					106 (27.7)
Madagascar	22 (5.8)	13 (3.4)	2 (0.5)	6 (1.6)	43 (11.3)
Tanzania	0	30 (7.9)	0	0	30 (7.9)
Senegal	0	6 (1.6)	0	14 (3.7)	20 (5.2)
Togo	13 (3.4)	0	0	0	13 (3.4)
South America					67 (17.5)
Peru	0	34 (8.9)	13 (3.4)	4 (1.0)	51 (13.4)
Brazil	0	0	0	11 (2.9)	11 (2.9)
Argentina	0	0	0	3 (0.8)	3 (0.8)
Chile	0	0	0	1(0.3)	1 (0.3)
Ecuador	0	0	0	1(0.3)	1 (0.3)
Europe					5 (1.3)
Romania	0	0	0	3 (0.8)	3 (0.8)
Republic of Ireland	0	0	0	2 (0.5)	2 (0.5)
North America				1 (0.3)	1 (0.3)
Canada	0	0	0	1 (0.3)	1 (0.3)

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Supplementary Table S5: Characteristics of individuals carrying carbapenemase-producing *Enterobacteriaceae* and corresponding resistance genes

Student	Age	Gender	Primary travel destination	Mission	Anatomical site	Species	Resistance gene
P028	21	F	Vietnam	School renovation	Vaginal	*Enterobacter cloacae*	bla_{Oxa48}
P077	21	F	South India	Humanitarian mission in an orphanage	Rectal	*Escherichia coli*	bla_{NDM}
P119	21	H	South India		Rectal	*E. coli*	bla_{NDM}
P121	19	F	South India		Rectal	*E. coli*	bla_{Oxa48}
P138	18	F	South India		Rectal	*E. coli*	bla_{NDM}
P013	19	H	North India	Supply of medical equipment and health advice	Rectal	*E. coli*	bla_{NDM}
P051	19	F	North India		Rectal	*E. coli*	bla_{Oxa48}
P146	21	F	North India		Rectal	*E. cloacae*	bla_{Oxa48}
P146	21	F	North India		Rectal	*E. coli*	bla_{NDM}
P147	20	H	North India		Rectal	*E. coli*	bla_{Oxa48}
P151	20	F	North India		Rectal	*E. coli*	bla_{Oxa48}

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Supplementary Table S6: Correlation between the acquisition of colistin resistance genes and the acquisition of MDR bacteria

		Acquisition of colistin resistance genes n (%)	OR [95%CI]	p*
Acquisition of at least one MDR bacteria	Yes	9 (12.3)	2.24 [0.92-5.48]	0.07
	No	13 (5.9)		
Acquisition of an ESBL-E	Yes	8 (11.9)	2.05 [0.82-5.13]	0.12
	No	14 (6.2)		
Acquisition of a CPE	Yes	1 (11.1)	1.56 [0.19-13.12]	0.68
	No	21 (7.4)		

p* Pearson's chi-square

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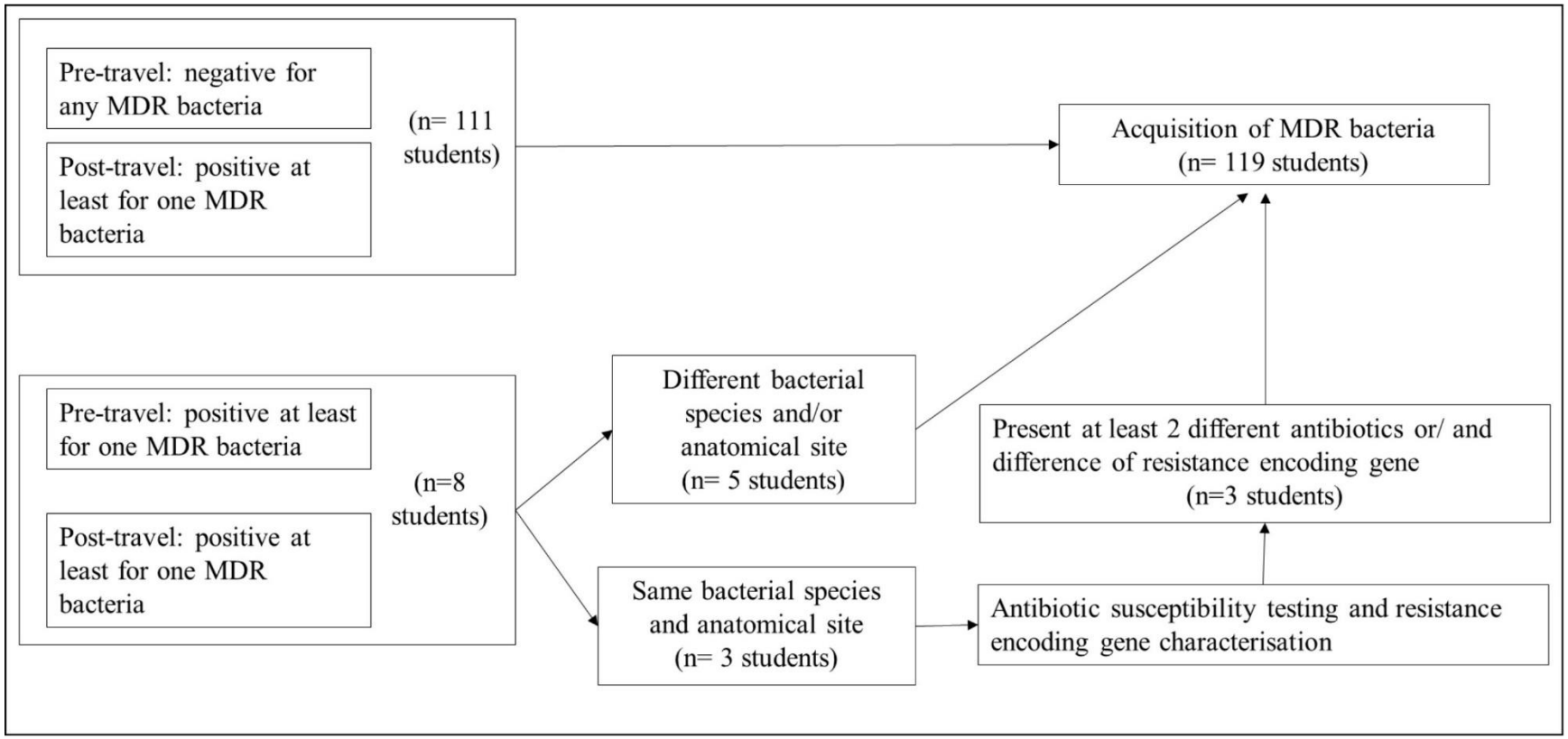

Supplementary Figure 1: Algorithm used to assess the acquisition of MDR bacteria among medical student abroad

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Student	Anatomical site	Species	AMX	AMC	TZP	FEP	CRO	FOS	IPM	EPM	AMK	GEM	DOX	STX	CIP	Resistance gene	IPM CMI (mg/L)	EPM CMI (mg/L)
P028	Vaginal	*Enterobacter cloacae*														bla_{Oxa48}	8	8
P146	Rectal	*E. cloacae*														bla_{Oxa48}	4	4
P013	Rectal	*Escherichia coli*														bla_{NDM}	16	512
P051	Rectal	*E. coli*														bla_{Oxa48}	32	3
P077	Rectal	*E. coli*														bla_{NDM}	32	32
P119	Rectal	*E. coli*														bla_{NDM}	16	512
P121	Rectal	*E. coli*														bla_{Oxa48}	3	1
P138	Rectal	*E. coli*														bla_{NDM}	32	512
P146	Rectal	*E. coli*														bla_{NDM}	32	512
P147	Rectal	*E. coli*														bla_{Oxa48}	2	4
P151	Rectal	*E. coli*														bla_{Oxa48}	4	8

Supplementary Figure 2: Antibiotic resistance patterns of 11 Carbapenemase-producing *enterobacteriaceae* isolates recovered from vaginal and rectal swab return-samples from 10 medical students. Red squares, resistance; green squares, susceptibility; orange squares, intermediate. The minimal inhibitory concentration values of imipenem and ertapenem are provided. AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; FEP, cefepime; CRO, ceftriaxone; FOS, fosfomycin; IPM, imipenem; EPM, ertapenem; AMK amikacine; GEN, gentamicin; DOX, doxycycline; SXT, sulfamethoxazole - trimethoprim; CIP, ciprofloxacin.

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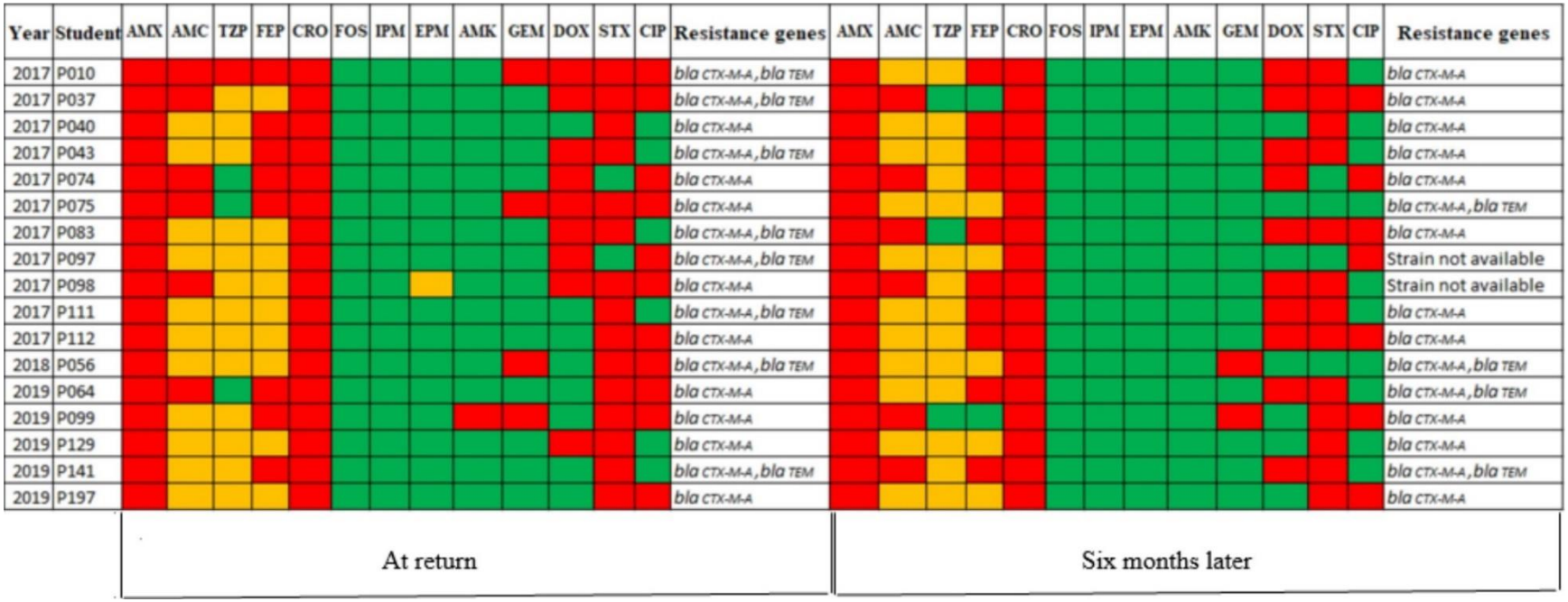

Supplementary Figure 3: Antibiotic resistance patterns of 17 ESBL-E *Escherichia coli* isolates recovered from rectal swab return-samples and 17 ESBL-E *E. coli* isolates recovered from rectal swabs six months later from 17 medical students. Red squares, resistance; green squares, susceptibility; orange squares, intermediate. AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; FEP, cefepime; CRO, ceftriaxone; FOS, fosfomycin; IPM, imipenem; EPM, ertapenem; AMK amikacine; GEN, gentamicin; DOX, doxycycline; SXT, sulfamethoxazole - trimethoprim; CIP, ciprofloxacin.

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