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► To cite this version:

Naouale Maataoui, Aurélie Mayet, Sandrine Duron, Hervé Delacour, France Mentré, et al.. High acquisition rate of extended-spectrum β -lactamase-producing Enterobacteriaceae among French military personnel on mission abroad, without evidence of inter-individual transmission. *Clinical Microbiology and Infection*, 2018, 25 (5), pp.631.e1-631.e9. 10.1016/j.cmi.2018.07.030 . inserm-02093954

HAL Id: inserm-02093954

<https://inserm.hal.science/inserm-02093954>

Submitted on 22 Oct 2021

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1 **Original article**

2 **High Acquisition Rate of ESBL-Producing Enterobacteriaceae among French Military**
3 **Personnel on Mission Abroad, without Evidence of Inter-Individual Transmission**

4

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28

29 **ABSTRACT**

30 **Objectives.** Acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-
31 E) by Europeans traveling individually in high-endemicity countries is common. However, how the
32 different ESBL-E strains circulate in groups of travelers has not been studied. We investigated
33 ESBL-E transmission within several groups of French military personnel serving overseas for 4-6
34 months.

35 **Methods.** We conducted a prospective study among French military personnel assigned to
36 Afghanistan, French Guiana, or Côte d'Ivoire for 4 to 6 months. Fecal samples provided by
37 volunteers before leaving and after returning were screened for ESBL-E *Escherichia coli* isolates.
38 ESBL-*E. coli* from each military group was characterized by repetitive element palindromic
39 polymerase chain reaction (rep-PCR) fingerprinting followed, in the Afghanistan group, by whole-
40 genome sequencing (WGS) if similarity was $\geq 97\%$.

41 **Results.** Among the 189 volunteers whose samples were negative before departure, 72 (38%) were
42 positive after return. The highest acquisition rates were observed in the Afghanistan (29/33, 88%)
43 and Côte d'Ivoire (39/80, 49%) groups. Acquisition rates on return from French Guiana were much
44 lower (4/76, 5%). WGS of the 20 strains from the Afghanistan group that clustered by rep-PCR
45 identified differences in sequence type, serotype, resistance genes, and plasmid replicons. Moreover,
46 single-nucleotide polymorphism (SNP) differences across acquired strains from a given cluster
47 ranged from 30 to 3641, suggesting absence of direct transmission.

48 **Conclusions.** ESBL-*E. coli* acquisition was common among military personnel posted overseas.
49 Many strains clustered by rep-PCR but differed by WGS and SNP analysis, suggesting acquisition
50 from common external sources rather than direct person-to-person transmission.

51

52 INTRODUCTION

53 The dissemination of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-
54 E) within the community is of great concern, notably in middle- and low-income countries, many of
55 which are in tropical regions. Europeans traveling to the tropics often acquire ESBL-E, returning
56 home as intestinal carriers [1–3]. Intestinal colonization is asymptomatic but associated with a risk of
57 subsequent infection, particularly when the bacterial load is high [4].

58 ESBL-E acquisition rates depend heavily on the geographic area of travel. Rates of up to 80%
59 have been reported after stays on the Indian subcontinent [5–7]. Risk factors for acquisition have
60 been studied extensively in individual civilian travelers [1,6–10]. Studies also reported ESBL-E
61 carriage by 13% to 57% of soldiers repatriated after injury [11,12]. However, data are meager on
62 ESBL-E carriage in healthy soldiers [13], and acquisition rates upon return from deployment
63 overseas are unknown. Moreover, all studies in healthy travelers investigated the acquisition rate in
64 each individual. Acquisition dynamics in people traveling in groups have never been studied.
65 Interindividual transmission has been demonstrated among household members [14–16], but whether
66 the same applies to a group of travelers living in close contact is unknown. During deployment in
67 endemic areas, soldiers are at risk both of acquiring ESBL-E from external sources and of
68 interindividual transmission within the group, whose members live in close contact and share many
69 facilities.

70 The objective of this study was to assess the ESBL-E acquisition rate among French military
71 personnel serving in three tropical regions (Afghanistan, French Guiana, and Côte d’Ivoire) for 4-6
72 months and to develop hypotheses about acquisition routes based on the results of PCR-based and
73 next-generation sequencing (NGS) methods to assess strain similarity.

74 **METHODS**

75

76 The study was registered on ClinicalTrials.gov (NCT01591538), approved by the
77 ethics committee of the Saint-Louis University Hospital in Paris, and by the Agence
78 Nationale de Sécurité du Médicament, France (ID RCB n°2011-A01569-32, 2011). The
79 participants volunteered for this cohort study and provided written informed consent before
80 study inclusion.

81

82 **Study design and setting**

83 Participation in this prospective study was proposed to all 120-150 members of each of three
84 French military units. Between March and December 2012, these units were assigned to group
85 missions in Afghanistan, French Guiana, and Côte d'Ivoire. Inclusion criteria were age ≥ 18 years;
86 having given informed consent; being able to provide a fecal sample; being an army service member
87 deployed overseas, and supported by the French military health service; and not participating in
88 another study during the same period.

89 Each participant completed a questionnaire before and after the mission and provided a fecal
90 sample within 10 days before leaving and within 2 days after returning (Figures S1 and S2).

91

92 **Microbiological methods**

93 Fecal samples were kept at 4°C and studied within 48 h after arrival at the laboratory.

94 *ESBL-E screening*

95 ESBL-E were detected using ChromID® ESBL (BioMérieux, Marcy l'Etoile, France). All
96 distinct colony morphotypes were identified by mass spectrometry (Biotyper, Bruker Daltonics,
97 Bremen, Germany) and tested for antibiotic susceptibility (amoxicillin, amoxicillin-clavulanate,
98 piperacillin-tazobactam, ceftazidim, cefotaxim, cefepime, imipenem, gentamicin, amikacin, nalidixic
99 acid, levofloxacin, ciprofloxacin, fosfomycin and cotrimoxazole) using the disc diffusion method, as
100 recommended by EUCAST (www.eucast.org). All ESBL-E were stored at -80°C until further testing.

101 *PCR typing*

102 Genomic DNA was extracted using the UltraClean[®] Microbial DNA Isolation Kit (Mo Bio
103 Laboratories, Carlsbad, CA, USA). The ESBL genes, including *bla*_{CTX-M1 group}, *bla*_{CTX-M9 group}, *bla*_{TEM},
104 and *bla*_{SHV}, were screened by PCR and sequenced as described elsewhere [17]. Phylogenetic groups
105 were identified by multiplex PCR as described previously [18]. Genetic relatedness was assessed by
106 repetitive extragenic palindromic polymerase chain reaction (rep-PCR) using the DiversiLab system
107 (BioMerieux, Marcy l'Etoile, France) [19]. Strains with $\geq 97\%$ similarity were considered
108 indistinguishable [20].

109 *Whole-genome sequencing (WGS)*

110 DNA libraries were prepared using the Illumina Nextera Kit (Illumina, Little Chesterford,
111 UK). Pooled libraries were sequenced on the MiSeq benchtop sequencer (Illumina[®]). De-novo
112 assemblies were created with a pipeline provided within the Sequana project
113 (<http://github.com/sequana/sequana>), which used SPAdes software [21]. The SPAdes assembler was
114 tuned to minimize assembly error (using the careful option) and contigs <500 bp were removed. The
115 quality of the de-novo assemblies was estimated using standard metrics (e.g., N50) computed using
116 QUAST software [22]. Reads used to construct the assemblies were remapped against the assembly
117 contigs to visualize coverage and detect assembly errors.

118 Parsnp software was used to construct a maximum likelihood phylogenetic tree, as described
119 previously [23]. The Center for Genomic Epidemiology website was consulted to identify
120 antimicrobial resistance genes, plasmid replicon types *in silico*, serotypes, and sequence type (ST)
121 profiles (<http://www.genomicepidemiology.org>).

122 Single-nucleotide polymorphisms (SNPs) were detected with a second pipeline (variant
123 calling) available in the Sequana project. This pipeline was designed according to the best practices
124 recommended by the Broad Institute [24]. Insertions/deletions (indels) were realigned using GATK
125 Indel Realigner software, duplicated reads were ignored, and aligned reads with a mapping quality
126 score <30 were removed (BWA score) [25]. Variant calling was performed with Freebayes using
127 non-default options except for the ploidy option, which was set at 1 [26]. All variants were filtered to

128 keep only those that had a Freebayes score >100, a frequency of 80%, a minimal depth of 10, and a
129 minimal ratio between the forward and reverse strand of 0.2.

130

131 **Statistical analysis**

132 We calculated the ESBL-E acquisition rates in participants whose fecal samples were negative
133 before the mission. Differences in acquisition rates across the three geographic groups were assessed
134 using the McNemar test. Univariate and multivariate analyses were performed to estimate factors
135 associated with ESBL-E acquisition. The study variables were age, sex, previous travel outside
136 continental France, prior antibiotic use, antibiotic use during the mission, malaria prophylaxis,
137 intestinal symptoms during the mission, hospitalization during the mission, contact with hospitalized
138 patients during the mission, country of deployment, and living conditions (military base or bivouac).
139 Variables associated with *P* values <0.2 by univariate analysis were included in a multivariate
140 logistic regression model with ESBL-E acquisition as the outcome. The covariates included in the
141 model were malaria prophylaxis during the mission (yes/no), area of deployment (Afghanistan, Côte
142 d'Ivoire, or French Guiana), and living conditions (military base and bivouac as two dichotomous
143 variables). *P* values ≤ 0.05 were considered significant for all analyses.

144 RESULTS

145

146 Study population

147 Overall, 272/420 (64.8%) of the departing soldiers volunteered to participate. Among them, 71
148 (26%) were excluded from the analysis because they did not provide fecal samples collected at the
149 required times (n=39) or were lost to follow-up (n=32). This left 201 (47.9%) soldiers for the
150 analysis, among whom 37 went to Afghanistan, 80 to French Guiana, and 84 to Côte d'Ivoire (Figure
151 1). Mean age was 25.4±5.1 years (range, 18-45 years), 97% were male, and mean overseas length of
152 stay was 5.3±1.1 months (range, 3.9-6.7 months). Soldiers in the Afghanistan group more often took
153 antibiotics during the mission, more often experienced intestinal symptoms, and less often took
154 malaria prophylaxis than those in the other two groups. In French Guiana, soldiers were younger and
155 more often lived in bivouacs (Table S1). The food and beverages ingested by soldiers from each
156 location are summarized in Table S2.

157

158 ESBL-E acquisition

159 Of the 201 participants, 12 (6%) had pre-departure fecal samples positive for ESBL-E,
160 including 7 who remained positive and 5 who were negative upon return. Of the 189 participants who
161 were negative before departure, 72 (38%) were positive upon return. Acquisition rates ranged from
162 5% (4/76; 95% confidence interval [95% CI], 2%-13%) in French Guiana, to 49% (39/80; 95% CI,
163 39%-62%) in Côte d'Ivoire, and 88% (29/33; 95% CI, 32%-46%) in Afghanistan ($P<0.0001$).

164

165 Factors associated with ESBL-E acquisition

166 Among the factors identified by univariate analysis (malaria prophylaxis, contact with
167 hospitalized patients, living conditions (military base or bivouac), and location of the mission
168 (Afghanistan or Côte d'Ivoire), only location of the mission remained significantly associated with
169 ESBL-E acquisition by multivariate analysis (Table 1); compared to French Guiana, serving in
170 Afghanistan or Côte d'Ivoire was significantly associated with acquisition. Odds ratios were 75.3

171 (95% CI, 13.0-434.5; $P<0.001$) for Afghanistan and 11.8 (95% CI, 2.78-50.4; $P=0.001$) for Côte
172 d'Ivoire. Multivariate analysis performed within each group showed that factors independently
173 associated with a lower acquisition rate were living in a bivouac in the Afghanistan group and older
174 age in the Côte d'Ivoire group (Tables S3 to S5).

175

176 **Microbiological results**

177 A total of 117 ESBL-E isolates (12 from pre and 105 from post-mission samples) were
178 identified in 84 volunteers (mean 1.4 isolates/volunteer). Among these, 52, 8, and 57 were isolated
179 from the Afghanistan, French Guiana, and Côte d'Ivoire groups, respectively. *E. coli* contributed
180 92% (108/117) of the isolates and *K. pneumoniae* 8% (9/117). Among ESBLs, CTX-M group 1
181 dominated, with 103 (88%) isolates (CTX-M-15, $n=96$, 82%; and CTX-M-1, $n=7$, 6%). CTX-M
182 group 9 was found in only 13 (11%) isolates (CTX-M-14, $n=6$, 5%; CTX-M-27, $n=5$, 4%; and CTX-
183 M-65, $n=2$, 2%) (Figures 2 and 3). SHV-42 was found in a single isolate. No difference was observed
184 in CTX-M distribution among isolates from Afghanistan and Côte d'Ivoire. No carbapenemase-
185 producing isolate was identified.

186 *E. coli* phylogroups were unevenly distributed, with no differences between the Afghanistan
187 and Côte d'Ivoire groups. Phylogroup A dominated (43%) over phylogroups D (17%), B1 (15%), B2
188 (11%), F (10%), E (3%), and C (1%). Resistance rates were 77% (90/117) for nalidixic acid, 48%
189 (56/117) for levofloxacin, 51% (60/117) for ciprofloxacin, 26% (30/117) for gentamicin, 11%
190 (13/117) for amikacin, 5% (4/117) for fosfomycin, and 85% (100/117) for cotrimoxazole.

191

192 **Rep-PCR and clonal relatedness**

193 Rep-PCR was performed on 48, 7, and 53 ESBL-producing *E. coli* isolates recovered before or
194 after deployment in Afghanistan, French Guiana, and Côte d'Ivoire, respectively.

195 In the Afghanistan group, rep-PCR showed that 28/48 (58%) of the isolates (2 before and 26
196 after the mission) were singletons. The remaining 20 (42%) isolates clustered in pairs (clusters I, III,
197 V, VI, and VII) or in groups of three (cluster IV), or seven (cluster II) (Figure 2). In all, of the 33

198 participants with positive samples, 16 (48%) carried strains that appeared similar by rep-PCR. In no
199 participants, including the 4 with positive samples at both time points, did a pre-mission strain cluster
200 with a post-mission strain.

201 In the French Guiana group, all 7 isolates (4 pre- and 3 post-mission) were singletons.

202 In the Côte d'Ivoire group, 24/53 (45%) isolates (2 before and 22 after the mission) were
203 singletons and 29/53 (55%) clustered in pairs (clusters I, II, III, V, VII, and IX) or in groups of three
204 (cluster IV), four (cluster X), or five (clusters VI and VIII) (Figure 3). In all, of the 43 participants
205 with positive samples, 24 (56%) carried strains that appeared similar by rep-PCR. In no participants,
206 including the 3 with positive samples at both time points, did a pre-mission strain cluster with a post-
207 mission strain.

208 WGS was performed on the 20 ESBL-producing *E. coli* isolates from the Afghanistan group
209 that had $\geq 97\%$ similarity by rep-PCR (Figure 4). This unit was chosen because it showed the highest
210 acquisition rate and because operational insecurity in Afghanistan caused the soldiers to live in more
211 confined conditions, involving closer contacts, compared to those deployed in Côte d'Ivoire. Isolates
212 belonging to the same rep-PCR cluster grouped together. However, within each rep-PCR cluster,
213 WGS consistently evidenced between-strain differences of various magnitudes. The largest
214 differences were observed within the rep-PCR cluster IV, in which the strains had different serotypes
215 and sequence types (STs) and hosted different resistance genes and plasmid replicons. In cluster VI,
216 all isolates had the same STs, but differences were found for the serotypes and resistance genes or
217 plasmid replicons. In clusters I, II (with all ST131 strains), and VII, resistance genes and plasmid
218 replicons were different. In cluster III, only the resistance genes, and in cluster V only the replicons,
219 were different. Of note, all ST131 strains in cluster II had the O25:H4 serotype and carried *fimH30*,
220 although these strains belonged to two different clades, C1 (H30-R) and C2 (H30-Rx).

221 SNPs were identified by comparing the core genome of isolates from each cluster. The
222 smallest number of SNPs was 3, between the U1-100-Dep and U1-104-Dep strains in cluster VII.
223 Both were pre-mission strains that were not found in post-mission samples from any other members

224 of the Afghanistan group. By contrast, numbers of SNPs between acquired strains belonging to the
225 same rep-PCR cluster ranged from 30 (cluster III) to 3641 (cluster IV) (Figure 4).

226 **DISCUSSION**

227 Our first important result is that ESBL-E acquisition by French military personnel serving in
228 Afghanistan, French Guiana, and Côte d'Ivoire was common but that acquisition rates varied widely,
229 with the highest being found in Afghanistan (88%).

230 Because of the absence of tourism in Afghanistan, no data are available on ESBL-E
231 prevalence and acquisition in healthy people returning from this country. However, the ESBL-E
232 acquisition rate in our Afghanistan group is consistent with the rates reported in travelers to other
233 countries of the same region, such as India, where rates can reach 85% [7]. Similarly, the 49%
234 acquisition rate observed in soldiers from Côte d'Ivoire is in agreement with those found in travelers
235 to sub-Saharan Africa [7,8]. Persistence of carriage after returning was not studied here but has been
236 reported to be fairly short-lived. Thus, 3 months after returning, ESBL-E colonization rates in healthy
237 travelers ranged from 5% to 8% [7,8]. The mission in French Guiana resulted in a low 5% ESBL-E
238 acquisition rate, in keeping with previous data in French travelers to this district [7]. French Guiana
239 has unusual characteristics for the region, being a French department with European standards of
240 medical care and economic welfare.

241 As reported previously in travelers, CTX-M-15 was the most frequently isolated enzyme
242 (82%), confirming its predominant role in the current CTX-M enzyme pandemic [3,27].

243 In contrast to other studies in travelers, neither antibiotic use during the mission nor gastrointestinal
244 symptoms were associated with an increased ESBL-E acquisition rate [7,28]. Our results confirm that
245 doxycycline used for malaria prophylaxis in French military personnel was not associated with
246 increased ESBL-E acquisition rates [27].

247 Our second most important finding is that WGS and SNP analysis showed no evidence of
248 direct person-to-person transmission. Therefore, the most likely hypothesis is acquisition from
249 external sources. WGS was performed on strains from soldiers deployed in Afghanistan, because
250 they had the highest acquisition rate and lived in closer conditions than those deployed in Côte
251 d'Ivoire, due to the greater operational risks. We found that isolates that clustered by rep-PCR had
252 different STs, serotypes, resistance genes, and/or plasmid replicons after WGS. Moreover, SNP

253 analysis showed that acquired isolates from the same rep-PCR cluster differed by 30 to 3641 SNPs,
254 suggesting absence of direct person-to-person transmission. Indeed, when 14 strains from a single *E.*
255 *coli* clone that persisted for 3 years within a household were sequenced, a maximal difference of 20
256 SNPs was observed and the mutation rate was estimated at 1.1 per genome per year [15]. In another
257 study, during a common-source outbreak that disseminated widely in Germany and France, *E. coli*
258 O104:H4 isolated in 7 individuals differed by a maximum of 19 SNPs [29]. The absence of cross-
259 transmission during the missions was also suggested by the fact that none of the pre-departure
260 isolates were detected in any study participant after the mission.

261 As ESBL strains were not acquired through direct interindividual transmission, the most
262 probable hypothesis is acquisition from external sources. Studies in travelers also support acquisition
263 from the environment, food, and beverages [7,8]. In Afghanistan, the soldiers lived in closed spaces
264 with good hygiene standards, making acquisition from environmental sources unlikely. Acquisition
265 therefore probably occurred from food and/or beverages. Moreover, the multivariate analysis in the
266 Afghanistan group showed a lower risk of ESBL-E acquisition in soldiers living in bivouacs
267 compared to those living in military bases. One of the main differences between these living
268 conditions was the source of food. In bivouacs, soldiers chiefly ate field ration packs, whereas on
269 many military bases they were also able to purchase food outside. The officers informed us that
270 soldiers often bought meals from the many street vendors posted around the military infrastructure
271 (personal communication). Food from street vendors has been shown elsewhere to be a major source
272 of ESBL-producing strains [30]. However, we could not confirm this hypothesis since the food sold
273 by the street vendors was not sampled during the study.

274 Our study has several limitations. First, no samples were obtained during the mission abroad.
275 Therefore, we cannot exclude that some participants acquired ESBL-E during the mission then lost
276 the organism before returning to France. Second, our cohort is not representative of the general
277 population since it was composed almost exclusively of men. Third, not all soldiers were sampled,
278 and only ESBL-E isolates from the Afghanistan group were compared by WGS, so that further
279 studies are needed to assess whether our findings apply to other regions. Last, we focused our work

280 on strain circulation and, therefore, compared only strains, so that we cannot rule out ESBL enzyme
281 transmission by plasmid transfer.

282

283 In conclusion, acquisition rates of intestinal ESBL-E were high in French military personnel
284 serving for several months in Afghanistan and Africa. In the group with the highest acquisition rate,
285 rep-PCR and WGS results argued against direct person-to-person transmission. The most likely
286 hypothesis was acquisition from common external sources. Control of ESBL-E exposure during
287 deployment is currently based on the same guidelines used to prevent diarrhea under poor sanitary
288 conditions (hand and personal hygiene, safe food and water supply, surface biocleaning) combined
289 with appropriate antibiotic use. Improved understanding of the routes of ESBL-E dissemination from
290 the local environment, including the food supply, is essential to design effective preventive strategies.

291

292 **Transparency declaration**

293 A D is employee of Da Volterra. A A is scientific advisor for Da Volterra. All other authors report no
294 potential conflicts.

295

296 **Acknowledgments**

297 The results of this study have been reported in part at the European Congress of Clinical
298 Microbiology and Infectious Diseases (ECCMID 2017, Vienna, Austria).

299

300 **Funding**

301 The clinical trial was sponsored and promoted by Da Volterra, represented by Dr Annie Ducher,
302 Chief Medical Officer (regulatory procedures, printing of CRFs, sampling equipment and reagents,
303 database management, initial analyses). The French military health service (public funds) was the
304 investigator (data collection, microbiological analyses and complementary statistical analyses).
305 INSERM UMR 1137, Paris Diderot performed the phenotypic and genotypic microbiological
306 analyses.

307

308

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310

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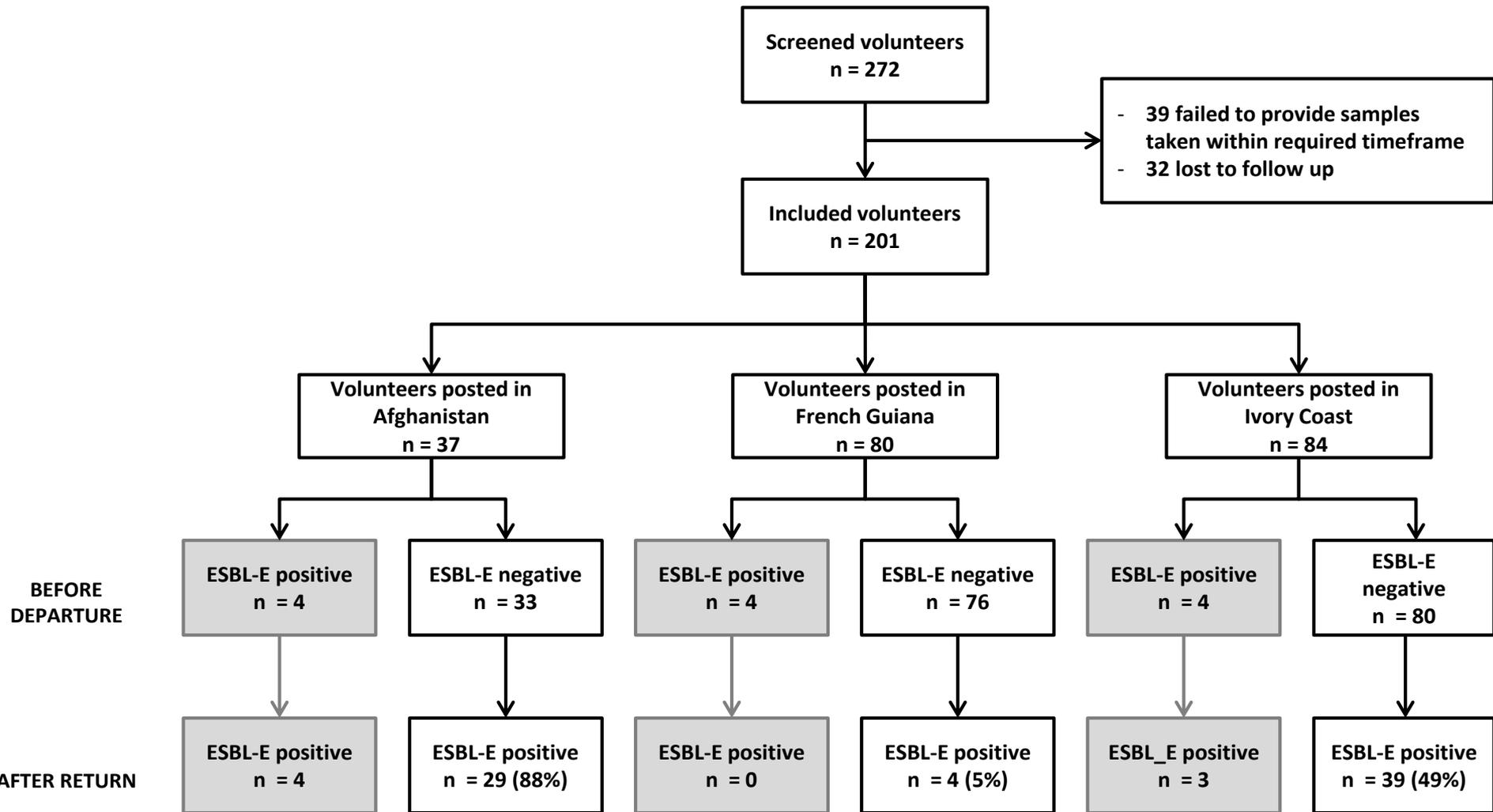
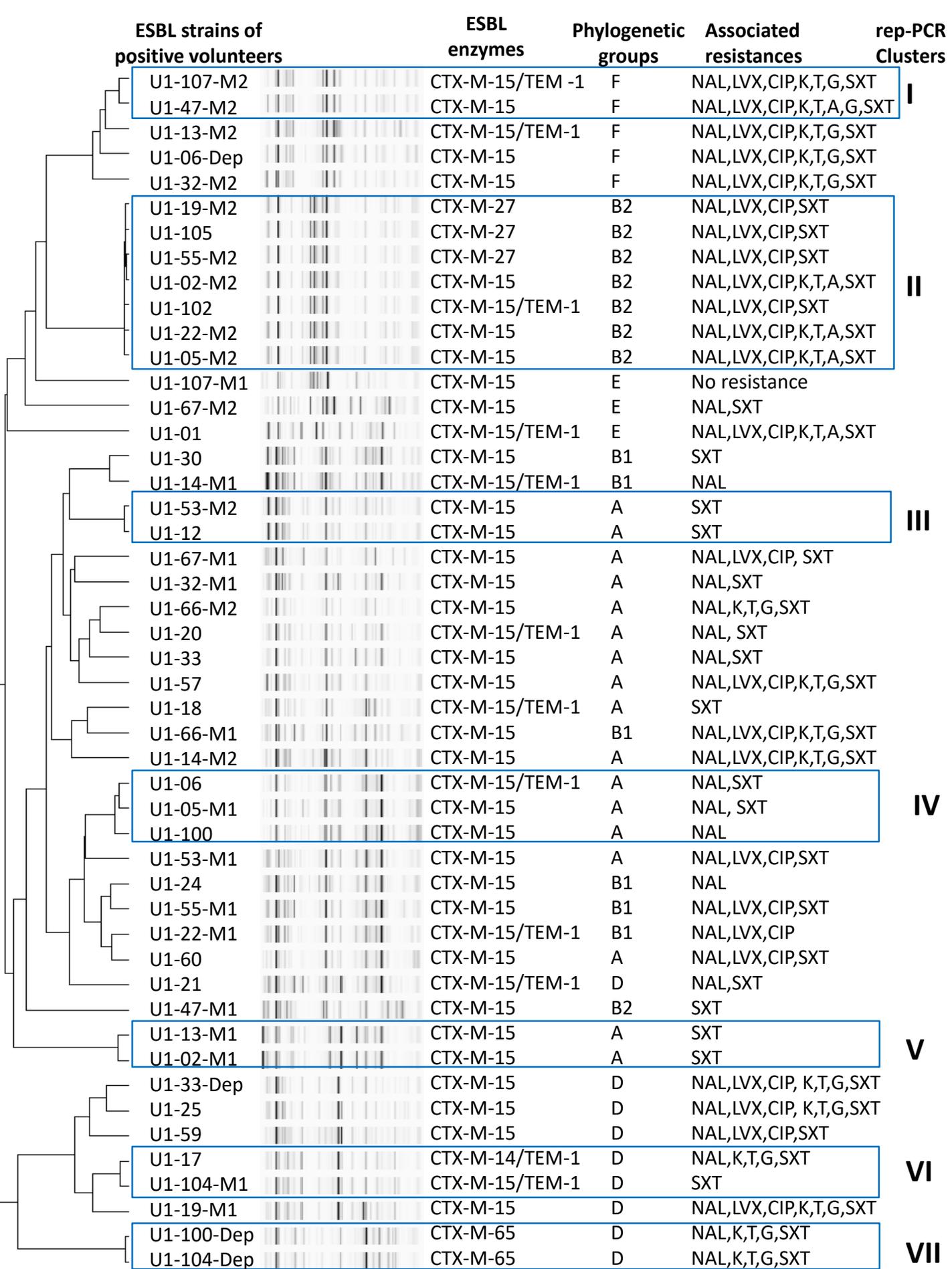


Figure 1 : Flow chart of the study
 ESBL-E, extended-spectrum beta-lactamase-producing Enterobacteriaceae



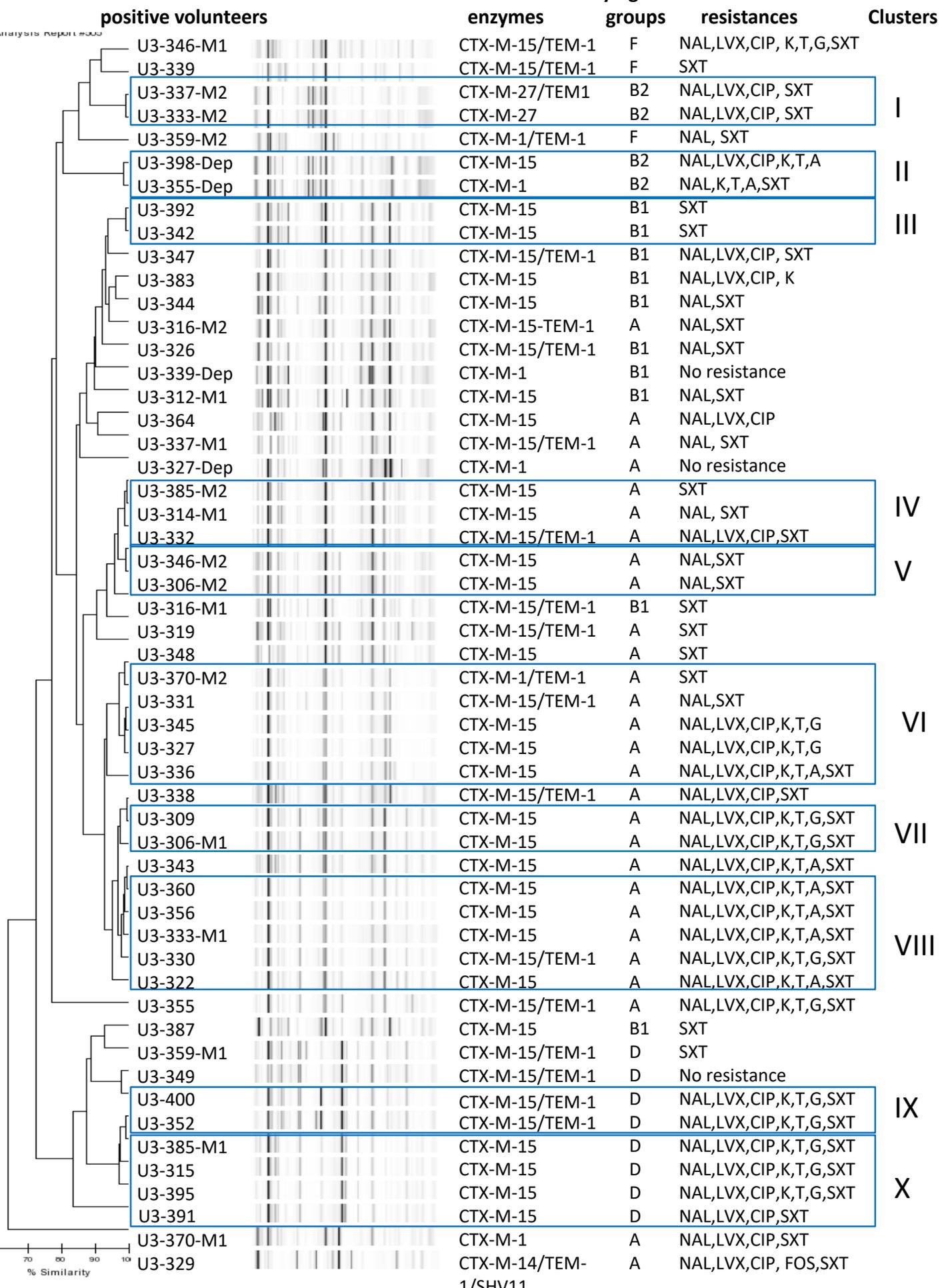


Table 1: Univariate and multivariate analyses to identify risk factors for acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae by soldiers with negative samples before the mission

The data are n (%) unless otherwise specified.

95%CI, 95% confidence interval

ESBL-E: extended spectrum beta-lactamase-producing Enterobacteriaceae

Variable	All participants	Non ESBL-E carriers	ESBL-E carriers	Univariate analysis		Multivariate analysis	
				Univariate odds ratio [95%CI]	<i>P</i> value	Adjusted odds ratio [95%CI]	<i>P</i> value
Age, years, mean (SD)	26.1 (5.2)	26.0 (5.1)	26.2 (5.4)	1.0 [1.0-1.1]	0.75		
Sex							
Male	178 (97.3)	110 (98.2)	68 (95.8)	1.0			
Female	5 (2.7)	2 (1.8)	3 (4.2)	2.4 [0.4-14.9]	0.34		
Previous travel outside continental							
France							
No	158 (85.9)	96 (85.0)	62 (87.3)	1.0			
Yes	26 (14.1)	17 (15.0)	9 (12.7)	0.8 [0.3-2.0]	0.65		
Prior antibiotic use							
No	175 (95.1)	106 (93.8)	69 (97.2)	1.0			
Yes	9 (4.9)	7 (6.2)	2 (2.8)	0.4 [0.1-2.2]	0.31		
Antibiotic use during the mission (other than malaria prophylaxis)							
No	159 (87.4)	101 (89.4)	58 (84.1)	1.0			
Yes	23 (12.6)	12 (10.6)	11 (15.9)	1.6 [0.7-3.8]	0.30		
Malaria prophylaxis							
No	16 (8.7)	6 (5.3)	10 (14.3)	1.0		1.0	
Yes	167 (91.3)	107 (94.7)	60 (85.7)	0.34 [0.12-0.97]	0.04	0.64 [0.12-3.32]	0.59
Intestinal symptoms during the mission							
No	89 (48.9)	57 (50.4)	32 (46.4)	1.0			
Yes	93 (51.1)	56 (49.6)	37 (53.6)	1.2 [0.6-2.1]	0.60		
Hospitalization during the mission							

No	174 (95.6)	107 (94.7)	67 (97.1)	1.0			
Yes	8 (4.4)	6 (5.3)	2 (2.9)	0.5 [0.1-2.7]	0.45		
Contact with a hospitalized patient							
during the mission							
No	147 (81.7)	96 (85.0)	51 (76.1)	1.0			
Yes	33 (18.3)	17 (15.0)	16 (23.9)	1.8 [0.8-3.8]	0.14		
Country of deployment							
French Guiana (reference)	76 (41.3)	72 (63.7)	4 (5.6)	1.0		1.0	
Côte d'Ivoire	75 (40.8)	37 (32.7)	38 (53.5)	18.5 [6.1-55.8]	<0.001	11.8 [2.78-50.4]	0.001
Afghanistan	33 (17.9)	4 (3.6)	29 (40.9)	130.5 [30.6-557.1]	<0.001	75.3 [13.0-434.5]	<0.001
Living conditions							
Military base							
No	34 (18.5)	31 (27.4)	3 (4.2)	1.0		1.0	
Yes	150 (81.5)	82 (72.6)	68 (95.8)	8.6 [2.5-29.3]	0.001	2.96 [0.47-18.7]	0.25
Bivouac							
No	102 (55.4)	41 (36.3)	61 (85.9)	1.0		1.0	
Yes	82 (44.6)	72 (63.7)	10 (14.1)	0.1 [0.04-0.2]	<0.001	0.81 [0.24-2.76]	0.74