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Original article

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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

METHODS

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

Study design and setting

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

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

Microbiological methods

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

ESBL-E screening

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

PCR typing

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

Whole-genome sequencing (WGS)

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

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

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

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

Statistical analysis

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

RESULTS

Study population

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

ESBL-E acquisition

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

Factors associated with ESBL-E acquisition

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

(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 d'Ivoire. Multivariate analysis performed within each group showed that factors independently associated with a lower acquisition rate were living in a bivouac in the Afghanistan group and older age in the Côte d'Ivoire group (Tables S3 to S5).

Microbiological results

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

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

Rep-PCR and clonal relatedness

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

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

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

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

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

WGS was performed on the 20 ESBL-producing *E. coli* isolates from the Afghanistan group that had ≥97% similarity by rep-PCR (Figure 4). This unit was chosen because it showed the highest acquisition rate and because operational insecurity in Afghanistan caused the soldiers to live in more confined conditions, involving closer contacts, compared to those deployed in Côte d'Ivoire. Isolates belonging to the same rep-PCR cluster grouped together. However, within each rep-PCR cluster, WGS consistently evidenced between-strain differences of various magnitudes. The largest differences were observed within the rep-PCR cluster IV, in which the strains had different serotypes and sequence types (STs) and hosted different resistance genes and plasmid replicons. In cluster VI, all isolates had the same STs, but differences were found for the serotypes and resistance genes or plasmid replicons. In clusters I, II (with all ST131 strains), and VII, resistance genes and plasmid replicons were different. In cluster III, only the resistance genes, and in cluster V only the replicons, were different. Of note, all ST131 strains in cluster II had the 025:H4 serotype and carried *fimH*30, although these strains belonged to two different clades, C1 (H30-R) and C2 (H30-Rx).

SNPs were identified by comparing the core genome of isolates from each cluster. The smallest number of SNPs was 3, between the U1-100-Dep and U1-104-Dep strains in cluster VII. 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).

DISCUSSION

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

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

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

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

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

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

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

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

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

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

Transparency declaration

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

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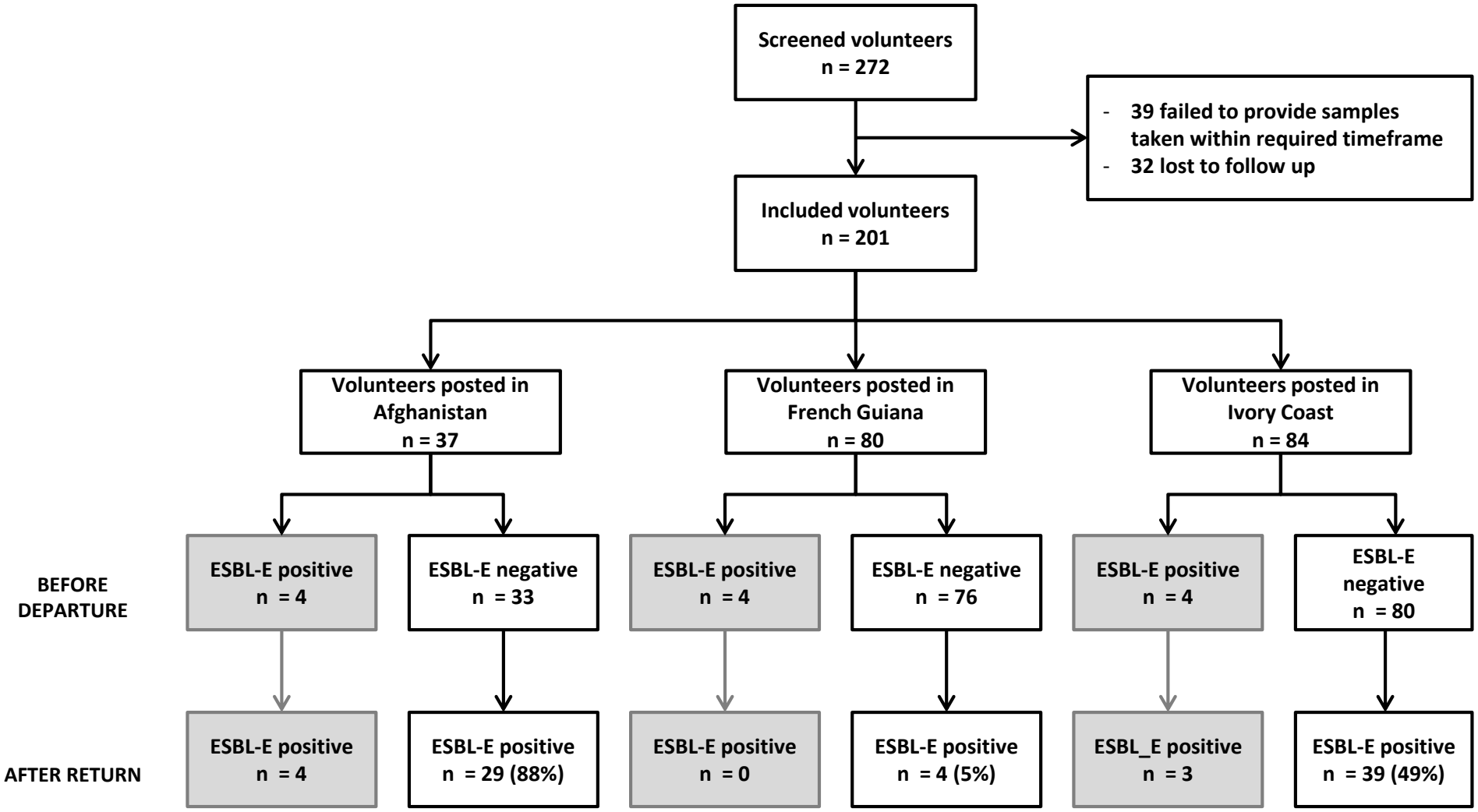
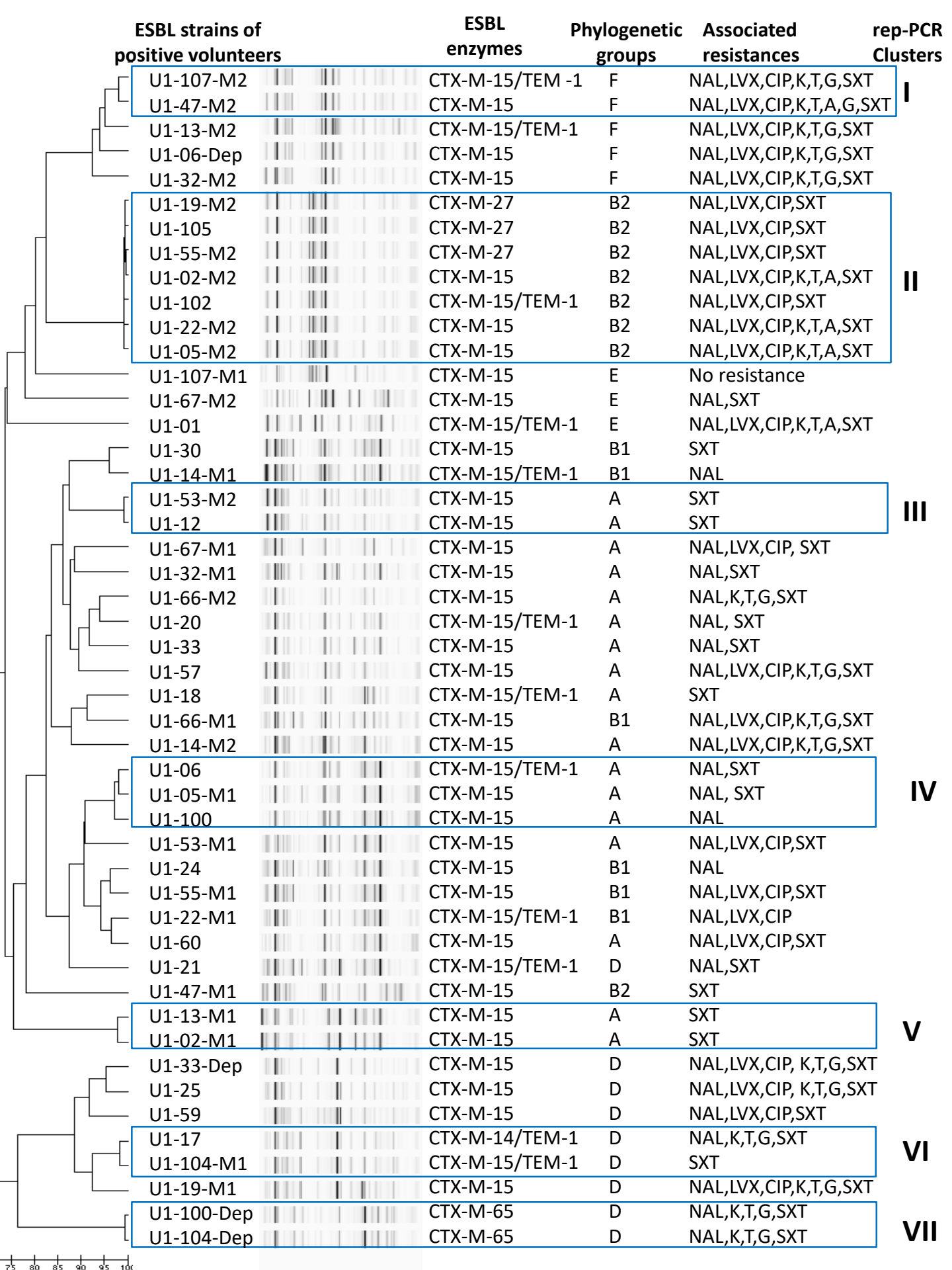
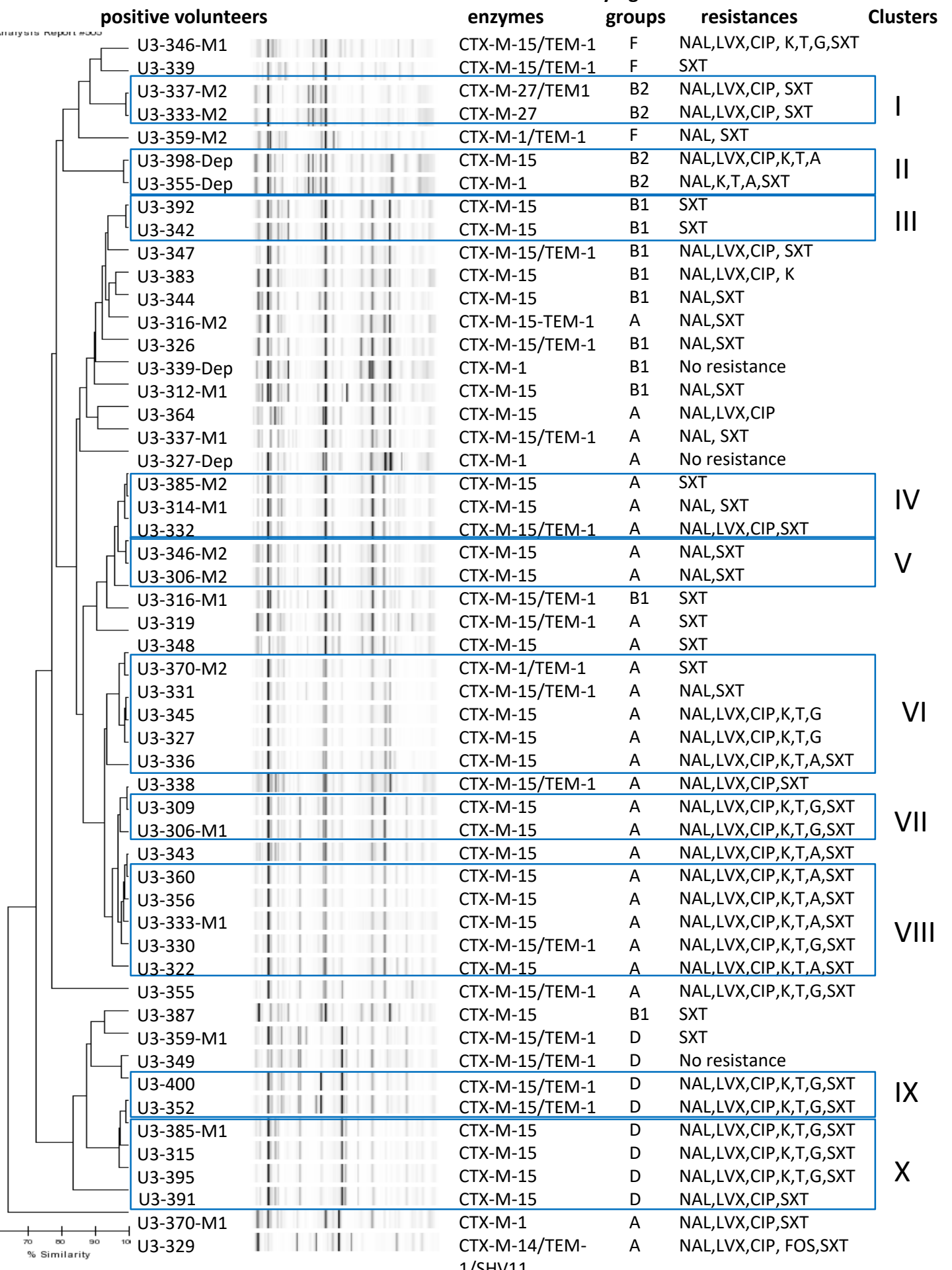


Figure 1 : Flow chart of the study

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


ESBL strains of positive volunteers
ESBL enzymes
Phylogenetic groups
Associated resistances
rep-PCR Clusters
U1-107-M2 CTX-M-15/TEM -1 F NAL,LVX,CIP,K,T,G,SXT
U1-47-M2 CTX-M-15 F NAL,LVX,CIP,K,T,A,G,SXT
I
U1-13-M2 CTX-M-15/TEM-1 F NAL,LVX,CIP,K,T,G,SXT
U1-06-Dep CTX-M-15 F NAL,LVX,CIP,K,T,G,SXT
U1-32-M2 CTX-M-15 F NAL,LVX,CIP,K,T,G,SXT
U1-19-M2 CTX-M-27 B2 NAL,LVX,CIP,SXT
U1-105 CTX-M-27 B2 NAL,LVX,CIP,SXT
U1-55-M2 CTX-M-27 B2 NAL,LVX,CIP,SXT
U1-02-M2 CTX-M-15 B2 NAL,LVX,CIP,K,T,A,SXT
U1-102 CTX-M-15/TEM-1 B2 NAL,LVX,CIP,SXT
U1-22-M2 CTX-M-15 B2 NAL,LVX,CIP,K,T,A,SXT
U1-05-M2 CTX-M-15 B2 NAL,LVX,CIP,K,T,A,SXT
II
U1-107-M1 CTX-M-15 E No resistance
U1-67-M2 CTX-M-15 E NAL,SXT
U1-01 CTX-M-15/TEM-1 E NAL,LVX,CIP,K,T,A,SXT
U1-30 CTX-M-15 B1 SXT
U1-14-M1 CTX-M-15/TEM-1 B1 NAL
U1-53-M2 CTX-M-15 A SXT
U1-12 CTX-M-15 A SXT
III
U1-67-M1 CTX-M-15 A NAL,LVX,CIP, SXT
U1-32-M1 CTX-M-15 A NAL,SXT
U1-66-M2 CTX-M-15 A NAL,K,T,G,SXT
U1-20 CTX-M-15/TEM-1 A NAL, SXT
U1-33 CTX-M-15 A NAL,SXT
U1-57 CTX-M-15 A NAL,LVX,CIP,K,T,G,SXT
U1-18 CTX-M-15/TEM-1 A SXT
U1-66-M1 CTX-M-15 B1 NAL,LVX,CIP,K,T,G,SXT
U1-14-M2 CTX-M-15 A NAL,LVX,CIP,K,T,G,SXT
U1-06 CTX-M-15/TEM-1 A NAL,SXT
U1-05-M1 CTX-M-15 A NAL, SXT
U1-100 CTX-M-15 A NAL
IV
U1-53-M1 CTX-M-15 A NAL,LVX,CIP,SXT
U1-24 CTX-M-15 B1 NAL
U1-55-M1 CTX-M-15 B1 NAL,LVX,CIP,SXT
U1-22-M1 CTX-M-15/TEM-1 B1 NAL,LVX,CIP
U1-60 CTX-M-15 A NAL,LVX,CIP,SXT
U1-21 CTX-M-15/TEM-1 D NAL,SXT
U1-47-M1 CTX-M-15 B2 SXT
U1-13-M1 CTX-M-15 A SXT
U1-02-M1 CTX-M-15 A SXT
V
U1-33-Dep CTX-M-15 D NAL,LVX,CIP, K,T,G,SXT
U1-25 CTX-M-15 D NAL,LVX,CIP, K,T,G,SXT
U1-59 CTX-M-15 D NAL,LVX,CIP,SXT
U1-17 CTX-M-14/TEM-1 D NAL,K,T,G,SXT
U1-104-M1 CTX-M-15/TEM-1 D SXT
VI
U1-19-M1 CTX-M-15 D NAL,LVX,CIP,K,T,G,SXT
U1-100-Dep CTX-M-65 D NAL,K,T,G,SXT
U1-104-Dep CTX-M-65 D NAL,K,T,G,SXT
VII
75 80 85 90 95 100


positive volunteers
enzymes
groups
resistances
Clusters
U3-346-M1 CTX-M-15/TEM-1 F NAL,LVX,CIP, K,T,G,SXT
U3-339 CTX-M-15/TEM-1 F SXT
U3-337-M2 CTX-M-27/TEM1 B2 NAL,LVX,CIP, SXT
U3-333-M2 CTX-M-27 B2 NAL,LVX,CIP, SXT
I
U3-359-M2 CTX-M-1/TEM-1 F NAL, SXT
U3-398-Dep CTX-M-15 B2 NAL,LVX,CIP,K,T,A
U3-355-Dep CTX-M-1 B2 NAL,K,T,A,SXT
II
U3-392 CTX-M-15 B1 SXT
U3-342 CTX-M-15 B1 SXT
III
U3-347 CTX-M-15/TEM-1 B1 NAL,LVX,CIP, SXT
U3-383 CTX-M-15 B1 NAL,LVX,CIP, K
U3-344 CTX-M-15 B1 NAL,SXT
U3-316-M2 CTX-M-15-TEM-1 A NAL,SXT
U3-326 CTX-M-15/TEM-1 B1 NAL,SXT
U3-339-Dep CTX-M-1 B1 No resistance
U3-312-M1 CTX-M-15 B1 NAL,SXT
U3-364 CTX-M-15 A NAL,LVX,CIP
U3-337-M1 CTX-M-15/TEM-1 A NAL, SXT
U3-327-Dep CTX-M-1 A No resistance
U3-385-M2 CTX-M-15 A SXT
U3-314-M1 CTX-M-15 A NAL, SXT
U3-332 CTX-M-15/TEM-1 A NAL,LVX,CIP,SXT
IV
U3-346-M2 CTX-M-15 A NAL,SXT
U3-306-M2 CTX-M-15 A NAL,SXT
V
U3-316-M1 CTX-M-15/TEM-1 B1 SXT
U3-319 CTX-M-15/TEM-1 A SXT
U3-348 CTX-M-15 A SXT
U3-370-M2 CTX-M-1/TEM-1 A SXT
U3-331 CTX-M-15/TEM-1 A NAL,SXT
U3-345 CTX-M-15 A NAL,LVX,CIP,K,T,G
U3-327 CTX-M-15 A NAL,LVX,CIP,K,T,G
U3-336 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
VI
U3-338 CTX-M-15/TEM-1 A NAL,LVX,CIP,SXT
U3-309 CTX-M-15 A NAL,LVX,CIP,K,T,G,SXT
U3-306-M1 CTX-M-15 A NAL,LVX,CIP,K,T,G,SXT
VII
U3-343 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
U3-360 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
U3-356 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
U3-333-M1 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
U3-330 CTX-M-15/TEM-1 A NAL,LVX,CIP,K,T,G,SXT
U3-322 CTX-M-15 A NAL,LVX,CIP,K,T,A,SXT
VIII
U3-355 CTX-M-15/TEM-1 A NAL,LVX,CIP,K,T,G,SXT
U3-387 CTX-M-15 B1 SXT
U3-359-M1 CTX-M-15/TEM-1 D SXT
U3-349 CTX-M-15/TEM-1 D No resistance
U3-400 CTX-M-15/TEM-1 D NAL,LVX,CIP,K,T,G,SXT
U3-352 CTX-M-15/TEM-1 D NAL,LVX,CIP,K,T,G,SXT
IX
U3-385-M1 CTX-M-15 D NAL,LVX,CIP,K,T,G,SXT
U3-315 CTX-M-15 D NAL,LVX,CIP,K,T,G,SXT
U3-395 CTX-M-15 D NAL,LVX,CIP,K,T,G,SXT
U3-391 CTX-M-15 D NAL,LVX,CIP,SXT
X
U3-370-M1 CTX-M-1 A NAL,LVX,CIP,SXT
U3-329 CTX-M-14/TEM-1/SHV11 A NAL,LVX,CIP, FOS,SXT
70
80
90
% Similarity

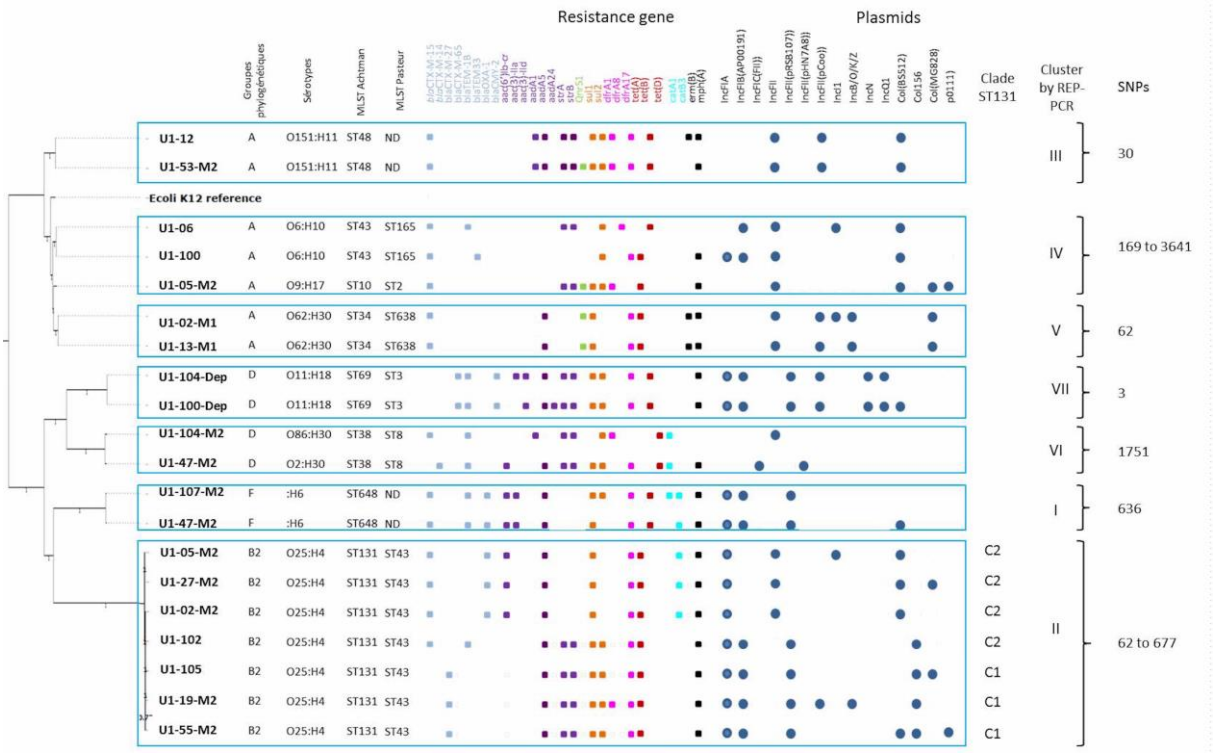

Resistance gene
Plasmids
Groupes phylogénétiques
Serotypes
MLST Achtman
MLST Pasteur
blaCTX-M-15
blaCTX-M-14
blaCTX-M-27
blaCTX-M-65
blaTEM-1B
blaTEM-33
blaOXA-1
blaOXY-2
aac(6')Ib-cr
aac(3)IIa
aac(3)IId
aadA1
aadA5
aadA24
strA
strB
qnrS1
sul1
sul2
dfrA1
dfrA8
dfrA17
tet(A)
tet(B)
tet(D)
catA1
catB3
erm(B)
mph(A)
IncFIA
IncFIB(AP001918)
IncFIC(FII))
IncFII
IncFII(pRSB107))
IncFII(pHN7A8))
IncFII(pCoo))
IncI1
IncB/O/K/Z
IncN
IncQ1
Col(BS512)
Col156
Col(MG828)
p0111)
Clade ST131
Cluster by REP-PCR
SNPs
U1-12 A O151:H11 ST48 ND III 30
U1-53-M2 A O151:H11 ST48 ND
Ecoli K12 reference
U1-06 A O6:H10 ST43 ST165 IV 169 to 3641
U1-100 A O6:H10 ST43 ST165
U1-05-M2 A O9:H17 ST10 ST2
U1-02-M1 A O62:H30 ST34 ST638 V 62
U1-13-M1 A O62:H30 ST34 ST638
U1-104-Dep D O11:H18 ST69 ST3 VII 3
U1-100-Dep D O11:H18 ST69 ST3
U1-104-M2 D O86:H30 ST38 ST8 VI 1751
U1-47-M2 D O2:H30 ST38 ST8
U1-107-M2 F :H6 ST648 ND I 636
U1-47-M2 F :H6 ST648 ND
U1-05-M2 B2 O25:H4 ST131 ST43 C2 II 62 to 677
U1-27-M2 B2 O25:H4 ST131 ST43 C2
U1-02-M2 B2 O25:H4 ST131 ST43 C2
U1-102 B2 O25:H4 ST131 ST43 C2
U1-105 B2 O25:H4 ST131 ST43 C1
U1-19-M2 B2 O25:H4 ST131 ST43 C1
U1-55-M2 B2 O25:H4 ST131 ST43 C1

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]	*P* value	Adjusted odds ratio [95%CI]	*P* 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