Intestinal Colonization with Multidrug-Resistant Enterobacteriaceae: Screening of Swiss Military Deployed to Kosovo

Esther Kuenzli

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Thomas Bü del, Odette J. Bernasconi

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Patricia Schlagenhauf

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Peter Florek

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Nejla Gülteki̇n

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Christoph Hatz

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Andrea Endimiani

PII: S2213-7165(19)30235-8
DOI: https://doi.org/10.1016/j.jgar.2019.08.027
Reference: JGAR 1039

To appear in:

Received Date: 19 August 2019

Please cite this article as: Kuenzli E, Büdel T, Bernasconi OJ, Schlagenhauf P, Florek R, Gültekin N, Hatz C, Endimiani A, Intestinal Colonization with Multidrug-Resistant Enterobacteriaceae: Screening of Swiss Military Deployed to Kosovo, *Journal of Global Antimicrobial Resistance* (2019), doi: https://doi.org/10.1016/j.jgar.2019.08.027

Intestinal Colonization with Multidrug-Resistant Enterobacteriaceae: Screening of Swiss Military Deployed to Kosovo

Running title: Screening of Swiss military for MDR Enterobacteriaceae

Esther Kuenzli,[1, 2, 3] Thomas Büdel,[4] Odette J. Bernasconi,[4] Patricia Schlagenhauf,[1] Peter Florek,[5] Nejla Gültekin,[6] Christoph Hatz,[1, 2, 3, 7] Andrea Endimiani [4*]

[1]Division of Communicable Diseases, Epidemiology, Biostatistics and Prevention Institute, WHO Collaborating Centre for Travellers' Health, University of Zurich, Zurich, Switzerland;

[2]Swiss Tropical and Public Health Institute, Basel, Switzerland;

[3]University of Basel, Basel, Switzerland;

[4]Institute for Infectious Diseases, University of Bern, Bern;

[5]Armed Forces International Command SWISSINT;

[6]Centre of Competence for Military and Disaster Medicine, Federal Department of Defence, Civil Protection and Sport DDPS, Swiss Armed Forces;

[7]Cantonal Hospital St. Gallen, Department of Infectious Diseases and Hospital Epidemiology, St. Gallen, Switzerland

***Corresponding author:** Prof. Andrea Endimiani MD, PhD, FAMH**,** Institute for Infectious Diseases, University of Bern Friedbühlstrasse 51, CH-3001, Bern, Switzerland Phone: +41-31-632-8632; Fax: +41-31-632-8766 Emails: andrea.endimiani@ifik.unibe.ch; aendimiani@gmail.com

Sir,

International travel is a known risk factor for intestinal colonization with extended-spectrum cephalosporin-resistant (ESC-R) Enterobacteriaceae [1], thereby contributing to their spread from high to low endemicity countries like Switzerland. This phenomenon is also observed in military personnel stationed in high endemicity areas. For instance, French soldiers deployed to Afghanistan and Côte d'Ivoire showed colonization rates with ESC-R *Escherichia coli* >50% [2]. In Switzerland, military personnel have been deployed for peacekeeping missions to various regions for decades.

In the present study, the extent of intestinal colonization with ESC-R- and/or colistin-resistant (COL-R) Enterobacteriaceae in military staff deployed to Kosovo from November 2017 to April 2018 was analyzed. Stools were collected before and after the service (within one week). As previously done, samples were enriched overnight in Luria-Bertani broth containing cefuroxime (3 mg/L) or colistin (2 mg/L). From each tube, aliquots were plated on ChromID ESBL/Carba (bioMérieux) or CHROMagar Orientation plus colistin (4 mg/L) and vancomycin (8 mg/L), respectively, and incubated overnight [3]. At least five colonies were selected from each positive agar plate for further analyses. Species identification was achieved using the MALDI-TOF MS (Bruker). MICs for antibiotics were obtained implementing the Sensititre GNX2F microdilution plates and interpreted using the 2019 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (www.eucast.org). Whole-genome sequencing was performed with NovaSeq 6000 (Illumina) and reads were analyzed using the tools of the Center for Genomic Epidemiology (CGE; www.genomicepidemiology.org/) [1, 3].

Both pre- and post-deployment stools were available for 21 participants. As shown in Table 1, two subjects were already colonized with COL-R *E. coli* strains before going to Kosovo: one carried the *mcr-1.2* gene in a 33kb IncX4 plasmid that is frequently reported worldwide (data not shown) [3], while the other one showed amino acid substitutions in the chromosomal PmrA/B two-component system. Upon return to Switzerland, three (14.3%) deployed persons (including one earlier colonized with the *mcr-1.2*-positive strain) screened positive for ESC-R *E. coli.* Such

strains were of different STs (ST2540, ST69, and ST484) not belonging to hyperepidemic lineages, and all carried the *bla*$_{\text{CTX-M-15}}$ along with other antimicrobial resistance genes (Supplementary File S1). Strains with reduced susceptibility to colistin, carbapenems and/or fluoroquinolones were not detected (Table 1).

The gut colonization prevalence with ESC-R Enterobacteriaceae recorded in the present study was similar to the one found in a study involving German soldiers deployed internationally between 2007-2015 (4.7%) and among French soldiers sent to French Guiana during 2012 (5.3%) [2, 4]. However, it was considerably lower than the incidence among French army persons returning from Côte d'Ivoire and Afghanistan (49% and 88%, respectively) in 2012 [2]. Based on our results, we speculate that the recorded colonization rates in soldiers returning to their home countries do not fully mirror the prevalence of antimicrobial resistance in the region of deployment. Unfortunately, data on colonization rates with ESC-R *E. coli* in the local population in Kosovo are not available. However, in 2017 the surrounding regions in Eastern and South Eastern Europe showed up to 40% of *E. coli* from clinical samples to be ESC-R (https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017). This is much higher than the 10% found in Switzerland during the same time period (www.anresis.ch). Therefore, colonization rates in military personnel returning from Kosovo could have been predicted to be much higher than the 14% actually found in our participants. This unexpected low colonization rate in the Swiss military personnel could have several reasons. It is possible that Kosovo has overall lower resistance rates than the neighboring countries. More likely, the low rates found in these subjects could be due to their different hygienic conditions compared to the local population, thus preventing them from acquiring multidrug-resistant bacteria from food chain, animals, and/or environment. Looking at our participants, we note for instance that half of them ate more than 50% of their meals inside the military compound (Table 1). This behavior is very different to what is observed in travelers visiting tropical and subtropical

countries who show considerably higher rates of gut colonization due to their closer contact and more frequent interaction with the local human and non-human settings [1].

Our data indicate that deployment-related colonization rates with ESC-R *E. coli* are slightly higher than those recorded in the healthy population in Switzerland (~7%) [5], but considerably lower than those observed in international travelers (up to 75%) [1]. Therefore, the impact of military personnel returning from international deployment on the spread of antimicrobial resistance is probably less important than that of travelers. Nevertheless, larger studies assessing military personnel returning from different countries need to be carried out to properly assess the impact of these subjects on the worldwide spread of antimicrobial resistance.

DECLARATIONS

Funding: This work was funded by a collaborative research agreement between the Zürich Competence Centre for Medical Military Biology and the Swiss Armed Forces and partially by the NRP-72, "*National Research Programme, Antimicrobial Resistance*" (Swiss National Science Foundation; grant No. 177378 to AE).

Competing Interests: None

Ethical Approval: Ethical approval was obtained by the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz (BASEC 2017-01161).

ACKNOWLEDGEMENTS

We thank Markus Hilty and Mathieu Clément (Institute for Infectious Diseases, University of Bern) for support. We are grateful to all participants who kindly donated their stool samples.

REFERENCES

[1] Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A, et al. Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. The Lancet Infectious Diseases. 2017;17:78-85.

[2] Maataoui N, Mayet A, Duron S, Delacour H, Mentre F, Laouenan C, 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. 2019;25:631 e1- e9.

[3] Büdel T, Künzli E, Clément M, Bernasconi OJ, Fehr J, Mohammed AH, et al. Polyclonal gut colonization with extended-spectrum cephalosporin- and/or colistin-resistant Enterobacteriaceae: a normal status for hotel employees on the island of Zanzibar, Tanzania. Journal of Antimicrobial Chemotherapy (doi:101093/jac/dkz296). 2019.

[4] Frickmann H, Wiemer D, Frey C, Hagen RM, Hinz R, Podbielski A, et al. Low Enteric Colonization with Multidrug-Resistant Pathogens in Soldiers Returning from Deployments-Experience from the Years 2007-2015. PLoS One. 2016;11:e0162129.

[5] Pires J, Kuenzli E, Hauser C, Tinguely R, Kasraian S, Atkinson A, et al. Intestinal colonisation with extended-spectrum cephalosporin-resistant Enterobacteriaceae in different populations in Switzerland: prevalence, risk factors and molecular features. Journal of global antimicrobial resistance. 2018;12:17-9.

Table 1. Demographic data of participants, colonization status before and after travelling, and characteristics (phenotypic and molecular) of recovered strains

ID	Age	Sex	Phenotypic and molecular characteristics of ESC- and/or COL-R strains (if any) [a]		Potential risk factors during deployment			
			Stools collected before deployment	Stools collected after deployment	Antibiotic use	Hospitalization	Diarrhea	Food outside military compound (times/week)
ID6	24	M	Negative	Negative	no	no	no	7
ID10	26	M	Negative	Negative	no	no	yes	6
ID12	27	M	Negative	Negative	no	no	yes	1
ID13	31	M	Negative	Negative	no	no	yes	1
ID22	47	M	Negative	Negative	na	na	na	na
ID25	25	F	*E. coli*: COL (4), CTX (≤1), FEP (≤2), MEM (≤1), CIP (1), CN (≤1), DOX (8), SXT (4) ST69-like; *mcr-1.2*, bla_{TEM-1B}, *aph(3'')-Ib*, *aph(6)-Id*, *aadA1*, *aadA2*, *mdf(A)*, *cmlA1*, *sul2*, *sul3*, *tet(A)*, *dfrA5*, GyrA (S83L), ParC (S80R), [PmrB: H2R, S138N, D283G]; Col440II, FIB, FII, Q1, X1, X4	*E. coli*: COL (≤0.25), CTX (16), FEP (≤2), MEM (≤1), CIP (≤0.25), CN (≤1), DOX (4), SXT (1) ST2540; $bla_{CTX-M-15}$, *aadA5*, *mdf(A)*, *tet(A)*, *dfrA17*, [PmrB: D283G, Y358N]; FII, FIA, FIB, HI1A, HI1B	na	na	na	na
ID26	25	F	Negative	Negative	na	na	na	na
ID29	49	F	Negative	*E. coli*: COL (≤0.25), CTX (16), FEP (≤2), MEM (≤1), CIP (≤0.25), CN (≤1), DOX (≤2), SXT (≤0.5) ST69; $bla_{CTX-M-15}$, *qnrS1*, *mdf(A)*, [PmrB: H2R, S138N, D283G]; FII, X4	no	no	yes	3
ID30	29	F	Negative	Negative	no	no	yes	7
ID31	33	F	Negative	Negative	yes	no	no	7
ID34	27	M	Negative	Negative	no	no	yes	1
ID37	32	M	Negative	Negative	no	no	no	0
ID38	31	F	Negative	Negative	no	no	yes	1
ID39	30	F	Negative	*E. coli*: COL (≤0.25), CTX (32), FEP (≤2), MEM (≤1), CIP (≤0.25), CN (≤1), DOX (≤2), SXT (≤0.5) ST484; $bla_{CTX-M-15}$, *qnrS1*, *mdf(A)*; FIC	no	no	no	7
ID40	29	F	Negative	Negative	yes	no	1	na
ID42	28	F	*E. coli*: COL (>4), CTX (≤1), FEP (≤2), MEM (≤1), CIP (≤0.25), CN (≤1), DOX (≤2), SXT (≤0.5) ST420-like; *mdf(A)*, [PmrA: T31S, I128N, G144S, ; PmrB: H2R, E123D, T156K, D283G, V351I]; Col156, FIB, FII	Negative	no	no	yes	1
ID64	26	M	Negative	Negative	na	na	na	na
ID65	25	M	Negative	Negative	no	no	yes	3
ID66	23	M	Negative	Negative	no	no	no	1
ID69	29	M	Negative	Negative	no	no	1	0

ID70	28	M	Negative	Negative	no	no	no	4

Note. M, male; F, female; na, not available; COL, colistin; CTX, cefotaxime; FEP, cefepime; MEM, meropenem; CIP, ciprofloxacin; CN, gentamicin; DOX, doxycycline, SXT, trimethoprim-sulfamethoxazole

[a] We show: bacterial species with antimicrobial phenotype (MIC, mg/L), sequence type (ST), antimicrobial resistance genes, and replicon type plasmid detected by WGS and implementing the CGE analysis. Among the unknown mutations (according to the CGE analysis), only those for *pmrA* and *pmrB* have been reported in square parentheses (as amino acid substitutions)