Intestinal Colonization with Extended-Spectrum Cephalosporin- and Colistin-Resistant *Enterobacteriaceae* in HIV-Positive Individuals in Switzerland: Molecular Features and Risk Factors

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Short running title: HIV-positive colonized with MDR *E. coli*

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Sir,

The increasing rates of gut colonization by extended-spectrum cephalosporin-resistant (ESC-R-) and/or colistin-resistant *Enterobacteriaceae* (COL-R-Ent) carrying the plasmid-mediated *mcr-1* gene in healthy humans raises serious concerns [1, 2]. Nevertheless, colonization prevalence and risk factors associated with HIV+ individuals are unknown. This population undergoes a decrease in CD4+ T cells in the gut-associated lymphoid tissue which is linked with a microbiota dysbiosis possibly favoring colonization [3]. The aim of this pilot study was to better understand this phenomenon to improve the management of these patients.

Between March 2015 and April 2016, 101 HIV+ individuals on suppressive anti-retroviral therapy (ART) donated a stool sample and filled out a questionnaire (Table S1 and S2). To detect ESC-R-, carbapenem-, and/or COL-R-Ent, stools were enriched in broth and plated on selective agar plates [1, 2]. Species identification was achieved by using the MALDI-TOF MS (Bruker Daltonics, Leipzig, Germany). MICs were obtained using the Sentitre^{TM} GNX2F plate (Trek Diagnostic Systems, Independence, OH, USA). β-lactamase genes were identified using both CT103XL microarray (Check-Points, Wageningen, Netherlands) and PCR/sequencing [1, 2]. COL-R-Ent were screened for *mcr-1/2* and PmrAB two-component system with PCR/sequencing [1]. Whole genome sequencing was carried out with MinION (Oxford Nanopore, Oxford, UK) [1]. Clonality was assessed with standard MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli) and phylogenetic grouping [2]. The PBRT kit (Diatheva, Cartoceto, Italy) was used to type plasmids. Conjugation was performed using *E. coli* JF33 [1]. Univariate analysis was performed to compare colonized and non-colonized subjects (GraphPad Prism software, version 7.0; La Jolla, CA, USA). Continuous variables were analyzed using Mann–Whitney U test, whereas categorical variables with Fisher's exact test.

Seven volunteers (6.9%) resulted colonized with ESC-R *E. coli* (other *Enterobacteriaceae* were not detected). This data is consistent with that reported in the healthy population in Switzerland and in other European countries [1, 2]. However, when looking into *Enterobacteriaceae* causing

infections in HIV+ subjects, the prevalence of ESC-R-Ent dramatically increased to 50%, though these studies were either conducted in countries with high occurrence of these multidrug-resistant organisms (MDROs) or have enrolled individuals not under ART [4 , 5].

Most ESC-R *E. coli* recovered in the present study were CTX-M-15 producers and associated with F plasmids (Table 1) [2]. Each isolate yielded an individual sequence type (ST), including the high risk clones (HiRC) ST131, ST73, ST405 and ST410 that might be associated with the frequent contact of HIV+ people with the healthcare facilities [2].

Four volunteers (4.0%) were also colonized with COL-R *E. coli* (other *Enterobacteriaceae* were not detected), of which one (31349) was of B1-ST5 and *mcr-1*-positive. The remaining three strains failed to demonstrate a plasmid-mediated mechanism of colistin resistance after conjugation experiments [1]. Analysis of the PmrAB system indicated that these strains had amino acid substitutions possibly driving colistin resistance (Table 1) [1]. Demographic, clinical and epidemiological data about the four volunteers colonized with COL-R *E. coli* are shown in Table S1. Notably, volunteer 31349 was a 58 year-old male who in the last year was hospitalized in Switzerland and received antibiotics, but he did not traveled anywhere.

Screening of raw genome data by PlasmidFinder-1.3 and ResFinder-2.1 indicated that *E. coli* strain 31349 possessed IncF, IncL, and IncX4 plasmids together with the *mcr-1* (no other resistance genes were detected) [1]. *Mcr-1* was located in a 33.3 Kb plasmid that shared >99% identity (≤30 mismatches) with IncX4 plasmids reported in *E. coli* from pig faeces in China (GenBank: KX254343) and from river water in Switzerland (GenBank: KZ129783). This is the first report of *mcr-1*-carrying *Enterobacteriaceae* in the gut of HIV+ people and highlights the global dissemination of this life-threatening resistance mechanism [1]. Despite the fact this COL-R strain carrying *mcr-1* was pan-susceptible to all antibiotics, the detection of this gene in a population carrying ESBL-producing HiRC is of great concern because these MDROs can further acquire the *mcr-1* and disseminate across different settings.

Regarding the univariate analysis, only the total CD4 cells count at the time of stool sampling was significantly lower among the colonized than those non-colonized subjects (median 301 *vs*. 706 cells/µl; P=0.02; Table S2 and Figure S1). This observation may have important implications for empirical therapy when HIV+ individuals have serious bacterial infections. However, given the small sample size, our study lacks statistical power. This hinders meaningful multivariable analysis and potentially other associations in the univariate analysis. Additionally, it is possible that our results are representative only for a country (Switzerland) with low-prevalence of ESC-R *E. coli* colonization in the general population alongside HIV+ people under successful ART. Finally, as already reported in the general population, travelling to South-East Asia (P=0.07) and hospitalization abroad (P=0.13) could also be potentially correlated with ESC-R-Ent colonization [1, 2]. It is important to note that we were unable to include a control group due to the lack of information about HIV status and CD4 cell count of healthy people.

This is the first study analyzing the presence of MDR *Enterobacteriaceae* in the intestinal tract of HIV+ people. When under ART, these subjects seem to acquire such pathogens as likely as the general population. However, the identification of HiRC underlines the potential for developing future difficult-to-treat extra-intestinal infections. Moreover, the identification of a strain carrying *mcr-1* on a plasmid with high similarity with others present in food animals and environment highlights potential sources of acquisition of these pathogens. Finally, a low total CD4 count might be an additional risk factor favoring intestinal colonization. Elucidating this phenomenon with a larger cohort will be crucial for a better management of HIV+ patients.

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COMPETING INTERESTS

None declared

ETHICAL APPROVAL

The study was approved by Kantonale Ethikkommission Bern (KEK): *Schweizerische HIV Kohortenstudie* (No. 21/88).

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