

1 **Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae***
2 **in Different Populations in Switzerland: Prevalence, Risk Factors and Molecular Features**

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14 **Running title:** Gut Colonization with cephalosporin-resistant *Enterobacteriaceae* in Switzerland

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23 Sir,

24 The worldwide increase of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-
25 Ent) colonizing the gut of healthy humans is alarming as this is a risk factor to develop future extra-
26 intestinal infections. Colonization has been linked to previous antibiotic consumption and travel to
27 high prevalence areas, but little is known about other populations including those with work-related
28 exposure or with particular predisposing conditions [1, 2]. Therefore, in this study we aimed to
29 estimate the prevalence and identify risk factors for intestinal colonization with ESC-R-Ent in
30 volunteers from different populations in Switzerland.

31 Between July 2013 and November 2016, 337 volunteers living in Switzerland were enrolled in
32 the study. These belonged to the following groups: A, HIV-positive (HIV+) individuals included in
33 the Swiss HIV Cohort (<http://www.shcs.ch/>) (n=101); B, personnel of the human clinical laboratory
34 of the Institute for Infectious Diseases, University of Bern (n=18); C, personnel from veterinary
35 clinics and clinical or research laboratories from the Vetsuisse Faculty, University of Bern (n=164);
36 D, health-care workers from the Department of Infectious Diseases, Bern University Hospital
37 (n=14); and E, healthy volunteers not belonging to any of the previous groups (n=40). Volunteers
38 filled in an epidemiological questionnaire.

39 Stools were enriched overnight in Luria-Bertani broth in different conditions to detect ESC-R-Ent
40 [3]. At least five colonies were tested per each positive growth on selective plates. Species
41 identification was obtained by using the MALDI-TOF MS (Bruker Daltonics, Bremen, Germany).
42 Antimicrobial susceptibility tests (i.e., MICs) were obtained using the microdilution Sentitre™
43 GNX2F plate (TREK Diagnostic Systems, Independence, Ohio, USA) and interpreted with the
44 2016 EUCAST breakpoints (v6.0; www.eucast.org). β -lactamase genes (*bla*) were identified using
45 the CT103XL microarray (Check-Points, Wageningen, The Netherlands) with subsequent PCR and
46 sequencing. Bacterial genotypes were established with MLST (<http://mlst.ucc.ie/mlst/dbs/Ecoli>)
47 and phylogenetic group (PhG) determination for *E. coli* isolates [3]. Statistical analysis was
48 performed comparing colonized and non-colonized individuals' lifestyle factors using GraphPad

49 Prism version 7.0 (La Jolla, California, USA). Continuous variables were analyzed using Mann-
50 Whitney *U* test, whereas categorical variables with Fisher's exact test. P values below 0.05 were
51 considered statistically significant. Odds Ratios (ORs) and Confidence Intervals (CIs) 95% were
52 computed for categorical variables.

53 The overall prevalence of ESC-R-Ent colonization in our study was 7.1% (CI 95% 4.8%-
54 10.4%, n=24/337) and was as follows for each of the different groups: A, 6.9% (CI 95% 3.4%-
55 13.6%; n=7/101); B, 5.6% (CI 95% 0.3%-25.8%; n=1/18); C, 7.3% (CI 95% 4.2%-12.4%;
56 n=12/164); D, 0% (CI 95% 0%-21.5%; n=0/14); and E, 10% (CI 95% 4%-23.1%; n=4/40). Our
57 results are consistent with those identified in surrounding countries [1]. However, the data suggest
58 that the prevalence of ESC-R-Ent is steadily increasing as previous Swiss studies reported
59 colonization rates between 2.8%-5.8% [1, 4].

60 A total of 28 ESC-R-Ent were recovered, of which 89.3% (n=25) were *E. coli*, and the remaining
61 were *K. pneumoniae*, *E. cloacae* and *E. fergusonii* (each, n=1) (Table 1). Three individuals were
62 found to be colonized with more than one ESC-R-Ent: two subjects possessed two different *E. coli*
63 and another one had two *E. coli* and one *E. fergusonii*. Overall, for the ESC-R-Ent resistance to
64 non- β -lactams was high given that 53.6% were resistant to doxycycline, 46.4% to trimethoprim-
65 sulphamethoxazole, 39.3% to aminoglycosides, and 32.1% to fluoroquinolones. Apart from for
66 aminoglycosides, resistance to non- β -lactams was lower than previously reported in Switzerland
67 [4].

68 As shown in Table 1, ESBLs were the most frequent resistance mechanism identified (96.4%), with
69 CTX-M-15 being the most common (67.9%). *E. coli* population structure revealed that most isolates
70 belong to PhG A (36%), but also to B2 or D (28% each), and to lesser extent B1 (8%). Interestingly,
71 MLST identified High Risk Clones (HiRC, 46.4%), including B2-ST131, B2-ST73, B2-ST127, D-
72 ST648, D-ST405, D-ST69, A-ST10 and A-ST410. The identification of HiRC in the gut is
73 concerning since these bacteria frequently cause infections, posing a threat for the carriers' health
74 [5].

75 A total of 202 (59.9%) questionnaires were returned. All participants from groups A, D and E
76 provided a questionnaire, whereas from groups B and C we received one and 46, respectively.
77 Among all studied factors, only hospitalization abroad in the past five years was significantly
78 associated with ESC-R-Ent colonization (OR 15.5, CI 95% 2.2-101.8, P=0.02), whereas travel
79 abroad, antibiotic intake, diet type, nor pets were associated with colonization (Supplementary
80 Table 1). Moreover, no particular group was associated with an increased risk of being colonized
81 with ESC-R-Ent (P=0.78). This is surprising as we would expect that people working in human or
82 veterinary hospital/laboratory environments would be more likely to be exposed to and colonized
83 with ESC-R-Ent. However, our findings are partially in line with a previous meta-analysis, which
84 demonstrated that neither lifetime hospitalization nor hospitalization in the past year or contact with
85 pets were predictors of colonization [1]. The identification of “hospitalization abroad” as a risk
86 factor for colonization is most likely due to the high prevalence of ESC-R-Ent in foreign hospitals
87 compared to Switzerland.

88 Although the studied population is not representative of the entire country, our results suggest
89 that the prevalence of ESC-R-Ent colonizing the gut of healthy people is increasing in Switzerland,
90 a trend also observed in surrounding countries. Moreover, hospitalization abroad seems to
91 contribute significantly to this phenomenon. Additionally, the high proportion of HiRCs found in
92 the gut of healthy people should raise awareness for the role of this niche in the dissemination of
93 life-threatening pathogens.

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98 **COMPETING INTERESTS**

99 None declared

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101 **ETHICAL APPROVAL**

102 HIV positive individuals: Kantonale Ethikkommission Bern: Schweizerische HIV Kohortenstudie
103 [No. 21/88]; personnel from the IFIK and Department of Infectious Diseases: evaluated as part of a
104 risk assessment by the hospital's infection prevention program; personnel from the Vetsuisse
105 Faculty: signed a written consent; remaining healthy individuals: Ethikkommission Nordwest- und
106 Zentralschweiz (EKNZ 239/12).

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108 **AUTHOR CONTRIBUTION**

109 Conception and design (AE); volunteer recruitment (EK, CH, CS, AR, HF, VP, JM, CH);
110 acquisition of data (JP, EK, CH, CS, RT, SK); analysis of data (JP, AA, AE); drafting the work (JP,
111 AE); critical revision of the work (JP, EK, CH, CS, AR, HF, VP, JM, AE); final approval of the
112 manuscript (all authors).

113

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136 **Table 1.** Molecular and phenotypic features of recovered ESC-R-Ent within the different groups

Group	ID volunteer	Main β -lactamase ^a	Species	PhG-ST ^b	Antimicrobial susceptibility test results (MICs, $\mu\text{g/mL}$) ^c
A	32094	CTX-M-15	<i>E. coli</i>	B ₂ -ST127	FOT (16), TAZ (2), FEP (8), AZT (4), GEN (>16), TOB (8)
		CTX-M-15	<i>E. coli</i>	D ₂ -ST405 (CC405)	TIM (64), FOT (>64), TAZ (16), FEP (16), AZT (>32), TOB (>16), AMI (\leq 4), CIP (>4), LEV (>16), SXT (>8/152), DOX (16)
	32117	CTX-M-15	<i>E. coli</i>	A ₁ -ST410 (CC23)	TIM (32), FOT (>64), TAZ (16), FEP (8), AZT (>32), CIP (>4), LEV (>16), SXT (>8/152), DOX (16)
	32315	CTX-M-15	<i>E. coli</i>	B ₂ -ST131 (CC131)	TIM (32), FOT (32), TAZ (8), FEP (4), AZT (16), TOB (>16), CIP (>4), LEV (8)
	31643	CTX-M-14	<i>E. coli</i>	B ₂ -ST73 (CC73)	TIM (128), FOT (>32), TAZ (4), FEP (>32), AZT (32)
	32300	CTX-M-15	<i>E. coli</i>	B1-ST5173	FOT (32), TAZ (4), FEP (4), AZT (8)
	31570	CTX-M-15	<i>E. coli</i>	A ₀ -ST189 (CC165)	TIM (128), FOT (32), TAZ (4), AZT (16), DOX (8)
	32388	CTX-M-8	<i>E. coli</i>	B ₂ -1170	TIM (32), FOT (8), TAZ (2), FEP (4)
B	KM1	CTX-M-1	<i>E. coli</i>	D ₂ -ST38 (CC38)	TAZ (\geq 32), FEP (4), DOX (4)
C	2292581	CTX-M-14-like	<i>E. coli</i>	D ₁ -ST31 (CC31)	FOT(8), DOX (8)
	2330192	CTX-M-1-like	<i>E. coli</i>	A ₁ -ST1312	FOT (16), FEP (4), AZT (4), DOX (16),
	2349998	CTX-M--1-like	<i>E. coli</i>	A ₁ -ST1312	FOT (16), FEP (4), AZT (8), DOX (16)
	2354728	CMY-2-like	<i>E. coli</i>	B ₂ -ST131 (CC131)	TIM (64), FOT (16), TAZ (\geq 32), AZT (8), DOX (8), MIN (4)
	2378367	CTX-M-15-like	<i>E. coli</i>	B ₂ -ST131 (CC131)	TIM (32), FOT (\geq 64), TAZ (8), FEP (4), AZT (\geq 32), DOX (4), MIN (4)
		CTX-M-15-like	<i>E. coli</i>	A ₀ -STNEW	TIM (16), FOT (\geq 64), TAZ (8), FEP (4), AZT (\geq 32)
	2382871	CTX-M-15-like	<i>E. coli</i>	D ₂ -ST405 (CC405)	TIM (32), TAZ (16), FOT (\geq 64), AZT (\geq 32), GEN (\geq 16), TOB (4), CIP (\geq 4), DOX (\geq 32), MIN (\geq 32)
	2474813	CTX-M-15	<i>K. pneumoniae</i>	-	TIM (64), FOT (32), TAZ (16), FEP (4), AZT (\geq 32), TOB (4), CIP (\geq 4), LEV (4), SXT (\geq 8/152), DOX (16), MIN (4)
	2498698	CTX-M-1-like	<i>E. coli</i>	A ₁ -ST10 (CC10)	FOT (8), AZT (8), GEN (\geq 16), TOB (4), SXT (\geq 8/152), DOX (\geq 32), MIN (8)
		CTX-M-1-like	<i>E. coli</i>	B ₁ -ST641 (CC86)	TIM (32), FOT (16), TAZ (4), FEP (8), AZT (8), GEN (\geq 16), TOB (2), SXT (\geq 8/152), DOX (\geq 32), MIN (\geq 32)
		CTX-M-1-like	<i>E. fergusonii</i>	-	FOT (\geq 64), FEP (4), AZT (8), GEN (\geq 16), TOB (4), STX (\geq 8/152)
	2501192	CTX-M-15-like	<i>E. coli</i>	B ₁ -ST205 (CC205)	TIM (32), FOT (32), TAZ (8), FEP (8), AZT (\geq 32), MER (4), CIP (\geq 4), LEV (\geq 16), SXT (\geq 8/152), DOX (16)
	2502424	CTX-M-14-like	<i>E. coli</i>	A ₁ -ST10 (CC10)	TIM (64), FOT (8)
	2503490	SHV-12-like	<i>E. cloacae</i>	-	TIM (32), TAZ (4), FOT (\geq 64), AZT (\geq 32), GEN (\geq 16), TOB (\geq 16), AMI (8), GEN (\geq 16), TOB (\geq 16), SXT (\geq 8/152), DOX (\geq 32), MIN (\geq 32)
2506378	CTX-M-14-like	<i>E. coli</i>	D ₁ -ST69 (CC69)	FOT (8), DOX (\geq 32), MIN (16),	
E	29	CTX-M-14	<i>E. coli</i>	D ₂ -ST648 (CC648)	TIM (32), FOT (\geq 64), AZT (4), TOB (8), CIP (\geq 4), LEV (\geq 16), SXT (\geq 8/152), DOX (\geq 32), MIN (8)
	50	CTX-M-15	<i>E. coli</i>	B ₂ -ST131 (CC131)	TIM (32), FOT (32), TAZ (8), FEP (4), AZT (16), GEN (\geq 16), TOB (\geq 16), LEV (8), CIP (\geq 4), SXT (\geq 8/152), DOX (4)
	59	CTX-M-15	<i>E. coli</i>	A ₁ -ST59	FOT (32), TAZ (4), AZT (8), CIP (2), LEV (4), SXT (\geq 8/152),
	HV1	CTX-M-15	<i>E. coli</i>	A ₁ -ST1312	FOT (8), AZT (4), SXT (\geq 8/152)

137 **Notes.** A, HIV+ individuals; B, personnel from human clinical laboratories; C, personnel from veterinary clinical or research laboratories; and E, others. TIM, ticarcillin/clavulanic acid; FOT, cefotaxime; TAZ, ceftazidime; FEP, cefepime; AZT, aztreonam; MERO, meropenem; AMI, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; SXT, Trimethoprim/sulfamethoxazole; DOX, doxycycline, MIN, minocycline; ST, sequence type; CC, clonal complex.

141 ^a Only β -lactamases conferring resistance to extended-spectrum cephalosporins are shown

142 ^b Phylogenetic group and ST are only shown for *E. coli*

143 ^c MIC values were interpreted according to the EUCAST criteria (Version 6.0; www.eucast.org), except for doxycycline and minocycline for which CLSI guidelines (document M100-S26) were used. Values presented represent only those from intermediate or resistant categories