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ORIGINAL ARTICLE

Novel insights into pivotal risk factors for rectal carriage of extended-spectrum-β-lactamase-producing enterobacterales within the general population in Lower Saxony, Germany

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Abstract

Aims: To estimate the prevalence of extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E) carriage in the general population of Lower Saxony, Germany, and to identify risk factors for being colonized.

Methods and Results: Participants were recruited through local press and information events. Detection of ESBL-E by culture was conducted using ESBL-selective chromagar plates containing third-generation cephalosporins. Identification of pathogens was performed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)_technology on Vitek mass spectrometry. Antibiotic susceptibility testing was conducted by microdilution (Vitek II) and an ESBL confirmation assay was carried out using a combination disk test. Of 527 randomly collected stool samples from healthy volunteers, 5.5% were tested positive for ESBL-E. Post-stratification for age and gender yielded a similar population estimate (5.9%). People traveling abroad and taking antibiotics had the greatest rectal ESBL-E carriage.

Conclusions: Potential risk factors (eg, working in healthcare facilities, recent inpatient stay) did not attribute to rectal ESBL-E carriage as other factors (eg, travelling, taking antibiotics). Rectal ESBL-E carriage within the general population seems to be high.

Significance and Impact of the Study: The known risk factors for carriage with MDRO might not be fully applicable to ESBL-E and require further examination in order to develop effective strategies for the prevention of ESBL-E dissemination within the general population.

KEYWORDS
general population, hygiene, infection control, multidrug-resistant organisms (MDRO), multi-resistant pathogens

INTRODUCTION

The prevalence of multidrug-resistant organisms (MDRO) with resistance characteristics against multiple currently used antibiotics has notably increased over the recent years both in Germany as well as worldwide (Marx, 2016). Infections with MDRO may lead to higher patient morbidity and mortality as well as elevated health expenditures (de Kraker et al., 2011), which highlights the consequences on the patient level but also the peremptorily emerging macrosocial problems. Even though the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) on all *Staphylococcus aureus* isolates from clinical specimens decreased in recent years, the prevalence of MDR-GNB is reported to have increased substantially (Maechler et al., 2017). Possible explanations for the current rise of MDR-GNB carriage might be the progressive production and use of antibiotics in human and veterinary medicine, the growing number of asylum seekers from countries with high MDR-GNB prevalence (Krüger et al., 2016), the ascending quantity of international travels and mobility increases (Meyer et al., 2012), the rising number of long-term care facilities (Gruber et al., 2013), and a frequent contact with livestock (Ewers et al., 2012). Further aspects are foodborne transmission and human-to-human transmission in households as well as pet-to-human transmission in households, and transmission in healthcare facilities such as hospitals (Köck et al., 2021). Extended-spectrum-β-lactamases (ESBL) are among the most common resistance mechanisms (Bradford, 2001). A study from 2014 found an intestinal carriage of ESBL-producing *Escherichia coli* in 6.3% of 3344 study participants representing the Bavarian community (Valenza et al., 2014). These results lead—together with similar findings of further current studies—to the assumption that there might be a reservoir of ESBL-E outside of known risk groups, as, e.g., hospitals and nursing homes, which leads to increasing carriage within the general population (Ben-Ami et al., 2009). Such a reservoir would be of great impact with respect to screening regimes and hygiene considerations as well as for the prevention of MDRO-related infections in general (Krüger et al., 2016; Valenza et al., 2014).

Even though MDR-GNB have justifiably been identified as a bacterial challenge of the twenty-first century (World Health Organization, 2020), most of the currently conducted studies focus on examining the prevalence of MDR-GNB carriage in risk groups or patients who are already ill (Rohde et al., 2020). However, the ESBL-E prevalence within the common healthy general population has not yet been sufficiently investigated; most studies assessing ESBL carriage in the general population are rather old. The present study aims at estimating in a prospective way the ESBL-E prevalence and associated risk factors for carriage in the healthy general population not related to healthcare in a North-Western European region. Another goal of the current study is to identify quintessential risk factors for rectal ESBL-E carriage.

METHODS

Study participants

Only participants with a place of residence in the western part of the federal state Lower Saxony, Germany were included in this study. Participants were recruited through diverse information events organized within a regional network of healthcare providers for MDRO prevention in the city of Osnabrueck, Lower Saxony, as well as quality circles of hygiene specialists from local hospitals and the Laboratory Medical Practice Osnabrueck. Further, the study was promoted in the local press to facilitate the widest possible distribution within the target population. Interested participants received a test kit including an information, a letter of consent, a questionnaire assessing potential risk factors (eg, travelling abroad, hospital stay), and a specimen tube for selfcollection of stool samples together with a sample bag for sending the sample to the laboratory. In total, 1800 test kits were distributed. Informed consent was obtained from all individual participants included in the study. Sample transport was free of charge for all participants. Sample collection and analyses have been performed from December 2018 to December 2019.

Microbiological analyses

Materials are listed in Table S1 according to the corresponding work step. The stool samples were suspended in sterile NaCl-solution and detection of ESBL-E by culture was conducted using ESBL-selective chromagar plates (Brilliance™ ESBL chromagar plates, order number P05302A, Thermo Fisher Scientific Inc.) before and after broth-enrichment by standard procedures published previously (Dahms et al., 2015; Hamprecht et al., 2016;

Krüger et al., 2016). All grown colonies on the selective plates were identified and differentiated with MALDI-TOF-Technology by the use of a Vitek® mass spectrometry (MS) system (bioMérieux Deutschland GmbH) following a previously described protocol (Hamprecht et al., 2016). In parallel, antibiotic susceptibility testing (AST) of all grown colonies of interest was conducted with a Vitek® 2 system (bioMérieux Deutschland GmbH) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (Hamprecht et al., 2016). An ESBL confirmation assay was carried out using a combination disk test (CDT) according to the guidelines of EUCAST breakpoint tables version 9.0 (2019).

Statistical analyses

The first goal of the present study was to estimate the prevalence of asymptomatic MDR-GNB carriage (including pseudomonas and acinetobacter) in the general healthy population. Due to the anonymized collection of specimens, it cannot entirely be ruled out that more than one person from one household participated. However, due to the vast spread of test tubes amongst the population, this likely will not frequently have been the case. In addition to computing the raw prevalence in our sample, we used multilevel regression and poststratification (MRP) to get an adjusted prevalence estimate. It has been shown in the past that utilizing this approach can reduce bias in epidemiological estimates (Loux et al., 2019). It mitigates the problem that samples can differ from the general population with respect to certain characteristics (eg, proportion of women, proportion of old people) by aligning sample proportions with population proportions after data collection (poststratification) by the means of weighting. In the present study, Bayesian multilevel logistic regression was used with ESBL-E test result as dependent variable (positive vs. negative finding) and potential risk factors (eg, age and gender) as predictors in order to get the ESBL-E prevalence estimate for each group. The resulting regression coefficients were then weighted by the relative size of each population stratum in order to estimate the overall prevalence in the population. Information about the size of population strata was obtained from census data and other surveys (Supplement S2).

The second objective of the study concerns the identification of risk factors for MDR-GNB carriage. Several potential risk factors were assessed via questionnaire and we computed the raw prevalence of ESBL-E for each group (eg, antibiotics use vs. no antibiotics use). In addition, adjusted prevalence estimates were computed for several risk factors utilizing the MRP approach described above. Strata were based on the risk factor of interest, age and/or gender. Finally, odds ratios were estimated for each risk factor by running separate Bayesian logistic regression analyses with one risk factor at a time. Odds ratios larger than 1 mean, that the odds for rectal ESBL-E carriage are larger in the risk group (eg, antibiotics use) than in the reference group (eg, no use of antibiotics). In contrast, values smaller than 1 imply that the odds for rectal ESBL-E carriage are larger in the reference group.

In our analyses, we accounted for the fact that 34 participants (two people with positive ESBL-E findings) did not answer all questions (eg, age, meat consumption). In order to ensure comparability of the results across analyses missing data were imputed. Specifically, each missing value was imputed 10 times through fully conditional specification (van Buuren, 2018). The reported results are based on the pooled results of all 10 data sets. For R code and additional information, see Supplement S3.

Ethical statement

Ethics approval was obtained from the ethics committee of the Osnabrueck University (file number: 4/71043.5).

RESULTS

In total, 531 (29.5%) out of 1800 distributed test kits were sent back by the participants. Out of the 531 returned test kits, 4 (0.8%) were excluded due to the fact that the participants did not have a place of residence in Lower Saxony, Germany. Of the remaining stool samples ($n = 527$), 30 (5.5%) were tested positive for ESBL-E. The 30 positive samples were from 30 different persons, so that the percentages shown are actually the prevalence of carriage rather than just the proportion of positive samples in all samples. Within the samples, carriage of multidrug-resistant *E. coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* was evidenced (Table 1). Thereby, *E. coli* dominated noticeably. Out of 527 samples, ESBL-producing *E. coli* was detected 28 (5.3%) times; in 13 (46.4%) of 28 cases, ESBL-producing *E. coli* had an additional resistance mechanism against fluorochinolones, so that they were classified as 3MRGN (multidrug-resistant gram-negative bacteria) according to the *German Commission for Hospital Hygiene and Infection Prevention* (KRINKO) MDR-GNB classification. Other detected co-resistances are listed in Table 1. ESBL-producing *K. pneumoniae* was found 2 (0.4%) times within the 527 samples; in 1 (50.0%) of the 2 cases, ESBL-producing *K. pneumoniae* was found with the additional fluorochinolone-resistance characteristic. ESBL-producing *C. freundii* with the additional 3MRGN resistance characteristic was present in 1 (0.2%)

TABLE 1 Isolates of extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E) in the present study

		Isolates	
Bacterial species	**Co-resistance**	**%**	n/n_{total}
Escherichia coli	None	1.9	10/527
Escherichia coli	CTX	0.9	5/527
Escherichia coli	FC	2.4	13/527
Escherichia coli	FC, CTX	1.3	7/527
Escherichia coli	FC, GEN	0.2	1/527
Escherichia coli	FC, CTX, GEN	0.6	3/527
Klebsiella pneumoniae	CTX	0.2	1/527
Klebsiella pneumoniae	FC, CTX	0.2	1/527
Citrobacter freundii	FC	0.2	1/527
All		5.5	30/527

Abbreviations: *C. freundii*: *Citrobacter freundii*; CTX: Cotrimoxazol;*E. coli*: *Escherichia coli*; FC: Fluorchinolon; GEN: Gentamicin; *K. pneumoniae*: *Klebsiella pneumoniae*; n.a.: not applicable.

out of the 527 samples. In 29 (96.7%) of the 30 MDR-GNB-positive samples only one bacterial species was found, whereas in 1 (3.3%) of the 30 MDR-GNB-positive samples, ESBL-producing *E. coli*, as well as ESBL-producing *K. pneumoniae*, were found jointly. No carbapenem-resistant enterobacterales, acinetobacter or pseudomonas were found.

Evaluation of the questionnaires revealed that out of the ESBL-E-positive participants ($n = 30$), 16 (53.3%) were female, 13 (43.3%) were male and 1 (3.3%) did not disclose the gender. The age of the ESBL-E-positive population ranged from 4 months to 70 years with the mean age being 41.47 ± 20.16 years. 23 (76.7%) of the 30 ESBL-E-positive participants stated that they have travelled abroad (within the last 12 months), 18 (60.0%) declared to consume meat on a regular basis (>3 times per week), 13 (43.3%) stated to have had a bath in natural waters (ie, rivers, lakes) (within the last 12 months), 12 (40.0%) stated that they have a pet, 11 (36.7%) stated to have taken antibiotics (within the last 12 months), 10 (33.3%) stated that they work in the healthcare system, 5 (16.7%) stated that they work in the agricultural sector, 4 (13.3%) stated that they had a hospital stay (within the last 12 months) and none stated that they work in the wastewater industry.

The relative frequency of risk factors in the study sample ($n = 527$), as well as the ESBL-E-prevalence in the respective group, are outlined in Table 2. In general, the corrected prevalence estimates (multi-level regression and poststratification) do not differ strongly from raw prevalence estimates. Corrected estimates are not reported for all potential risk factors because information about the combined distribution (eg, pet keeping + gender + age) in the population was not always available. The risk factors of travelling abroad and the use of antibiotics as well as the combination of these two determinants emerge as predominant risk factors for rectal ESBL-E carriage.

In Figure 1, the numbers of positive ESBL-E findings in different age groups are displayed. It could be shown that rectal ESBL-E carriage is present amongst a wide variety of age groups. The age group ranging from 20 to 29 years comprises the most persons colonised with ESBL-E. Figure 2 depicts the number of persons with and without the risk factor of having had a hospital stay and an accompanying rectal ESBL-E carriage across the age groups. The results indicate that rectal ESBL-E carriage does not differ strongly between persons having a hospital stay and people not having a hospital stay. The number of persons who used and who did not use antibiotics are shown in Figure 3 together with positive ESBL-E findings in these groups. rectal ESBL-E carriage seems to be greater in persons taking antibiotics (see age groups 20–29 or 50–59). This pattern of results holds after poststratifying for age and gender (see Table 2).

DISCUSSION

Within the study sample ($n = 527$)—which represents the general population in parts of western Lower Saxony—a relevant rectal ESBL-E carriage rate of 5.5% could be determined. MRP—which was used in order to get an adjusted prevalence estimate—revealed an adjusted rate of rectal ESBL-E carriage for the general population of 5.9% (Table 2). The fact that raw and adjusted estimates are quite similar points in favour of the notion that the results are robust. The positive ESBL-E quota within this study in the general population corresponds with previously published German data collected in inpatients and outpatients (Eckmanns et al., 2014; Valenza et al., 2014). Furthermore, our findings are well comparable with rectal ESBL-E carriage data recorded in other European countries (Geser et al., 2012; Nicolas-Chanoine et al., 2013; Vendrik et al., 2021). The comparable distribution within European countries could lead to the assumption that ESBL-E acquisition might be traced back to similar environmental conditions for instance as a consequence of common agricultural and water policies within the European Union environmental regulations, as one train of thought. Correspondingly, rectal ESBL-E carriage in countries outside of the European Union (eg, India, Nepal and Egypt) widely varies from the observed rectal ESBL-E carriage in Germany in terms of a much higher quota (Abdul Rahman & El-Sherif, 2011; Subramanya et al., 2021; Vento et al., 2013). Considering the tremendous dissimilarities between the healthcare

TABLE 2 Relative frequency of risk factors in the sample ($n = 527$), extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E)-prevalence in the respective groups and odds ratios (OR) comparing the prevalence across groups

Group				
No.	**Explanation**	**Sample percentage**	**MDR-GNB prevalence (%)**	**OR (95% credibility intervals)**
0	All	100	5.5 (5.9)[III]	
1a	Women	62.8	4.8 (5.1)[IV]	0.74 (0.36; 1.50)
1b	Men	37.2	6.7 (6.9)[IV]	
2a	Age ≤35	31.0	7.9 (7.1)[V]	0.99 (0.97; 1.01)
2b	Age >35 and ≤55	31.9	4.8 (5.6)[V]	
2c	Age >55	37.0	4.1 (5.0)[V]	
3a	Travelling abroad[I]	63.9	6.5 (6.7/6.7)[VI]	1.69 (0.80; 3.78)
3b	No travelling abroad[I]	36.1	3.7 (4.1/4.3)[VI]	
4a	Regular meat consumption[II]	64.4	5.0	0.81 (0.40; 1.68)
4b	No regular meat consumption[II]	35.6	6.4	
5a	Bathing in natural waters[I]	46.2	5.3	0.95 (0.47; 1.90)
5b	No bathing in natural waters[I]	53.8	5.6	
6a	Pet keeping	42.4	4.9	0.84 (0.41; 1.70)
6b	No pet keeping	57.6	5.9	
7a	Use of antibiotics[I]	22.5	9.3 (9.0)[IV]	1.93 (0.91; 3.97)
7b	No use of antibiotics[I]	77.5	4.4 (4.8)[IV]	
8a	Working in the healthcare system	36.2	4.7 (5.0)[III]	0.80 (0.38; 1.66)
8b	Not working in the healthcare system	63.8	5.9 (6.2)[III]	
9a	Working in the agricultural sector	5.6	13.7	2.08 (0.67; 5.66)
9b	Not working in the agricultural sector	94.4	5.0	
10a	Having a hospital stay[I]	16.9	3.4 (4.2/4.1)[VI]	0.62 (0.21; 1.58)
10b	No hospital stay[I]	83.1	5.9 (6.0/6.2)[VI]	
11a	Working in the wastewater industry	0.6	0.0	-
11b	Not working in the wastewater industry	99.4	5.5	
12a	3a & 7a	15.2	10.0	-
12b	3a & 7b	48.7	5.5	
12c	3b & 7a	7.3	7.8	
12d	3b & 7b	28.8	2.6	

Note: Mean percentage based on 10 imputations is reported in the middle columns because the results did not vary substantially between imputations (1.6% as the largest difference for sample percentage, and 0.8% as the largest difference for MDR-GNB prevalence across imputations). Values in parentheses represent prevalence estimates based on MRP, which account for the fact that the distribution of certain variables (eg, gender) in our sample differs from the distribution in the population. Both adjusted prevalence estimates and odds ratios are based on Bayesian logistic regression. OR for age is based on the metric age variable rather than artificial age groups in order to avoid information loss. OR for the wastewater industry was not estimated since there were only a few people working in this area. Number of missing values before imputation: Gender = 13, Age = 10, Traveling abroad = 7, Meat consumption = 10, Bathing in natural waters = 7, Pets = 8, Antibiotics = 6, Job in the healthcare system = 6, Job in the agricultural sector = 6, Hospital stay = 8, Job in the wastewater industry = 6. The imputations were carried out using the R package *mice*.

Abbreviations: [II]>3 times per week; [III]Age and gender differences were considered together in the poststratification. [IV]Age differences were considered in the poststratification; [I]within the last 12 months; No. = number; OR = Raw odds ratio together with 95% credible intervals; [V]Gender differences were considered in the poststratification; [VI]Age differences and gender differences were considered separately in the poststratification, the age-adjusted estimate is reported first, followed by the gender-adjusted estimate.

systems on a global scale, the above findings support the hypothesis that ESBL-E spread might follow a spectrum of pathways different from other MDRO, like MRSA; the latter seemingly related to the state of the healthcare systems, whereas ESBL-E is not or to a much lesser extent.

The present carriage rate of ESBL-E noticeably exceeds the carriage rate of MRSA of 0.5% within the general population in Germany (Becker et al., 2017; Köck et al., 2016). MRSA is widely known as a problematic pathogen as well as a significant originator of nosocomial infections with a

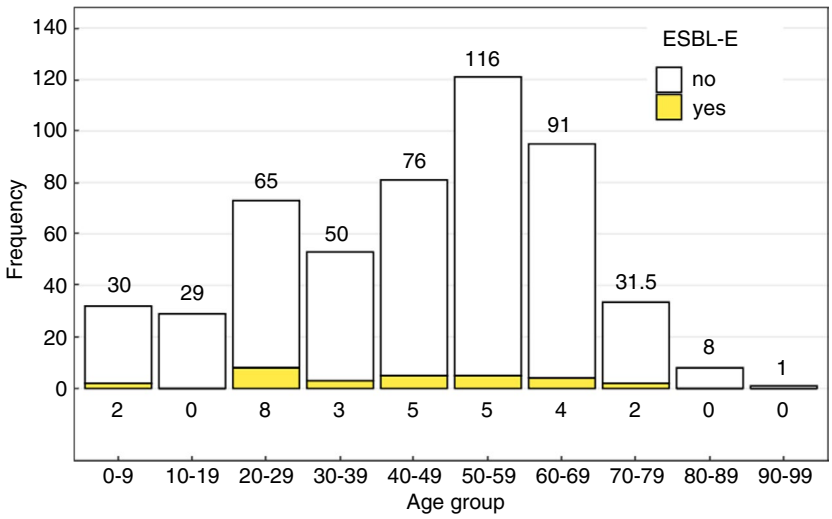

FIGURE 1 Age distribution in the sample. The number of positive extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E) findings in different age groups is reported. The results are based on median values from multiple imputations (10 age values were missing in the original data). Hence, decimal numerals are provided for the age category 70–79

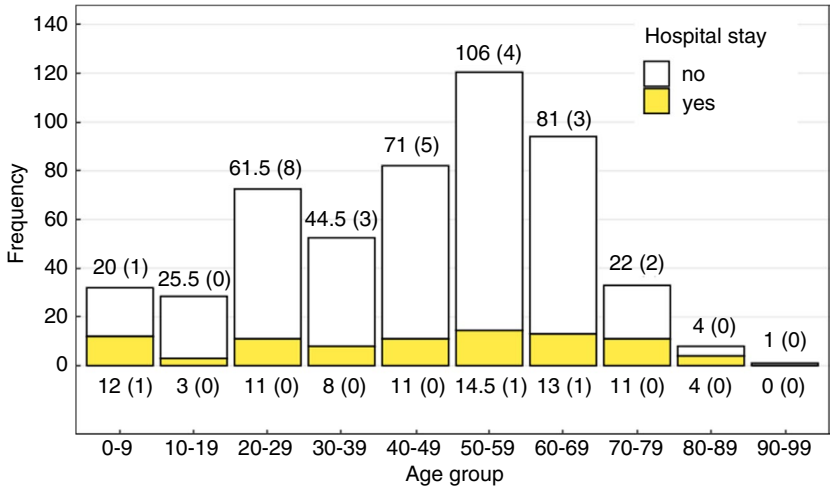

FIGURE 2 Number of people with/without hospital stay in different age groups. The number of positive extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E) findings is reported in parentheses. The results are based on median values from multiple imputations (10 age values and 8 hospital stay values were missing in the original data). Hence, decimal numerals are given for some age categories

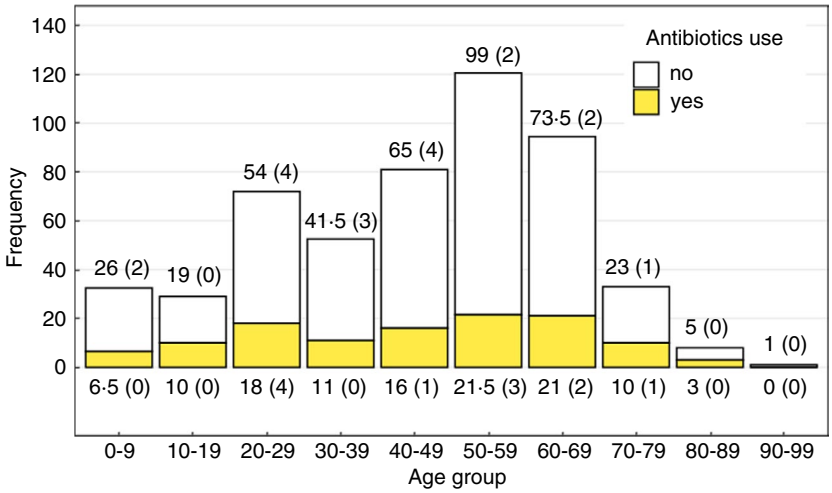

FIGURE 3 Number of people with/without antibiotics use in different age groups. The number of positive extended-spectrum-β-lactamase (ESBL)-producing enterobacterales (ESBL-E) findings is reported in parentheses. The results are based on median values from multiple imputations (10 age values and 6 antibiotics use values were missing in the original data). Hence, decimal numerals are given for some age categories

potentially detrimental course of disease (de Kraker et al., 2011), which is also extensively communicated within specialist publications as well as the lay press. On the contrary, ESBL-E seems to be widely overlooked in the public perception. The pertinence of rectal ESBL-E carriage seems to have been underestimated in the public health policy and health policy perception which leads to the fact that—contrary to MRSA—far less developed structures exist for tackling the increasing spread of ESBL-E within the general public and the environment on a global scale. Also, the risk of an endogenous infection after carriage of multi-drug-resistant *E. coli* as well as *K. pneumoniae*

should be considered (Denkel et al., 2020). This is based on the fact that within this study sample ESBL-producing *E. coli* dominated noticeably (Table 1). Data from the Laboratory Medical Practice Osnabrueck, Germany (J. Esser, unpublished) concerning urinary infection analytics in 2019 reveal that out of 9984 submissions from outpatients within the same geographical catchment area as in the current study, 5.8% of all recognised *E. coli*—as a cause of the urinary tract infection—were ESBL-E (2.0% *E. coli* ESBL, 3.8% *E. coli* ESBL with co-resistance against ciprofloxacin). These numbers are substantiated by data from the Public Health Agency of Lower Saxony, Germany from the so-called ARMIN project (monitoring of antibiotic resistance in Lower Saxony) (Health Office of Lower Saxony, 2019). The ARMIN data from 2017 reveal 6.5% third-generation cephalosporin-resistant *E. coli* from outpatients urinary-tract material. They correspond to the findings of the present study and further demonstrate that enteral rectal ESBL-E carriage leads to clinical consequences in practice. Due to antibiotic resistance, treatment on a case-by-case basis is substantially more difficult since the calculated initial therapy often fails.

In order to prevent further dissemination of ESBL-E, it is indispensable to identify and interrupt the ways in which ESBL-E are entering the human intestinal flora. At the present time, typical risk factors for the acquisition of MDRO (age, mounting morbidity, hospital stays, metabolic diseases as diabetes mellitus or renal insufficiency) serve as a basis for predicting those aforementioned ways and for determining the screening and isolation measurements in hospitals. It is further known that rectal ESBL-E carriage is often attained whilst traveling abroad—especially traveling to Southeast Asia (Meyer et al., 2012). Also, human-to-human transmission (ie, in households, etc.) is an important transmission route (Köck et al., 2021). There is growing evidence that ESBL-E—as opposed to MRSA or other MDRO—is ingested in regular everyday life orally via food or water as well as on the road during travelling, which points out that humans might be part of a worldwide closed ESBL-E dissemination cycle (Westphal-Settele et al., 2018). As the aforementioned way is identified as the essential way of acquiring rectal ESBL-E carriage, it will be inevitable to establish measures which reduce the appearance of ESBL-E within the worldwide circulation. In this sense, the most basic and fundamental action would be the reduction of the use of antibiotics in human medicine as well as veterinary medicine and subsequently the antibiotic levels in wastewater and soil along with all the consequences for the environment.

In order to identify whether the aforementioned way of ingestion is of high relevance, the present study was conducted with participants of the general population in Lower Saxony, Germany. The negligible association with age (OR: 0.99 [95% CI: 0.97–1.01]) and the consistent occurrence of ESBL-E in all age groups—especially in children, adolescents and young adults—indicate that the known risk factors for the acquisition of MDRO appear not to be of relevance for the inspected study sample (Figure 1). Elsewise, it could have been expected that an increasing ESBL-E prevalence would have been found with increasing age. Also, the potential risk factor of having a hospital stay could not be confirmed in the present study. Specifically, there was no substantially increased ESBL-E occurrence in people with previous hospital stays (Figure 2) (OR: 0.62 [95% CI: 0.21–1.58]). It also was striking and surprising that ESBL-E prevalence rate was not greater for people working in the healthcare system—which entails contact with potentially ESBL-E colonized or infected people—than for other people (OR: 0.80 [95% CI: 0.38–1.66]) so that the transmission path of nosocomial contact/smear infection seems to have little importance with regard to ESBL-E acquisition for medical staff (Mellmann et al., 2016). Circulation of ESBL-E within stationary units might accordingly have a weaker importance in terms of ESBL-E acquisition for the otherwise healthy population. ESBL-E outbreaks in inpatients were regardless reported in several cases especially concerning *Klebsiella* spp. and *Acinetobacter* spp., which were found in low numbers or rather not at all, respectively, in our study participants (Table 1) (Steul et al., 2020).

Within the present study, the following predictors for rectal ESBL-E carriage were identified. A history of travels—which has been identified as a predictor for rectal ESBL-E carriage in previous studies (Meyer et al., 2012)—is accompanied by a noticeable increase of rectal ESBL-E carriage (ESBL-E prevalence rate of 6.5% for people who have travelled vs. 3.7% for people who have not travelled); especially travels to Asia likely act a part in this (Meyer et al., 2012). At this, specific recommendations for preventing Travellers' diarrhoea could take effect: *cook it*, *peel it*, *boil it or leave it*, which targets the oral uptake as a significant entranceway of ESBL-E. The intake of antibiotics (ESBL-E prevalence rate for people who have taken antibiotics of 9.3% vs. 4.4% for people who have not taken antibiotics) poses a risk factor for rectal ESBL-E carriage across all age groups (Figure 3). It could be postulated that due to the collateral damage of every treatment with antibiotics regarding the physiological intestinal flora, the susceptibility for orally ingested ESBL-E is increasing (Pilmis et al., 2020). The colonisation barrier of the intestine is thus impaired, which is why ESBL-E are absorbed by the intestinal flora after oral uptake. Our study provides preliminary hints that interactions between risk factors may be relevant—eg, travelling abroad and the use of antibiotics; the combination of these two most striking predictors

increases the risk for rectal ESBL-E carriage considerably (Table 2). Meat consumption was not accompanied by increased odds for rectal ESBL-E carriage (OR: 0.81 [95% CI: 0.40–1.68]), presumably due to the fact that also all other foods along the product chain might be contaminated with ESBL-E (Belmar Campos et al., 2014)—as, e.g., through irrigation of fruits and vegetables. Further, the factors of pet keeping (OR: 0.84 [95% CI: 0.41–1.70]) and bathing in natural waters (OR: 0.95 [95% CI: 0.47–1.90]) did not show a positive correlation with rectal ESBL-E carriage (Table S2). It must be noted that for the factors of working in the agricultural sector as well as working in the wastewater industry, no prevalence estimates based on MRP could be given due to the fact that the necessary population data were not available (Table 2). However, larger samples or samples with larger numbers of ESBL-E positive findings are needed in the future to further examine the carriage patterns described in this study.

Regarding the results of Table 2, a potential selection bias has to be discussed as the present dataset has indicators for skewness (ie, one-third of all participants were working in the healthcare sector and many of them took bathes in natural waters, such as lakes or rivers). To further investigate this imbalance of some risk factors we conducted the extensive poststratification with the outcome that—as already mentioned—raw and adjusted estimates are quite similar. This demonstrates the robustness of our raw findings.

It is fair to conclude that the present study highlights that target points for combating the continuous increase of ESBL-E are mostly of global nature. At this, withdrawal of selection pressure in all ecological systems through reduction of the global antibiotic consumption as well as an improved deactivation in water seems essential. Since no therapeutic intervention to remove rectal ESBL-E carriage is presently available, preventative measures appear highly relevant in order to effectively avoid rectal ESBL-E carriage.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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