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Gastric acid suppression, lifestyle factors and intestinal carriage of ESBL and carbapenemase-producing Enterobacterales: a nationwide population-based study

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Background: Gastric acid-suppressive therapy has been suggested to increase the risk for intestinal carriage of MDR Enterobacterales, but there is scarce community-based evidence substantiating this risk.

Objectives: To investigate if acid-suppressant use is associated with a risk of intestinal carriage of ESBL and carbapenemase-producing Enterobacterales (ESBL-E) in the open population, and to assess possible modifying factors.

Methods: Within the framework of a nationwide seroprevalence study, we identified a population-based cross-sectional cohort comprising 2746 adults (≥18 years), who provided stool specimens between February 2016 and June 2017. Specimens were tested by phenotypic assays and confirmatory genotype analysis to detect carriage of ESBL-E. Covariate data were extracted from self-administered questionnaires. ORs and 95% CIs were estimated using multivariable multilevel logistic regression, controlling for confounders informed by directed acyclic graphs.

Results: Among 2746 participants, 316 (11.5%) used acid suppressants; the prevalence of ESBL-E carriage was 7.4% (95% CI, 6.1%–8.6%). Current use of acid suppressants was not associated with ESBL-E carriage (adjusted OR [aOR], 1.05; 95% CI, 0.64–1.74); lifestyle and comorbidity did not modify this association. A higher BMI (≥25 kg/m^2) (aOR, 1.42 [95% CI, 1.02–1.98]), non-Western ethnic origin (aOR, 1.96 [95% CI, 1.34–2.87]), travel to Eastern-Mediterranean, Western-Pacific or South-East Asia regions (aOR, 3.16 [95% CI, 1.71–5.83]) were associated with ESBL-E carriage. Sensitivity analyses confirmed these results; spline analysis supported a BMI-associated risk.

Conclusions: In this open population study, current use of acid suppressants was not associated with ESBL-E carriage. Travel to high-endemic regions and non-Western ethnicity were confirmed as risk factors, while a higher BMI emerged as a potential new risk for ESBL-E carriage.

Introduction

Globally, the spread of ESBL and carbapenemase-producing Enterobacterales (ESBL-E) poses a major health threat.[1] Enteric colonization serves as the main source for human-to-human spread, and puts carriers at risk for difficult-to-treat infection.[2–5]

Recently, we performed a meta-analysis, covering over 29 000 patients, on the risk for enteric colonization associated with the use of gastric acid-suppressive therapy; this showed that the use of proton pump inhibitors (PPIs) nearly doubled the risk for MDR Enterobacterales carriage.[6] This association is biologically

plausible. PPIs cause a rise in gastric pH that permits increased passage of ingested microorganisms to the intestines, and disturb the gut microbiome by altering microbial diversity.[7,8] Possibly, this leads to altered colonization resistance.[9,10]

While the results of this meta-analysis support an association, studies specifically designed to quantify the risk in the open population are scarce. Most studies are hospital based, aimed at identifying predictors of carriage, and conducted in patients with more severe chronic diseases. Although our meta-analysis includes four community-based studies,[11–14] heterogeneity, small size,[12] use of surrogate outcomes,[13] and lack of control for dietary and lifestyle factors[11–14] preclude definitive conclusions about the role of PPIs used in the open population. Moreover, lifestyle factors and comorbidity differ between PPI and non-PPI users, and can affect the gut microbiome,[15–17] but whether such factors confound or modify the risk associated with PPI use remains unclear.

Hence, we aimed to investigate whether the use of acid suppressants is associated with a risk of intestinal carriage of ESBL-E in the context of lifestyle factors in a nationally representative general-population setting. Second, we assessed the influence of potential modifying factors. To do so, we used directed acyclic graphs (DAGs) to delineate the causal structures of the variables under study.[18,19]

Methods

We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies and recommendations for reporting DAGs.[20,21] We used deidentified data of the PIENTER-3 study for which ethics approval (no. M015-022) and consent from all participants were obtained; thus, this substudy was absolved from ethics review.

Design, data sources and population

We analysed data from a subcohort of the nationwide cross-sectional Dutch seroprevalence study (PIENTER-3), collected between 1 February 2016 and 19 June 2017, by the National Institute for Public Health and the Environment (RIVM).[22] We included all adults aged 18 years or older who provided a faecal specimen. This subcohort included predominantly healthy individuals, thereby minimizing confounding by disease severity. In addition, information on health and lifestyle factors was collected, which enabled analysis of their influence. Details of the design and sampling procedure are summarized in Method S1 (available as Supplementary data at *JAC* Online) and described elsewhere.[22] Briefly, the PIENTER-3 study comprises a stratified, 2-stage clustered probability sample of Dutch residents with oversampling of non-Western respondents. Data were collected with structured health questionnaires.

Outcome

The primary outcome was carriage of ESBL- or carbapenemase-producing Enterobacterales, defined as the presence of ≥1 MDR isolate in faecal specimens, identified by microbiological testing in accordance with EUCAST guidelines.[23]

Microbiological analysis

Faecal specimen aliquots were selectively enriched by overnight incubation at 37°C in 5 mL of in-house tryptic soy broth with ampicillin 50 mg/L;[11] 10 μL of the enrichment broth was inoculated on selective ESBL agar (EbSA, Cepheid Benelux, Apeldoorn, the Netherlands) and on MacConkey agar with a 10 μg ertapenem disc,[24] then incubated overnight at 37°C under aerobic conditions. All oxidase-negative variants (five colonies per morphotype) were subcultured and analysed. Specimens yielding growth-negative control agars (5% sheep blood) were excluded.

Antibiotic susceptibility testing and species determination were done with the VITEK 2 system and carbapenem Etests (VITEK-MS, VITEK 2-AST and Etest; bioMérieux, Marcy-L'Étoile, France).[23] ESBL production was confirmed by disc diffusion (Rosco Diagnostica, Taastrup, Denmark); carbapenemase production was confirmed by combination disc tests (Rosco Diagnostica) and CIM tests.[23,25] Confirmed isolates were stored in cryovials (8% glycerol) at −80°C.

Genotypic analysis

DNA was extracted using the QIAamp DNA mini kit (QIAgen, Venlo, the Netherlands). Carbapenemase gene families and ESBL gene groups were analysed by PCR (LightCycler 480 Real-time PCR, Roche, Almere, the Netherlands) ($bla_{CTX\text{-}M}$ groups 1, 2, 9, 8, bla_{TEM}, bla_{SHV}, bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{VIM}).[11] Carrier status was defined on the basis of the result of phenotypic confirmation when the genotype remained undetected.

Exposure assessment and covariates

A priori, we selected covariates for potential adjustment based on evidence from current literature. Covariate data were extracted from the questionnaires and included: (i) socio-demographic parameters (age, sex, ethnicity, municipality and urbanization level), socio-economic status (occupation in the healthcare sector, education level), (ii) lifestyle factors (BMI, alcohol use, smoking status and vegetarian diet), (iii) selected comorbidities (cancer, diabetes, bone-marrow or organ transplantation, rheumatoid arthritis and immune, renal, hepatic, gastrointestinal, lung, cardiovascular and neurological disease), (iv) international travel in the past 6 months (WHO region), (v) medication use (acid suppressants, antibiotics, immunomodulating agents or systemic corticosteroids, antineoplastic agents, metformin, statins, laxatives), and (vi) prior hospitalization (in the Netherlands and/or foreign). Details of the covariates are shown in Method S1.

We classified acid-suppressive therapy according to the WHO Anatomical Therapeutic Chemical (ATC) classification system. Because few participants used medications other than PPIs and for certain participants the acid suppressant used remained unspecified, two exposure groups were created: (i) current use of PPIs, and (ii) current use of any acid suppressant (PPI, histamine-2 antagonist, gastric antacid and/or unspecified). Dosages were not specified.

Data analysis

ORs and 95% CIs for the association of acid-suppression use and ESBL-E carriage were estimated by multilevel (mixed-effect) logistic regression analyses using adaptive Gauss quadrature estimation with complete cases. We fitted 2-level multilevel models with random intercepts to account for intermunicipality variation, i.e. individuals clustered within municipalities (Table S1). To control for the sampling method, we also incorporated design variables (i.e. age and sex) as fixed effects.[26]

Confounders were selected on the basis of *a priori* knowledge and evaluated using a causal DAG (Figure 1); the minimally sufficient adjustment set for the total effect was identified by backdoor-criterion.[18,19,27] Confounders included for risk adjustment were: age, sex, lifestyle (BMI, smoking and alcohol use), comorbidity, and prior hospital admission. To identify individual-level risk factors, we also assessed the independent associations of covariates in secondary analyses.

Stratification and sensitivity analysis

We performed subset analyses to assess the association of acid suppression with ESBL-E carriage across strata by exploring the influence of predisposing factors and known risk factors. We tested for interaction and

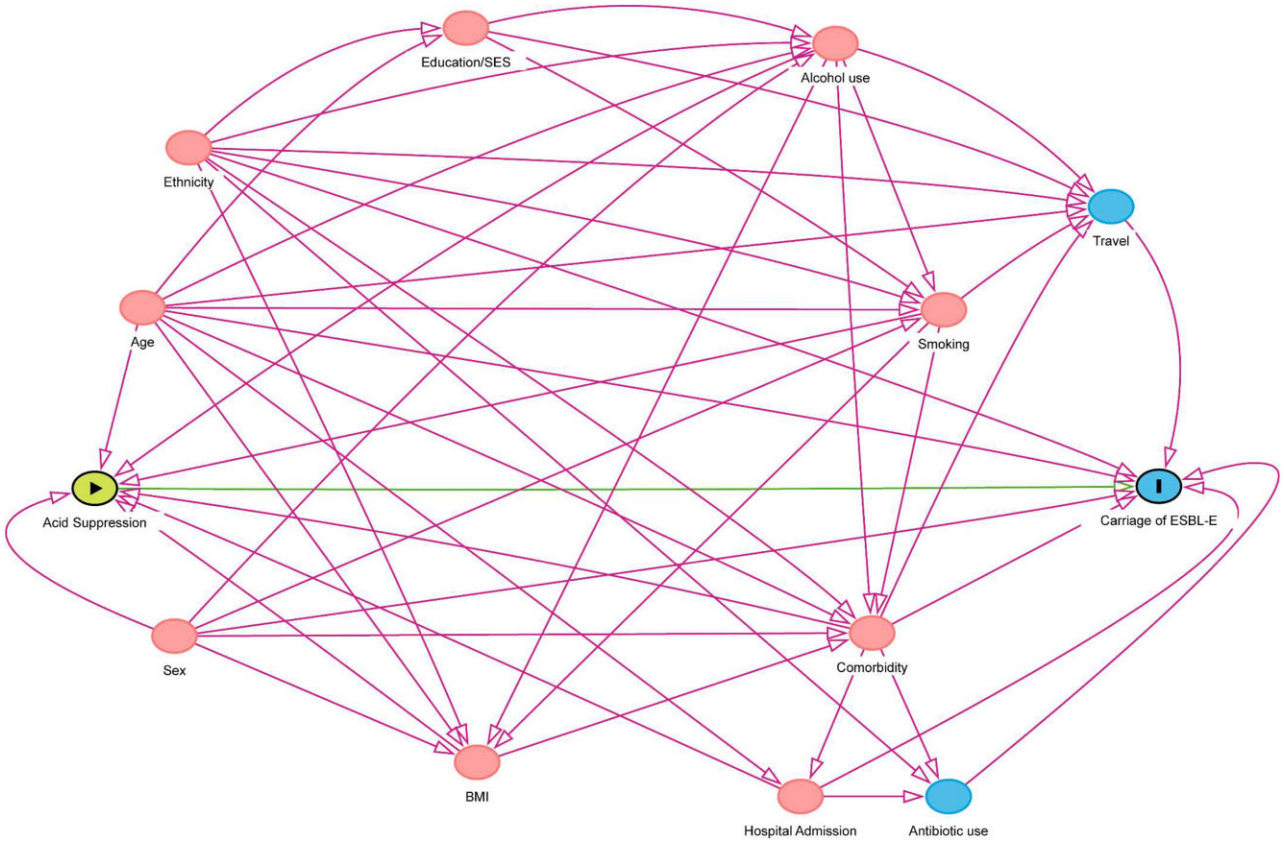

Figure 1. Causal DAG. The DAG was created with DAGitty, and depicts causal relationships between the covariates, and the exposure and outcome of the causal path of interest. Red circles indicate ancestors of the exposure and outcome (i.e. confounders); blue circles indicate ancestors of the outcome (i.e. causal determinants of the outcome); green lines indicate causal paths; and pink lines indicate biasing paths. SES, socio-economic status (e.g. occupation and income). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

computed stratum-specific ORs for *post hoc* defined subgroups (age, sex, ethnicity, education, lifestyle factors [BMI, smoking, alcohol use and diet], comorbidity, immunosuppression, travel and antibiotic use in the past 3 months).

We also performed three sensitivity analyses. First, to address misspecification bias, we repeated the multivariable-adjusted analyses using three restructured DAGs (Figure S1); these accounted for additional confounders (ethnicity, travel and diet). Second, we controlled for prior antibiotic use in addition to the adjustments informed by the DAGs. Third, to account for missingness, (missing data levels of 1%–10%) we fitted multiple logistic regression models with robust variance estimation after multiple imputation, using 20 imputed data sets by chained equations (Method S1).[28]

Multilevel regression analyses were conducted with Stata, version 16.0 (StataCorp, College Station, TX, USA) (Method S1).[22,26] To adjust for the multistage design and oversampling by ethnicity in PIENTER-3, prevalence estimates were weighted by age, sex, ethnicity and level of urbanization using census data (Statistics Netherlands, 31 December, 2016) (Method S1) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). DAGs were designed systematically; 'DAGitty' was used to identify statistical adjustment sets (Method S1).[27,29]

Results

Sample characteristics

A total of 2751 individuals was eligible for inclusion and provided faecal specimens; 5 adults (<1%) missed exposure data, thus yielding a final sample size of 2746 adults (Figure 2). Median age was 50 years (IQR 34–64; range 18–87), and 1582 participants (57.6%) were female. Acid-suppressive medications were used by 316 (11.5%) participants (Table 1). Detailed characteristics of participants are shown in Table S2. Users of acid suppressants were older (median age 65 [IQR 52–70] years versus 48 [33–63] years), had a higher comorbid disease burden (219 [69.3%] versus 835 [34.4%]), were more likely to be overweight or obese (BMI $\geq$25 kg/m^2: 205 [72.2%] versus 1149 [52.8%]) and were more likely to be former or current smokers (195 [62.9%] versus 1106 [49.2%]) (Table S3).

Prevalence

We detected 209 ESBL-E isolates in 195 carriers (7.1% [95% CI% 6.2%–8.1%]) (Figure S2); 14 participants carried >1 ESBL-E isolate. Most isolates were *Escherichia coli* (91%); $bla_{\text{CTX-M}}$ was the main resistance mechanism, detected in 86% of the isolates. Carbapenemase producers were found in two carriers (0.07% [95% CI, 0.02%–0.26%]) (one $bla_{\text{OXA-48-like}}$ and one PCR-negative) (Table S4).

The standardized prevalence of ESBL-E carriage was 7.4%, hence one in 13 adult participants carried $\geq$1 ESBL-E isolate (Table 2). Age-stratified (weighted) prevalence estimates are shown in Figure S3. We observed marked differences in carriage rate

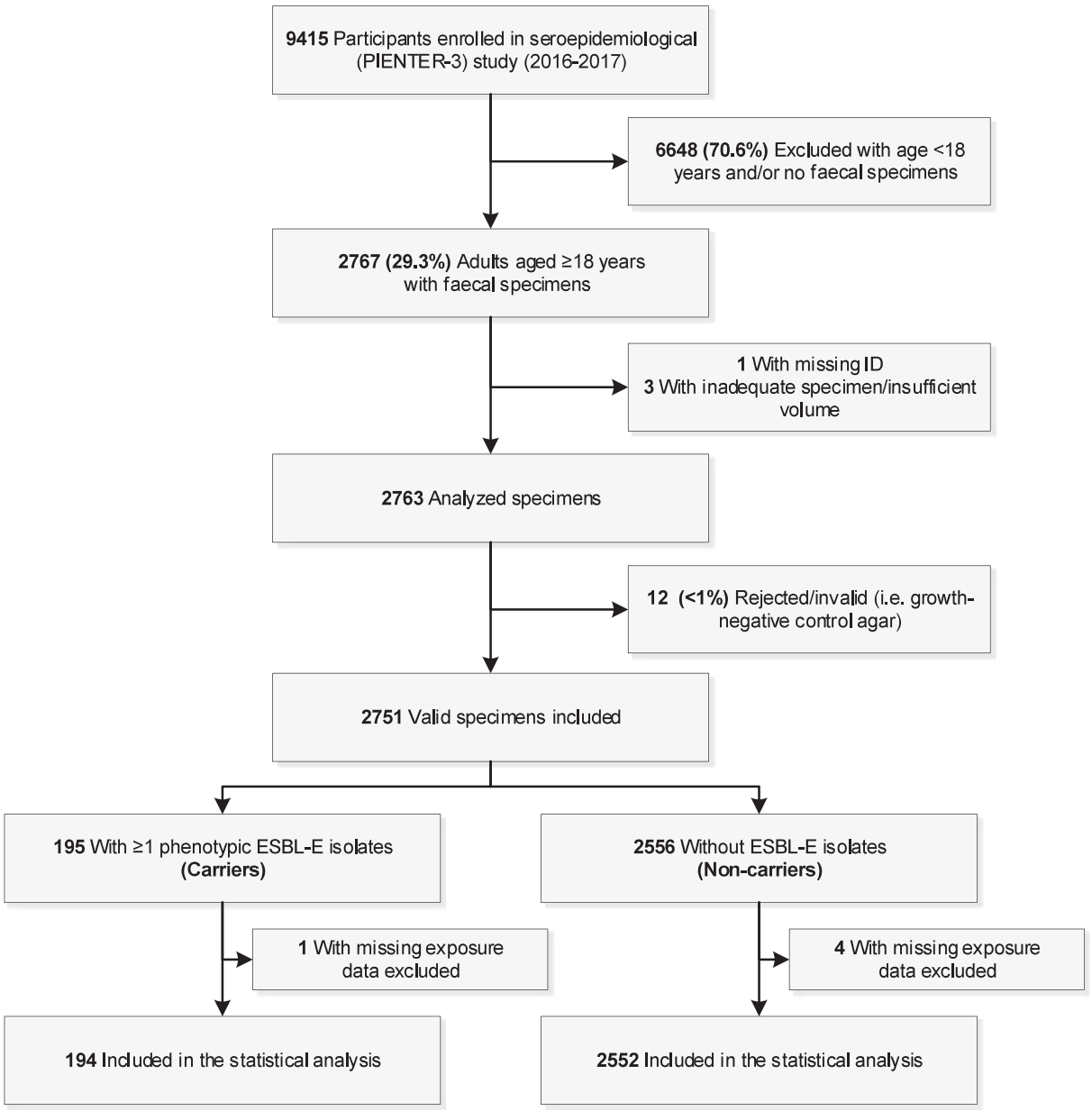

Figure 2. Study flow diagram. In the seroprevalence (PIENTER-3) study a multistage cluster sampling method was used to draw a sample of Dutch residents (1852 included in this study), including an oversampling of non-Western migrants (178 included in this study), persons living in low vaccination coverage areas (506 included in this study) and persons born in Suriname, Aruba and the former Dutch Antilles (215 included in this study). The study population for the main analysis ($N = 2746$) included participants with complete outcome and exposure data. For the standardized prevalence estimation, a subsample of the population ($N = 2751$) including the five participants with missing exposure data was used. ID, identifier.

between participants of different ethnicity: the prevalence was nearly twice as high in non-Western participants in comparison to Western participants (Table 2). Basic characteristics stratified by ethnicity are shown in Table S5.

Association of acid suppression with ESBL-E carriage

In the primary analysis, while adjusting for design variables, the use of any acid-suppressive medication as well as the use of PPIs were not associated with increased odds of ESBL-E carriage (Table 1). The lack of association between the use of acid suppressants or PPIs and ESBL-E carriage remained unchanged after a more robust adjustment, taking into account the complex causal structure of the plausible confounders in the DAG (Figure 1) (Table 3).

Secondary analyses

In secondary analyses adjusting for design variables, BMI $\geq 25\,kg/m^2$ (OR 1.48 [95% CI, 1.07–2.05]), non-Western ethnic origin (OR 1.96 [95% CI, 1.34–2.87]) and travel to the Eastern-

Table 1. Sample characteristics[a]

Characteristic	Total $N = 2746$	ESBL-E carrier, No. (%) $N = 194$	ESBL-E non-carrier, No. (%) $N = 2552$	Adjusted OR (95% CI)[b]
Age (years) median (IQR)	50.0 (34.0–64.0)	50.0 (30.0–64.0)	50.0 (35.0–65.0)	
Age group, years				
<40 (18–39)	906 (33.0%)	70 (36.1%)	836 (32.8%)	1 [Reference]
40–59	888 (32.3%)	59 (30.4%)	829 (32.4%)	0.84 (0.59–1.21)
≥60	952 (34.7%)	65 (33.5%)	887 (34.8%)	0.87 (0.60–1.24)
Sex				
male	1164 (42.4%)	79 (40.7%)	1085 (42.5%)	1 [Reference]
female	1582 (57.6%)	115 (59.3%)	1467 (57.5%)	1.05 (0.77–1.42)
Ethnicity				
Western	2301 (83.8%)	145 (74.7%)	2156 (84.5%)	1 [Reference]
non-Western[c]	445 (16.2%)	49 (25.3%)	396 (15.5%)	1.96 (1.34–2.87)
Educational level[d]				
high	820 (31.5%)	61 (32.6%)	759 (31.4%)	1 [Reference]
medium	908 (34.9%)	59 (31.6%)	849 (35.1%)	0.85 (0.58–1.23)
low	876 (33.6%)	67 (35.8%)	809 (33.5%)	1.11 (0.76–1.62)
Urbanization level[e]				
level 1–2 (large to extreme UA)	1272 (46.3%)	104 (53.6%)	1168 (45.7%)	1 [Reference]
level 3–4 (small to medium UA)	1095 (39.9%)	70 (36.1%)	1025 (40.2%)	0.76 (0.54–1.07)
level 5 (non-urbanized)	379 (13.8%)	20 (10.3%)	359 (14.1%)	0.61 (0.36–1.03)
Healthcare-related occupation				
yes	465 (16.9%)	38 (19.6%)	427 (16.7%)	1.16 (0.78–1.73)
no	2281 (83.1%)	156 (80.4%)	2125 (83.3%)	1 [Reference]
BMI, median (IQR)	25.4 (22.9–28.5)	25.9 (23.3–29.0)	25.3 (22.9–28.4)	
BMI (kg/m^2)[f]				
15.2–24.9 (normal)	1106 (45.0%)	66 (37.7%)	1040 (45.5%)	1 [Reference]
≥25.0 (overweight or obese)	1354 (55.0%)	109 (62.3%)	1245 (54.5%)	1.48 (1.07–2.05)
Smoking				
current or former smoker	1301 (50.9%)	89 (48.6%)	1212 (51.1%)	0.98 (0.72–1.34)
never smoked	1255 (49.1%)	94 (51.4%)	1161 (48.9%)	1 [Reference]
Alcohol use				
yes	1925 (75.8%)	129 (70.1%)	1796 (76.2%)	0.74 (0.52–1.05)
never	616 (24.2%)	55 (29.9%)	561 (23.8%)	1 [Reference]
Vegetarian diet				
yes	54 (2.1%)	3 (1.6%)	51 (2.1%)	0.66 (0.20–2.18)
no	2571 (97.9%)	183 (98.4%)	2388 (97.9%)	1 [Reference]
Comorbidity[g]				
yes	1054 (38.4%)	78 (40.2%)	976 (38.2%)	1.16 (0.84–1.58)
no	1692 (61.6%)	116 (59.8%)	1576 (61.8%)	1 [Reference]
Hospitalized during the last 6 months[h]				
yes	146 (5.4%)	14 (7.3%)	132 (5.2%)	1.45 (0.81–2.58)
no	2569 (94.6%)	178 (92.7%)	2391 (94.8%)	1 [Reference]
Travel during the last 6 months[i]				
no travel or travel within EUR	2435 (93.4%)	157 (87.2%)	2278 (93.9%)	1 [Reference]
travel to EMR, SEAR or WPR	75 (2.9%)	15 (8.3%)	60 (2.5%)	3.64 (2.01–6.61)
travel to AMR or AFR	97 (3.7%)	8 (4.4%)	89 (3.7%)	1.30 (0.62–2.74)
Antibiotic use in the past 3 months				
yes	648 (23.6%)	48 (24.7%)	600 (23.5%)	1.07 (0.76–1.50)
no	2098 (76.4%)	146 (75.3%)	1952 (76.5%)	1 [Reference]
Current medication use				

Continued

Table 1. *Continued*

Characteristic	Total $N = 2746$	ESBL-E carrier, No. (%) $N = 194$	ESBL-E non-carrier, No. (%) $N = 2552$	Adjusted OR (95% CI)[b]
immune-suppressing agents[j]	56 (2.0%)	3 (1.5%)	53 (2.1%)	0.75 (0.23–2.43)
NSAID/aspirin	158 (5.8%)	9 (4.6%)	149 (5.8%)	0.82 (0.41–1.66)
PPIs	279 (10.2%)	19 (9.8%)	260 (10.2%)	1.02 (0.62–1.69)
acid-suppressive medication[k]	316 (11.5%)	23 (11.9%)	293 (11.5%)	1.11 (0.70–1.78)

AFR, Africa region; AMR, Americas region; EMR, Eastern Mediterranean region; EUR, European region; SEAR, South-East Asia region; UA, urbanized area; WPR, Western Pacific region.
[a]Data are given as number (percentage) of participants unless otherwise specified. Data were missing for education (5.2%); BMI (10.4%); smoking (6.9%); alcohol use (7.5%); vegetarian diet (4.4%); hospital admission (1.1%); and travel (5.1%).
[b]Adjusted for age and sex.
[c]Defined using terminology of Statistics Netherlands based on the country of birth of the participant and his/her parents; non-Western ethnicity includes Morocco, Turkey, Surniname, Aruba, Netherlands Antilles and other non-Western countries.
[d]Low, no education, primary education or preparatory vocational; medium, secondary education or intermediate vocational; high, higher vocational or university level.
[e]Defined by surrounding address density per square km following definitions of Statistics Netherlands.
[f]Cut-off value according to the WHO.
[g]Asthma or non-asthmatic lung disease, cardiovascular disease, renal or hepatic disease, immune disorders, organ or bone-marrow transplantation, rheumatic arthritis, gastrointestinal disease, neurological comorbidity, diabetes mellitus type 1/2 and malignancy.
[h]Hospitalized in the Netherlands and/or abroad ($\geq$1 overnight hospital stay).
[i]Categorized using WHO regions and risk of acquisition based on literature.
[j]Antineoplastics, immunosuppressants and systemic corticosteroids.
[k]PPIs, histamine-2 receptor antagonists and gastric antacids.

Table 2. Prevalence of ESBL-E carriage, overall and by ethnic origin

Characteristic	No. of participants[a]	Prevalence, % (95% CI)
Overall standardized population estimate[b]	2029	7.4 (6.1–8.6)
Ethnic subgroup[c]		
Western ethnic origin	2305	8.4 (7.6–8.9)
non-Western ethnic origin	446	16.2 (10.3–23.7)

[a]Sample sizes are unweighted.
[b]Standardized by age-, sex-, ethnicity- and urbanization to national census data (Statistics Netherlands, 2016); the sample was restricted to the National Dutch sample and oversampling of non-Western migrants ($N = 2029$) to generate stable representative weighted estimates.
[c]Adjusted for complex design features (i.e. stratification and clustering) with corresponding Korn–Graubard 95% CIs.

Mediterranean, South-East Asia region or Western-Pacific (OR 3.64 [95% CI, 2.01–6.61]) yielded higher odds for ESBL-E carriage. Multivariable multilevel adjustment informed by the DAG (Figure 1) yielded similar results for the above risk factors. To test whether our BMI cut-off was robust, we also modelled restricted cubic splines (Figure S4); these confirmed higher odds for a BMI $\geq$25 kg/m^2.[30] Adjusted associations with ESBL-E carriage for the prespecified covariates are shown in Tables S6 and S7.

Stratification and sensitivity analyses

In the subgroup analyses, there were no interaction effects and in none of the strata an effect of acid suppression on carriage of ESBL-E was observed (Tables S8, S9 and S10). Sensitivity analyses using multiple imputation, and multivariable adjustments derived from three restructured DAGs (Figure S1), yielded findings similar to those of the primary and secondary analyses (Table 3 and Table S7). Additional control for prior antibiotic use, a well-known modifier of the gut microbiome, changed the associations little. Also, ancillary control for acid-suppressive therapy and education, for the factors BMI and non-Western ethnicity, respectively, did not change the results (Table S11).

Discussion

In this nationwide cross-sectional epidemiological study, we found no evidence for higher odds of ESBL-E carriage with gastric acid-suppressant use in the open population. The lack of association was robust and remained unchanged even under stratified and sensitivity analyses. By contrast, a high BMI ($\geq$25 kg/m^2), non-Western ethnic origin and travel to the Eastern-Mediterranean, South-East Asia or Western-Pacific in

Table 3. Multivariable-adjusted odds of ESBL-E carriage for acid suppression; adjustment by different DAGs

Exposure	Adjusted OR (95% CI)			
	DAG 1[a]	DAG 2[b]	DAG 3[c]	DAG 4[d]
Acid-suppressive therapy[e]	1.05 (0.64–1.74)	1.09 (0.68–1.77)	1.13 (0.69–1.85)	1.15 (0.69–1.93)
PPIs	0.95 (0.55–1.64)	1.00 (0.60–1.68)	1.03 (0.61–1.76)	1.03 (0.59–1.80)
Unexposed	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]

AFR, Africa region; AMR, Americas region; DAG, directed acyclic graph; EMR, Eastern Mediterranean region; EUR, European region; MSAS, minimally sufficient adjustment set; SEAR, South-East Asia region; WPR, Western Pacific region.
[a]DAG 1 MSAS: age (<40, 40–60, ≥60), sex (male, female), BMI (normal, overweight/obese), smoking (never, current or former smoker), alcohol use (yes, no), comorbidity (yes, no), hospital admission (yes, no).
[b]DAG 2 MSAS: age (<40, 40–60, ≥60), sex (male, female), ethnicity (Western, non-Western), smoking (never, current or former smoker), alcohol use (yes, no), comorbidity (yes, no).
[c]DAG 3 MSAS: age (<40, 40–60, ≥60), sex (male, female), ethnicity (Western, non-Western), diet (vegetarian, non-vegetarian), comorbidity (yes, no), international travel (no travel or within EUR, travel to EMR, SEAR or WPR, and travel to AMR or AFR).
[d]DAG 4 MSAS: age (<40, 40–60, ≥60), sex (male, female), ethnicity (Western, non-Western), BMI (normal, overweight/obese), comorbidity (yes, no), international travel (no travel or within EUR, travel to EMR, SEAR or WPR, and travel to AMR or AFR).
[e]PPIs, histamine-2 receptor antagonists and gastric antacids.

the recent past (within 6 months) were associated with higher odds of ESBL-E carriage.

The null finding associated with the use of acid suppressants appears inconsistent with the result from our previous meta-analysis, where we found an OR of 1.41 (95% CI, 1.07–1.87).[6] The CI for the exposure, however, overlaps broadly with the CI of the pooled result of our meta-analysis, hence the result of the present study may still be consistent with the meta-analysis. On the contrary, in the present study, we performed more robust risk adjustments than in previous epidemiological population-based studies—via DAGs we controlled for several confounders, and lifestyle, not accounted for in other studies.

Several explanations can be proposed for the lack of association. Firstly, there are differences in the studied populations. Within a hospital environment, PPI users are more likely to be exposed to resistant microorganisms than in the open community; this is an important aspect, because actual risk depends on sufficient and/or frequent exposure to ESBL-E during PPI use. Indeed, most studies in the meta-analysis are hospital-based; a previous study in the open Danish population, with low endemicity, also showed that hospitalization is a major risk factor for ESBL-E carriage.[6,13] In addition, other host factors in more vulnerable patient groups might predispose to enteric colonization during the use of acid suppressants.

Secondly, confounding by BMI could explain our divergent result. A higher BMI is known to increase the risk for gastro-oesophageal reflux disease; and may therefore increase the likelihood of PPI use.[31] Indeed, acid-suppression use was more frequent among overweight or obese participants in our present study. In addition, numerous studies have shown that obesity is associated with an altered composition of gut microbiota, in particular with lower microbial diversity—dysbiosis is considered to impair colonization resistance mediated by the microbiota.[9,32,33] Of note, in our study, while BMI did not modify the association of acid-suppression use with ESBL-E carriage, it remains possible that PPIs enhance the risk of carriage by reducing gastric acid secretion in persons with an altered gut microbiome related to obesity.

Interestingly, a higher BMI appeared as a risk factor for ESBL-E carriage: it was associated with 40% higher odds for carriage. We interpret this finding with caution, however, because the odds were small, and we are not aware of previous reports describing this association in a similar setting. In addition, microbiota-related changes have also been associated with obesity-related comorbidities.[33] The effect changed little even after control for known modifiers of the gut microbiota (i.e. age, antibiotics, acid suppressants); an association seems biologically plausible via the effects on the microbiota related to obesity.[32,33] The weak association is in line with an expected small risk based on the findings of a meta-analysis of 10 studies on the association of obesity with microbiota; Sze *et al.*[34] found evidence for a weak effect. Nevertheless, it cannot be ruled out that the association found in our study represents residual confounding or noise, although our DAGs suggest otherwise. This finding might, for example, indicate other dietary habits that we did not explore.[35]

Several of the risk factors we detected are consistent with those found in studies that evaluated determinants of ESBL-E carriage in the general population, supporting the validity of our findings.[11,14,36–38] First, the higher odds associated with travel to Asia was shown before.[11,14,36,37,39,40] Travel was not associated with acid-suppression use, congruent with findings from our recent meta-analysis in which the association for PPI use among travel cohorts was null.[6] Among acid-suppression users, travel was uncommon, possibly because PPI use correlates with older age, while travellers are younger; also in our study, most acid-suppression users (64%) were older (≥60 years). Second, the prevalence of ESBL-E carriage was nearly 2-fold in participants of non-Western ethnic origin compared with participants of Western origin; this accords with ethnic disparities in ESBL-E carriage found in a prior study that was designed to oversample ethnic groups.[36] Non-Western and Western participants differed with respect to socio-demographic and lifestyle factors. Therefore, non-Western ethnicity may be an indicator for greater burden of risk-increasing factors: non-Western participants were older, travelled more frequently to high-endemic regions, used more often antibiotics in

the recent past and had overall higher BMI; these indicators, however, did not fully explain the difference. The association between higher body weight, as measured by BMI, merits further exploration in studies where not only BMI, but also PPI use and gut microbiota composition are determined.

Strengths and limitations

A major strength of our study is that we used robust methods to adjust for confounding and to assess possible residual confounding. To our knowledge, no study has explored the effect of comprehensive lifestyle factors on ESBL-E carriage in a population-representative setting in the open population. In addition, we used stringent analyses and carefully constructed DAGs in order to visualize interference of possible unmeasured confounding; via these DAGs we did not detect major confounding issues.

This study has limitations. The use of observational data is always limited by the potential for unmeasured confounding, but we mitigated this limitation by the analyses mentioned under strengths. Second, our data are cross-sectional—relying on survey measurements—and therefore liable to recall bias and reverse causation. However, participants were not aware of our hypothesis at the time of data collection, hence recall and temporal bias seem unlikely; if any, recall bias would be non-differential between carriers and non-carriers of ESBL-E. Third, the self-reported measurements by use of questionnaires may have introduced some non-differential misclassification with respect to the exposure medications, since we did not validate their use with an additional source, i.e. pharmacy database. This is consistent with a prior report, published in the interim since our meta-analysis, that found no association of ESBL-E carriage with PPI use, and also used questionnaires only.[38] In contrast, two previous studies based on the general population that additionally ascertained medication use showed increased odds for carriage.[11,14] Although in our study the exposure was measured by self-report, which may better reflect true acid-suppression use (measuring both over-the-counter use and prescription use), non-differential misclassification may have diluted the odds. Finally, we were unable to control for more detailed dietary habits and, due to lack of data on dosages, we were unable to study a dose–response association.

Conclusions

In this population-based analysis, we found no evidence of higher odds for ESBL-E carriage with current use of gastric acid suppressants. We confirmed the well-known risk factors travel and non-Western ethnicity and describe a novel possible risk associated with higher BMI. The latter may play a role via alterations in gut microbiota composition and merits further study.

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Transparency declarations

None to declare.

Author contributions

Concept and design: K.v.D., F.R.M.v.d.K., S.C.d.G. and C.M.J.E.V.-G. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: R.P.J.W., K.v.D., S.C.d.G. and C.M.J.E.V.-G. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: R.P.J.W. and J.W.R.T. Obtained funding: C.M.J.E.V.-G. Supervision: K.v.D., S.C.d.G. and C.M.J.E.V.-G. All authors approved the final manuscript.

Supplementary data

Method S1, Tables S1 to S11 and Figures S1 to S4 are available as Supplementary data at *JAC* Online.

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