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Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Carbapenemase producing Enterobacteriaceae, Surveillance, Prevalence

TITLE:

Local prevalence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* intestinal carriers at admission in a Spanish University Hospital and co-expression of ESBL and OXA-48 carbapenemase in *Klebsiella pneumoniae*

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ABSTRACT

Objective: to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in a University Hospital in Spain.

Design: prevalence survey.

Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a University tertiary hospital in Madrid (Spain).

Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-2016 with a rectal swab taken at admission or as soon as possible within the first 48 hours.

Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers and prevalence of ESBL-E infections at admission.

Results: the ESBL-E carriers prevalence on admission was 7.70% (CI 95% 7.19-8.22). Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae* (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K. pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an active infection by ESBL-E at admission.

Conclusions: The prevalence found in our study is very similar to that found in the literature. However, we found a high percentage of simultaneous ESBL and CP producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of colonized patients, the ESBL-infection rate on admission was very low.

Key words: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*, carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study is one of the most prolonged in time and with the largest number of patients assessing colonization with multidrug resistant microorganisms, including adult participants of variable age groups and gender from a university hospital providing specialized assistance to 8.51% of the population of Madrid (Spain)
- The large number of patients included (10,643) gives strength to the results.
- Genes codifying ESBL and CP were characterized by PCR and sequencing. Unfortunately total characterization was not feasible in all isolates, only 24.73% of total ESBL producing isolates and 65.91% of total CP producing isolates.

FUNDING STATEMENT

- The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation strategies for ESBL carriers in medical and surgical hospital wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-N°282512).
- MH-G is supported with a contract from Instituto de Salud Carlos III of Spain (iP-FIS program, ref. IFI14/00022).

POTENTIAL CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

TEXT

BACKGROUND

The emergence of antimicrobial resistance represents a global challenge for healthcare due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization, prolonged hospitalization, ICU admission and previous use of cephalosporins and aminoglycosides were leading risk factors [3, 4].

The situation today is very different since their prevalence has increased dramatically in the community, especially in urinary tract infections, where these enzymes are more frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the inadequate empirical treatment, delaying the efficient antimicrobial treatment for example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the prevalence of microbial resistance in our geographic area and the epidemiological characteristics in order to establish the scope of the problem and analyze its evolution.

The aim of this study was to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an active surveillance screening program (R-GNOSIS project).

METHODS

Study design and settings

The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation

strategies for ESBL carriers in medical and surgical hospital wards, funded by the EU (FP7-HEALTH-2011-SINGLE STAGE-N°282512).

The University Hospital Ramón y Cajal is a public referral center, located in the North of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds) wards took part in the study.

Patients

Between March 3rd 2014 and April 3rd 2016, screening rectal swabs were obtained, after verbal consent, from all patients aged 18 and older, at admission or as soon as possible within the first 48 hours.

Patient involvement

All patients were informed of the aim of the study and the consequences of a positive result (contact isolation and needing a new rectal screening at any hospital admission in the future to check their status) and gave their verbal consent to participate. As soon as the microbiological result was known by the investigators, patients and their familiars were informed.

Laboratory analysis

The samples were seeded on ChromoID-ESBL and Chromo-ID CARBA/OXA-48 (BioMérieux, France) selective chromogenic-agar plates. Bacterial identification was performed using the MALDI-TOF-MS (Bruker-Daltonics, Germany) mass spectrometry. ESBL and carbapenemase (CP) production were phenotypically confirmed by the double-disk diffusion test, Hodge Test and KPC/MBL/OXA-48 Confirm and ESBL AmpC Screen Kits (Rosco Diagnostica, Germany). Antimicrobial susceptibility was studied with microdilution (MicroScan, Beckman, CA) and gradient strips (Etests, BioMérieux, France). Genes codifying ESBL (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) and CP (bla_{VIM}, bla_{KPC}, *bla*NDM, bla_{OXA-48}) were characterized by PCR and sequencing.

Ethics

The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines (ICH-GCP-Guidelines, CMPM/ICH/135/95) of the European Medicines Agency. It was granted authorization by the Ethics Committee of Clinical Research and waiver of the requirements to obtain informed consent from patients, being verbal consent considered sufficient (Ref. 251/13).

Specifications stipulated in the Personal Data Protection Act 15/1999, of 13 December were followed.

Statistical analyses

A descriptive analysis of the variables collected was conducted, the qualitative variables were expressed as percentages and the quantitative variables as measures of central tendency (mean and median) and dispersion (standard deviation). Pearson's Chi-squared test was used to compare proportions and the Student's T-test to compare means. All statistics analysis was performed using SPSS Statistics v19 (IBM®) software.

RESULTS

During the research period 12,590 admissions of 9,706 patients took place in the participating wards. In 84.5% of admissions, a rectal swab could be obtained within the first 48 hours of admission. Table 1.

TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and Neurosurgery wards and patients included in the study.

Ward	Admissions (n)	Swab at admission (n)	%
Gastroenterology	3,380	2,916	86.27
Pneumology	3,240	2,752	84.94
Urology	4,685	3,963	84.59
Neurosurgery	1,285	1,012	78.75

Total	**12,590**	**10,643**	**84.55**

Gender and mean age of included patients are shown in Table 2.

TABLE 2. Age and gender of the included patients.

Ward	Gender		Age (years)	
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)
Gastroenterology	1,732 (59.39)	1,184 (40.61)	66.53 (16.59)	69 (26.75)
Pneumology	1,625 (59.05)	1,127 (40.95)	70.72 (15.28)	74 (19)
Urology	3,009 (75.93)	954 (24.07)	66.89 (14.56)	69 (20)
Neurosurgery	533 (52.67)	479 (47.33)	60.23 (16.52)	61 (25)
Total	**6,899 (64.82)**	**3,744 (35.18)**	**64.91 (16.79)**	**67 (25)**

S.D.: standard deviation; I.R.: interquartile range.

The prevalence of ESBL-E fecal carriers at admission was 7.7% (Table 3). Table 3 shows the distribution of carriers by gender and ward, as well as their age (mean and median).

The majority of patients colonized with ESBL-E were male, just like the majority of hospital patients, the difference not being statistically significant. The mean age of colonized patients was higher than the mean age of the total number of hospitalized patients (69.29 -S.D.15.67 *vs* 64.91 -S.D. 16.79-), the difference being statistically significant (p = 0.0087).

The difference in prevalence of colonization at admission among the surveyed wards was statistically significant (p = 0.001). The highest prevalence was found in the Gastroenterology ward, with 9.05%, the difference being significant with the rest of wards (p = 0.01). When comparing the prevalence between medical wards

(Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery), the difference was not statistically significant.

A total of 845 multiresistant *Enterobacteriaceae* were isolated in 820 patients, as 25 patients were colonized by more than one microorganism at the time of admission (0.23%). Eighty-eight (10.41%) of the isolated *Enterobacteriaceae* were simultaneous ESBL and carbapenemase (CP) producers, 33.47% of these patients were known carriers, i.e., their clinical records included a previous positive culture for ESBL-E.

TABLE 3. ESBL-producing *Enterobacteriaceae* carriers at admission.

Hospital admission wards	Gender		Age (years)		Prevalence (%) CI 95%
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)	
Gastroenterology	160 (60.61)	104 (39.39)	66.33 (16.56)	67.5 (26.75)	9.05 (7.99-10.11)
Pneumology	122 (61.31)	77 (38.69)	74.78 (14.36)	79 (15)	7.23 (6.25-8.22)
Urology	234 (80.69)	56 (19.31)	69.82 (14.04)	72 (21)	7.32 (6.49-8.14)
Neurosurgery	44 (65.67)	23 (34.33)	62.27 (17.14)	66 (26)	6.62 (5.04-8.20)
Total	**560 (68.29)**	**260 (31.71)**	**69.29 (15.67)**	**72 (24.75)**	**7.70 (7.19-8.22)**

ESBL: extended-spectrum beta-lactamases; S.D.: standard deviation; I.R.: interquartile range ; CI: confidence interval.

The most frequently isolated ESBL-producer microorganism at admission was *E. coli* (77.51%; -n=655), followed by *K. pneumoniae* (20.71%, n=175), being only 1.78% other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C. amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%; *Acinetobacter* spp 0.12%). Among ESBL-*E. coli* isolates, 1.83% were simultaneous ESBL and CP producers (n=12). Among ESBL-*K. pneumoniae* isolates, 42.86% were simultaneous ESBL and CP producers (n=75). Only one patient was colonized by a different ESBL and CP producer, *K. oxytoca*.

The typing of 209 beta-lactamases (24.73% of total ESBL) and 58 carbapenemases was possible (65.91% of total CP). Most of ESBL (83.25%) belonged to the CTX-M group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining 16.75% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the typed CP, 91.38% were OXA-48 type (Table 5). In the case of 4 patients colonized simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K. pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae* respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of them and CTX-M-14 in 1, and OXA-48 in the case of CP.

TABLE 4. Distribution of ESBL strains isolated and typed in rectal swabs at hospital admission

Enzyme	Microorganism						
	ESBL *E. coli*	ESBL *K. pneum.*	ESBL *E. cloacae*	ESBL *C. freundii*	ESBL +CP *E. coli*	ESBL +CP *K. pneum.*	**Total (%)**
CTX-M	2	1	-	-	-	-	**3 (1.44%)**
CTX-M-1	9	4	-	-	-	-	**13 (6.22%)**
CTX-M-9	10	3	-	-	-	-	**13 (6.22%)**
CTX-M-14	24	1	-	-	2	-	**27 (12.92%)**
CTX-M-15	34	31	1	-	3	40	**109 (52.15%)**
CTX-M-27	6	-	-	-	-	-	**6 (2.87%)**
CTX-M-32	2	-	-	-	-	-	**2 (0.96%)**
CTX-M-55	1	-	-	-	-	-	**1 (0.48%)**
SHV	5	1	-	-	-	-	**6 (2.87%)**
SHV-2	-	1	-	-	-	-	**1 (0.48%)**
SHV-12	7	8	-	1	-	5	**21 (10.05%)**
SHV-28	-	5	-	-	-	1	**6 (2.87%)**
SHV-31	-	1	-	-	-	-	**1 (0.48%)**
Total	**100**	**56**	**1**	**1**	**5**	**46**	**209 (100%)**

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase.

TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal swabs at hospital admission

Enzyme	Microorganism		
	ESBL +CP *E. coli*	ESBL +CP *K. pneumoniae*	Total (%)
KPC-3	1	-	1 (1.72%)
NDM-1	-	1	1 (1.72%)
OXA-48	8	45	53 (91.38%)
VIM-1	-	3	3 (5.17%)
Total	9	49	58 (100%)

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase

Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of patients admitted during the research period and 6.59% of ESBL-E intestinal carriers. Of those 54 patients, all except one also showed a positive rectal swab, 90.74% of those (49 patients) with the same specie causing the infection, and 9.26% (5 patients) with a different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures) were urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

A total of 56 microorganisms were isolated in the 55 positive clinical cultures, as one of them was positive for two ESBL-E. The most frequently isolated microorganism was once again *E. coli* (67.86%), followed by ESBL and CP-*K. pneumoniae* (23.21%), ESBL-*K. pneumoniae* (7.14%); *K. oxytoca* was isolated in 1 culture (1.79%).

DISCUSSION

In our study, the prevalence of ESBL-E carriers at admission was 7.7%, ranging between 6.62% and 9.05% depending on the ward. The prevalence of ESBL-E carriers in healthy individuals as well as in ambulatory and hospitalized patients has been researched in a number of studies. In all of them, *E. coli* is always the most frequently isolated microorganism, similarly to our study (77.51%) [11-19]. In a meta-analysis published in 2016 which analyzed prevalence studies in healthy persons, and included 28,909 individuals from 66 studies, the mean global prevalence of colonization was 14%, with great variability among regions [19]. It was higher in Asia, with 46% and Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe, 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean prevalence was 2%, although it was admitted that there were very few studies for this region [20].

Our prevalence of intestinal carriers at admission is virtually the same to that found by a Dutch study recently published, which was 7.9% in patients coming from their homes and 8.6% in patients coming from long-term care facilities, a distinction not made in our research [21]. Studies in three different areas in Spain (Madrid, Barcelona and Zaragoza) show that the prevalence of carriers has increased in the last years, reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers among patients admitted to Emergency Units was 7.4%, also very similar to our figure [22].

In our facility, 10.41% of ESBL microorganisms were simultaneous carbapenemase producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the 58 carbapenemases typed (65.91% of total CP), the vast majority of them, 91.38% belonged to the OXA-48 type. This fact is especially important in the case of *K. pneumoniae* with 42.86% of them being ESBL and CP producers (91.84% OXA-48). ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed

at hospital admission (69.27% of them urinary tract infections). We did not find a similar study to compare our data with but we think this finding must be deeply analyzed.

Male gender has been identified as a risk factor for the intestinal colonization by ESBL-E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized patients were men, but they were also the majority of the total number of hospitalized patients, the difference not being statistically significant [11]. Age is another risk factor identified in the bibliography; in our study, the mean age of colonized patients was higher than the mean age of hospitalized patients (69.29 years *vs* 64.91 years), being the difference statistically significant in this case ($p = 0.0087$) [23, 24].

The prevalence of carriers at admission was higher in the Gastroenterology ward, despite being younger than the mean, with a difference statistically significant as compared to the rest of included wards. In other published studies, liver disease has been identified as a risk factor for intestinal colonization by ESBL-E, being the prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in patients with chronic liver disease one of the possible explanations [25, 26]. Another risk factor for ESBL-E carriage recently described in the literature is proton pump inhibitors (PPI) use, and these type of patients are often receiving PPIs and other medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide an explanation as risk factors for every patient were not recorded.

Beta-lactamase characterization was not feasible in all isolates, only 24.73% of total ESBL producing isolates. The main enzyme group was CTX-M, the most common according to the literature, followed by SHV, CTX-M-15 group prevailing with more than 52% [8, 12-14, 19, 21, 22, 24].

In the last years, ESBL-E infections have become an increasing concern; in the United States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur per year [29]. Infections by these bacteria are associated to higher mortality rates and higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However, few studies have associated the fact of being an intestinal carrier of ESBL-E with the

development of infections caused by these bacteria. A recent cohort study performed in patients with haematological malignancies found a 3.5-fold greater risk of developing bacteraemia by ESBL-E among colonized patients when compared to non-colonized patients; despite of the fact that mortality was similar in both groups, colonization was associated to longer hospital stays, shorter survival period and higher costs [31]. On the contrary, another similar study did not find correlation between ESBL-E colonization and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were diagnosed at admission and almost 70% were urinary tract infections. That means that 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of the colonized patients. Only in one patient with ESBL-E infection at admission no ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections were found in colonized patients, the total prevalence of infection is very low, and only in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract infection). In two cases patients died during hospital admission, although their infection had been fully resolved and death was caused by an underlying oncological disease.

This study, one of the most prolonged in time and with the largest number of patients, confirms once again the extension of ESBL-E intestinal colonization in the community showing, however, a low prevalence of infection. It is necessary to continue with the epidemiological surveillance of these microorganisms, in order to acquire a better knowledge of the implications of being an intestinal carrier of ESBL-E. The high percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply studied.

WORD COUNT

2,256

AUTHOR CONTRIBUTIONS

Conception and design of study: M Bonten, R Cantón, P Gastemeier, F Maechler.

Data collection: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa.

Analysis and/or interpretation of data: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa, JM Aranaz-Andrés, R Cantón.

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DATA SHARING STATEMENT

Extra data is available by emailing: cristina.diazagero@salud.madrid.org

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Reporting checklist for cross sectional study.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
	#12b	Describe any methods used to examine subgroups and interactions	6
	#12c	Explain how missing data were addressed	NA
	#12d	If applicable, describe analytical methods taking account of sampling strategy	NA
	#12e	Describe any sensitivity analyses	NA
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	7
	#13b	Give reasons for non-participation at each stage	7
	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7

	#14b	Indicate number of participants with missing data for each variable of interest	7
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
	#16b	Report category boundaries when continuous variables were categorized	7
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	10
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11-12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11-12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

BMJ Open

Local prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in Klebsiella pneumoniae: a prevalence survey in a Spanish University Hospital

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TITLE:

Local prevalence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in *Klebsiella pneumoniae:* a prevalence survey in a Spanish University Hospital

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ABSTRACT

Objective: to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in a University Hospital in Spain.

Design: prevalence survey.

Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a University tertiary hospital in Madrid (Spain).

Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-2016 with a rectal swab taken at admission or as soon as possible within the first 48 hours.

Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers and prevalence of ESBL-E infections at admission.

Results: the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19). Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae* (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K. pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an active infection by ESBL-E at admission.

Conclusions: The prevalence found in our study is very similar to that found in the literature. However, we found a high percentage of simultaneous ESBL and CP producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of colonized patients, the ESBL-infection rate on admission was very low.

Key words: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*, carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study is one of the most prolonged in time and with the largest number of patients assessing colonization with multidrug resistant microorganisms, including adult participants of variable age groups and gender from a university

hospital providing specialized assistance to 8.51% of the population of Madrid (Spain).

- The large number of patients included (10,643) gives strength to the results.
- Genes codifying ESBL and CP were characterized by PCR and sequencing. Unfortunately total characterization was not feasible in all isolates, only 24.67% of total ESBL producing isolates and 73.86% of total CP producing isolates.

FUNDING STATEMENT

- The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation strategies for ESBL carriers in medical and surgical hospital wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-N°282512).
- MH-G is supported with a contract from Instituto de Salud Carlos III of Spain (iP-FIS program, ref. IFI14/00022).

POTENTIAL CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

TEXT

BACKGROUND

The emergence of antimicrobial resistance represents a global challenge for healthcare due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization, prolonged hospitalization, ICU admission and previous use of cephalosporins and aminoglycosides were leading risk factors [3, 4].

The situation today is very different since their prevalence has increased dramatically in the community, especially in urinary tract infections, where these enzymes are more frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the inadequate empirical treatment, delaying the efficient antimicrobial treatment for example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the prevalence of microbial resistance in our geographic area and the epidemiological characteristics in order to establish the scope of the problem and analyze its evolution.

The aim of this study was to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an active surveillance screening program (R-GNOSIS project).

METHODS

Study design and settings

The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation

strategies for ESBL carriers in medical and surgical hospital wards, funded by the EU (FP7-HEALTH-2011-SINGLE STAGE-N°282512).

The University Hospital Ramón y Cajal is a public referral center, located in the North of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds) wards took part in the study.

Patients

Between March 3rd 2014 and April 3rd 2016, screening rectal swabs were obtained, after verbal consent, from all patients aged 18 and older, at admission or as soon as possible within the first 48 hours.

Patient involvement

All patients were informed of the aim of the study and the consequences of a positive result (contact isolation and needing a new rectal screening at any hospital admission in the future to check their status) and gave their verbal consent to participate; if the patient refused the swab was not taken. As soon as the microbiological result was known by the investigators, patients and their familiars were informed.

Laboratory analysis

The samples were seeded on ChromoID-ESBL and Chromo-ID CARBA/OXA-48 (BioMérieux, France) selective chromogenic-agar plates. Bacterial identification was performed using the MALDI-TOF-MS (Bruker-Daltonics, Germany) mass spectrometry. ESBL and carbapenemase (CP) production were phenotypically confirmed by the double-disk diffusion test, Hodge Test and KPC/MBL/OXA-48 Confirm and ESBL AmpC Screen Kits (Rosco Diagnostica, Germany). Antimicrobial susceptibility was studied with microdilution (MicroScan, Beckman, CA) and gradient strips (Etests, BioMérieux, France). Genes codifying ESBL (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) and CP (bla_{VIM}, bla_{KPC}, *bla*NDM, bla_{OXA-48}) were characterized by PCR and sequencing.

Ethics

The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines (ICH-GCP-Guidelines, CMPM/ICH/135/95) of the European Medicines Agency.

A waiver of written informed consent of individual patients in the participating wards was requested. This waiver was granted by the Ethics Committee of Clinical Research (Comité Ético de Investigación Clínica del Hospital Universitario Ramón y Cajal, Madrid, Spain) as well as by the Medical Direction on October 2013 (Ref. 251-13), since the study did not expose patients to any novel risk, and no investigational drugs, devices, or procedures were involved and verbal consent was considered sufficient.

The study included all standard safeguards for ensuring the confidentiality of patient information and specifications stipulated in the Personal Data Protection Act 15/1999, of 13 December were followed.

Statistical analyses

A descriptive analysis of the variables collected was conducted, the qualitative variables were expressed as percentages and the quantitative variables as measures of central tendency (mean and median) and dispersion (standard deviation). Pearson's Chi-squared test was used to compare proportions and the Student's T-test to compare means. All statistics analysis was performed using SPSS Statistics v19 (IBM®) software.

RESULTS

During the research period 12,590 admissions of 9,706 patients took place in the participating wards. In 84.5% of admissions, a rectal swab could be obtained within the first 48 hours of admission. Table 1.

TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and Neurosurgery wards and patients included in the study.

Ward	Admissions (n)	Swab at admission (n)	%
Gastroenterology	3,380	2,916	86.27
Pneumology	3,240	2,752	84.94
Urology	4,685	3,963	84.59
Neurosurgery	1,285	1,012	78.75
Total	**12,590**	**10,643**	**84.55**

Gender and mean age of included patients are shown in Table 2.

TABLE 2. Age and gender of the included patients.

Ward	Gender		Age (years)	
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)
Gastroenterology	1,732 (59.39)	1,184 (40.61)	66.53 (16.59)	69 (26.75)
Pneumology	1,625 (59.05)	1,127 (40.95)	70.72 (15.28)	74 (19)
Urology	3,009 (75.93)	954 (24.07)	66.89 (14.56)	69 (20)
Neurosurgery	533 (52.67)	479 (47.33)	60.23 (16.52)	61 (25)
Total	**6,899 (64.82)**	**3,744 (35.18)**	**64.91 (16.79)**	**67 (25)**

S.D.: standard deviation; I.R.: interquartile range.

The prevalence of ESBL-E fecal carriers at admission was 7.69% (Table 3). Table 3 shows the distribution of carriers by gender and ward, as well as their age (mean and median).

The majority of patients colonized with ESBL-E were male, just like the majority of hospital patients, the difference not being statistically significant. The mean age of colonized patients was higher than the mean age of the total number of hospitalized

patients (69.27 -S.D.15.68 *vs* 64.91 -S.D. 16.79-), the difference being statistically significant (p = 0.0087).

The difference in prevalence of colonization at admission among the surveyed wards was statistically significant (p = 0.001). The highest prevalence was found in the Gastroenterology ward, with 9.02%, the difference being significant with the rest of wards (p = 0.01). When comparing the prevalence between medical wards (Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery), the difference was not statistically significant.

A total of 843 multiresistant *Enterobacteriaceae* were isolated in 818 patients, as 25 patients were colonized by more than one microorganism at the time of admission (0.23%). Eighty-eight (10.44%) of the isolated *Enterobacteriaceae* were simultaneous ESBL and carbapenemase (CP) producers, 33.99% of these patients were known carriers, i.e., their clinical records included a previous positive culture for ESBL-E.

TABLE 3. ESBL-producing *Enterobacteriaceae* carriers at admission.

Hospital admission wards	Gender		Age (years)		Prevalence (%) CI 95%
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)	
Gastroenterology	159 (60.23)	104 (39.77)	66.78 (16.62)	67.2 (26.64)	9.02 (7.96-10.08)
Pneumology	122 (61.31)	77 (38.69)	74.78 (14.36)	79 (15)	7.23 (6.25-8.22)
Urology	234 (80.69)	56 (19.31)	69.82 (14.04)	72 (21)	7.32 (6.49-8.14)
Neurosurgery	44 (66.67)	22 (33.33)	62.45 (17.26)	66.67 (25.84)	6.52 (4.95-8.09)
Total	**559 (68.34)**	**259 (31.66)**	**69.27 (15.68)**	**72 (25)**	**7.69 (7.18-8.19)**

ESBL: extended-spectrum beta-lactamases; S.D.: standard deviation; I.R.: interquartile range ; CI: confidence interval.

The most frequently isolated ESBL-producer microorganism at admission was *E. coli* (77.70%; -n=655), followed by *K. pneumoniae* (20.64%, n=174), being only 1.66% other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C. amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%). Among ESBL-*E. coli* isolates, 1.83% were simultaneous ESBL and CP producers (n=12). Among ESBL-*K. pneumoniae* isolates, 43.10% were simultaneous ESBL and CP producers (n=75). Only one patient was colonized by a different ESBL and CP producer, *K. oxytoca*.

The typing of 208 beta-lactamases (24.67% of total ESBL) and 65 carbapenemases was possible (73.86% of total CP). Most of ESBL (83.17%) belonged to the CTX-M group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining 16.83% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the typed CP, 90.77% were OXA-48 type (Table 5). In the case of 4 patients colonized simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K. pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae* respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of them and CTX-M-14 in 1, and OXA-48 in the case of CP.

TABLE 4. Distribution of ESBL strains isolated and typed in rectal swabs at hospital admission

Enzyme	Microorganism						
	ESBL *E. coli*	ESBL *K. pneum.*	ESBL *E. cloacae*	ESBL *C. freundii*	ESBL +CP *E. coli*	ESBL +CP *K. pneum.*	**Total (%)**
CTX-M	1	-	-	-	-	-	**1 (0.48%)**
CTX-M-1	10	4	-	-	-	-	**14 (6.73%)**
CTX-M-9	10	3	-	-	-	-	**13 (6.25%)**
CTX-M-14	23	1	-	-	-	2	**26 (12.50%)**
CTX-M-15	35	31	1	-	3	40	**110 (52.88%)**
CTX-M-27	6	-	-	-	-	-	**6 (2.88%)**
CTX-M-32	2	-	-	-	-	-	**2 (0.96%)**
CTX-M-55	1	-	-	-	-	-	**1 (0.48%)**
SHV	1	1	-	-	-	-	**2 (0.96%)**
SHV-2	1	1	-	-	-	-	**2 (0.96%)**
SHV-12	10	8	-	1	-	5	**24 (11.54%)**
SHV-28	-	5	-	-	-	1	**6 (2.88%)**
SHV-31	-	1	-	-	-	-	**1 (0.48%)**
Total	**100**	**55**	**1**	**1**	**2**	**48**	**208 (100%)**

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase.

TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal swabs at hospital admission

Enzyme		Microorganism	
	ESBL +CP *E. coli*	ESBL +CP *K. pneumoniae*	Total (%)
KPC-3	1	-	1 (1.54%)
NDM-1	-	1	1 (1.54%)
OXA-48	11	48	59 (90.77%)
VIM-1	-	4	4 (6.15%)
Total	12	53	65 (100%)

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase

Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of patients admitted during the research period and 6.6% of ESBL-E intestinal carriers. Of those 54 patients, all except one also showed a positive rectal swab, 90.74% of those (49 patients) with the same specie causing the infection, and 9.26% (5 patients) with a different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures) were urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

A total of 56 microorganisms were isolated in the 55 positive clinical cultures, as one of them was positive for two ESBL-E. The most frequently isolated microorganism was once again *E. coli* (67.86%), followed by ESBL and CP-*K. pneumoniae* (23.21%), ESBL-*K. pneumoniae* (7.14%); *K. oxytoca* was isolated in 1 culture (1.79%).

DISCUSSION

In our study, the prevalence of ESBL-E carriers at admission was 7.69%, ranging between 6.52% and 9.02% depending on the ward. The prevalence of ESBL-E carriers in healthy individuals as well as in ambulatory and hospitalized patients has been researched in a number of studies. In all of them, *E. coli* is always the most frequently isolated microorganism, similarly to our study (77.70%) [11-19]. In a meta-analysis published in 2016 which analyzed prevalence studies in healthy persons, and included 28,909 individuals from 66 studies, the mean global prevalence of colonization was 14%, with great variability among regions [19]. It was higher in Asia, with 46% and Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe, 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean prevalence was 2%, although it was admitted that there were very few studies for this region [20].

Our prevalence of intestinal carriers at admission is virtually the same to that found by a Dutch study recently published, which was 7.9% in patients coming from their homes and 8.6% in patients coming from long-term care facilities, a distinction not made in our research [21]. Studies in three different areas in Spain (Madrid, Barcelona and Zaragoza) show that the prevalence of carriers has increased in the last years, reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers among patients admitted to Emergency Units was 7.4%, also very similar to our figure [22].

In our facility, 10.44% of ESBL microorganisms were simultaneous carbapenemase producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the 65 carbapenemases typed (73.86% of total CP), the vast majority of them, 90.77% belonged to the OXA-48 type. This fact is especially important in the case of *K. pneumoniae* with 43.10% of them being ESBL and CP producers (90.57% OXA-48). ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed

at hospital admission (69.27% of them urinary tract infections). We did not find a similar study to compare our data with but we think this finding must be deeply analyzed.

Male gender has been identified as a risk factor for the intestinal colonization by ESBL-E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized patients were men, but they were also the majority of the total number of hospitalized patients, the difference not being statistically significant [11]. Age is another risk factor identified in the bibliography; in our study, the mean age of colonized patients was higher than the mean age of hospitalized patients (69.27 years *vs* 64.91 years), being the difference statistically significant in this case (p = 0.0087) [23, 24].

The prevalence of carriers at admission was higher in the Gastroenterology ward, despite being younger than the mean, with a difference statistically significant as compared to the rest of included wards. In other published studies, liver disease has been identified as a risk factor for intestinal colonization by ESBL-E, being the prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in patients with chronic liver disease one of the possible explanations [25, 26]. Another risk factor for ESBL-E carriage recently described in the literature is proton pump inhibitors (PPI) use, and these type of patients are often receiving PPIs and other medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide an explanation as risk factors for every patient were not recorded.

Unfortunately total characterization was not feasible in all isolates due to budget issues so we decided to analyze a random selection. We were able to determine 24.67% of total ESBL producing isolates; that low percentage is a limitation of our study and the results could differ if all the ESBLs had been analyzed but they are compatible with the epidemiology described in the literature. The main enzyme group was CTX-M, the most common according to the literature, followed by SHV, CTX-M-15 group prevailing with 52.88% [8, 12-14, 19, 21, 22, 24].

In the last years, ESBL-E infections have become an increasing concern; in the United States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur per year [29]. Infections by these bacteria are associated to higher mortality rates and higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However, few studies have associated the fact of being an intestinal carrier of ESBL-E with the development of infections caused by these bacteria. A recent cohort study performed in patients with haematological malignancies found a 3.5-fold greater risk of developing bacteraemia by ESBL-E among colonized patients when compared to non-colonized patients; despite of the fact that mortality was similar in both groups, colonization was associated to longer hospital stays, shorter survival period and higher costs [31]. On the contrary, another similar study did not find correlation between ESBL-E colonization and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were diagnosed at admission and almost 70% were urinary tract infections. That means that 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of the colonized patients. Only in one patient with ESBL-E infection at admission no ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections were found in colonized patients, the total prevalence of infection is very low, and only in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract infection). In two cases patients died during hospital admission, although their infection had been fully resolved and death was caused by an underlying oncological disease.

This study, one of the most prolonged in time and with the largest number of patients, confirms once again the extension of ESBL-E intestinal colonization in the community showing, however, a low prevalence of infection. It is necessary to continue with the epidemiological surveillance of these microorganisms, in order to acquire a better knowledge of the implications of being an intestinal carrier of ESBL-E. The high percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply studied.

WORD COUNT

2,450

AUTHOR CONTRIBUTIONS

Conception and design of study: M Bonten, R Cantón, P Gastemeier, F Maechler.

Data collection: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa.

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DATA SHARING STATEMENT

Extra data is available by emailing: cristina.diazagero@salud.madrid.org

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Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
	#12b	Describe any methods used to examine subgroups and interactions	6
	#12c	Explain how missing data were addressed	NA
	#12d	If applicable, describe analytical methods taking account of sampling strategy	NA
	#12e	Describe any sensitivity analyses	NA
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	7
	#13b	Give reasons for non-participation at each stage	7
	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7

	#14b	Indicate number of participants with missing data for each variable of interest	7
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
	#16b	Report category boundaries when continuous variables were categorized	7
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	10
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11-12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11-12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

BMJ Open

Local prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in Klebsiella pneumoniae: a prevalence survey in a Spanish University Hospital

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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Carbapenemase producing Enterobacteriaceae, Surveillance, Prevalence

TITLE:

Local prevalence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in *Klebsiella pneumoniae:* a prevalence survey in a Spanish University Hospital

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ABSTRACT

Objective: to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in a University Hospital in Spain.

Design: prevalence survey.

Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a University tertiary hospital in Madrid (Spain).

Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-2016 with a rectal swab taken at admission or as soon as possible within the first 48 hours.

Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers and prevalence of ESBL-E infections at admission.

Results: the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19). Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae* (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K. pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an active infection by ESBL-E at admission.

Conclusions: The prevalence found in our study is very similar to that found in the literature. However, we found a high percentage of simultaneous ESBL and CP producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of colonized patients, the ESBL-infection rate on admission was very low.

Key words: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*, carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study is one of the most prolonged in time and with the largest number of patients assessing colonization with multidrug resistant microorganisms, including adult participants of variable age groups and gender from a university

hospital providing specialized assistance to 8.51% of the population of Madrid (Spain).

- The large number of patients included (10,643) gives strength to the results.
- Genes codifying ESBL and CP were characterized by PCR and sequencing. Unfortunately total characterization was not feasible in all isolates, only 24.67% of total ESBL producing isolates and 73.86% of total CP producing isolates.

FUNDING STATEMENT

- The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation strategies for ESBL carriers in medical and surgical hospital wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-N°282512).
- MH-G is supported with a contract from Instituto de Salud Carlos III of Spain (iP-FIS program, ref. IFI14/00022).

POTENTIAL CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

TEXT

BACKGROUND

The emergence of antimicrobial resistance represents a global challenge for healthcare due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization, prolonged hospitalization, ICU admission and previous use of cephalosporins and aminoglycosides were leading risk factors [3, 4].

The situation today is very different since their prevalence has increased dramatically in the community, especially in urinary tract infections, where these enzymes are more frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the inadequate empirical treatment, delaying the efficient antimicrobial treatment for example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the prevalence of microbial resistance in our geographic area and the epidemiological characteristics in order to establish the scope of the problem and analyze its evolution.

The aim of this study was to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an active surveillance screening program (R-GNOSIS project).

METHODS

Study design and settings

The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms: Studying Intervention Strategies), within the Work Package 5 Patient isolation

strategies for ESBL carriers in medical and surgical hospital wards, funded by the EU (FP7-HEALTH-2011-SINGLE STAGE-N°282512).

The University Hospital Ramón y Cajal is a public referral center, located in the North of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds) wards took part in the study.

Patients

Between March 3rd 2014 and April 3rd 2016, screening rectal swabs were obtained, after verbal consent, from all patients aged 18 and older, at admission or as soon as possible within the first 48 hours.

Patient involvement

Patients were not directly involved in the design and conception of the study. All patients were informed of the aim of the study and the consequences of a positive result (contact isolation and needing a new rectal screening at any hospital admission in the future to check their status) and gave their verbal consent to participate; if the patient refused the swab was not taken. As soon as the microbiological result was known by the investigators, patients and their familiars were informed.

Laboratory analysis

The samples were seeded on ChromoID-ESBL and Chromo-ID CARBA/OXA-48 (BioMérieux, France) selective chromogenic-agar plates. Bacterial identification was performed using the MALDI-TOF-MS (Bruker-Daltonics, Germany) mass spectrometry. ESBL and carbapenemase (CP) production were phenotypically confirmed by the double-disk diffusion test, Hodge Test and KPC/MBL/OXA-48 Confirm and ESBL AmpC Screen Kits (Rosco Diagnostica, Germany). Antimicrobial susceptibility was studied with microdilution (MicroScan, Beckman, CA) and gradient strips (Etests,

BioMérieux, France). Genes codifying ESBL (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) and CP (bla_{VIM}, bla_{KPC}, *bla*NDM, bla_{OXA-48}) were characterized by PCR and sequencing.

Ethics

The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines (ICH-GCP-Guidelines, CMPM/ICH/135/95) of the European Medicines Agency.

The Ethics Committee of Clinical Research (Comité Ético de Investigación Clínica del Hospital Universitario Ramón y Cajal, Madrid, Spain) formally reviewed and approved the study protocol on October 2013 (Ref. 251-13). A waiver of written informed consent of individual patients in the participating wards was requested and granted by the Committee as well as by the Medical Direction, since the study did not expose patients to any novel risk, and no investigational drugs, devices, or procedures were involved and verbal consent was considered sufficient.

The study included all standard safeguards for ensuring the confidentiality of patient information and specifications stipulated in the Personal Data Protection Act 15/1999, of 13 December were followed.

Statistical analyses

A descriptive analysis of the variables collected was conducted, the qualitative variables were expressed as percentages and the quantitative variables as measures of central tendency (mean and median) and dispersion (standard deviation). Pearson's Chi-squared test was used to compare proportions and the Student's T-test to compare means. All statistics analysis was performed using SPSS Statistics v19 (IBM®) software.

RESULTS

During the research period 12,590 admissions of 9,706 patients took place in the participating wards. In 84.5% of admissions, a rectal swab could be obtained within the first 48 hours of admission. Table 1.

TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and Neurosurgery wards and patients included in the study.

Ward	Admissions (n)	Swab at admission (n)	%
Gastroenterology	3,380	2,916	86.27
Pneumology	3,240	2,752	84.94
Urology	4,685	3,963	84.59
Neurosurgery	1,285	1,012	78.75
Total	**12,590**	**10,643**	**84.55**

Gender and mean age of included patients are shown in Table 2.

TABLE 2. Age and gender of the included patients.

Ward	Gender		Age (years)	
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)
Gastroenterology	1,732 (59.39)	1,184 (40.61)	66.53 (16.59)	69 (26.75)
Pneumology	1,625 (59.05)	1,127 (40.95)	70.72 (15.28)	74 (19)
Urology	3,009 (75.93)	954 (24.07)	66.89 (14.56)	69 (20)
Neurosurgery	533 (52.67)	479 (47.33)	60.23 (16.52)	61 (25)
Total	**6,899 (64.82)**	**3,744 (35.18)**	**64.91 (16.79)**	**67 (25)**

S.D.: standard deviation; I.R.: interquartile range.

The prevalence of ESBL-E fecal carriers at admission was 7.69% (Table 3). Table 3 shows the distribution of carriers by gender and ward, as well as their age (mean and median).

The majority of patients colonized with ESBL-E were male, just like the majority of hospital patients, the difference not being statistically significant. The mean age of colonized patients was higher than the mean age of the total number of hospitalized

patients (69.27 -S.D.15.68 *vs* 64.91 -S.D. 16.79-), the difference being statistically significant (p = 0.0087).

The difference in prevalence of colonization at admission among the surveyed wards was statistically significant (p = 0.001). The highest prevalence was found in the Gastroenterology ward, with 9.02%, the difference being significant with the rest of wards (p = 0.01). When comparing the prevalence between medical wards (Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery), the difference was not statistically significant.

A total of 843 multiresistant *Enterobacteriaceae* were isolated in 818 patients, as 25 patients were colonized by more than one microorganism at the time of admission (0.23%). Eighty-eight (10.44%) of the isolated *Enterobacteriaceae* were simultaneous ESBL and carbapenemase (CP) producers, 33.99% of these patients were known carriers, i.e., their clinical records included a previous positive culture for ESBL-E.

TABLE 3. ESBL-producing *Enterobacteriaceae* carriers at admission.

Hospital admission wards	Gender		Age (years)		Prevalence (%) CI 95%
	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)	
Gastroenterology	159 (60.23)	104 (39.77)	66.78 (16.62)	67.2 (26.64)	9.02 (7.96-10.08)
Pneumology	122 (61.31)	77 (38.69)	74.78 (14.36)	79 (15)	7.23 (6.25-8.22)
Urology	234 (80.69)	56 (19.31)	69.82 (14.04)	72 (21)	7.32 (6.49-8.14)
Neurosurgery	44 (66.67)	22 (33.33)	62.45 (17.26)	66.67 (25.84)	6.52 (4.95-8.09)
Total	**559 (68.34)**	**259 (31.66)**	**69.27 (15.68)**	**72 (25)**	**7.69 (7.18-8.19)**

ESBL: extended-spectrum beta-lactamases; S.D.: standard deviation; I.R.: interquartile range ; CI: confidence interval.

The most frequently isolated ESBL-producer microorganism at admission was *E. coli* (77.70%; -n=655), followed by *K. pneumoniae* (20.64%, n=174), being only 1.66% other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C. amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%). Among ESBL-*E. coli* isolates, 1.83% were simultaneous ESBL and CP producers (n=12). Among ESBL-*K. pneumoniae* isolates, 43.10% were simultaneous ESBL and CP producers (n=75). Only one patient was colonized by a different ESBL and CP producer, *K. oxytoca*.

The typing of 208 beta-lactamases (24.67% of total ESBL) and 65 carbapenemases was possible (73.86% of total CP). Most of ESBL (83.17%) belonged to the CTX-M group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining 16.83% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the typed CP, 90.77% were OXA-48 type (Table 5). In the case of 4 patients colonized simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K. pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae* respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of them and CTX-M-14 in 1, and OXA-48 in the case of CP.

TABLE 4. Distribution of ESBL strains isolated and typed in rectal swabs at hospital admission

Enzyme	Microorganism						
	ESBL *E. coli*	ESBL *K. pneum.*	ESBL *E. cloacae*	ESBL *C. freundii*	ESBL +CP *E. coli*	ESBL +CP *K. pneum.*	**Total (%)**
CTX-M	1	-	-	-	-	-	**1 (0.48%)**
CTX-M-1	10	4	-	-	-	-	**14 (6.73%)**
CTX-M-9	10	3	-	-	-	-	**13 (6.25%)**
CTX-M-14	23	1	-	-	-	2	**26 (12.50%)**
CTX-M-15	35	31	1	-	3	40	**110 (52.88%)**
CTX-M-27	6	-	-	-	-	-	**6 (2.88%)**
CTX-M-32	2	-	-	-	-	-	**2 (0.96%)**
CTX-M-55	1	-	-	-	-	-	**1 (0.48%)**
SHV	1	1	-	-	-	-	**2 (0.96%)**
SHV-2	1	1	-	-	-	-	**2 (0.96%)**
SHV-12	10	8	-	1	-	5	**24 (11.54%)**
SHV-28	-	5	-	-	-	1	**6 (2.88%)**
SHV-31	-	1	-	-	-	-	**1 (0.48%)**
Total	**100**	**55**	**1**	**1**	**2**	**48**	**208 (100%)**

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase.

TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal swabs at hospital admission

Enzyme		Microorganism	
	ESBL +CP *E. coli*	ESBL +CP *K. pneumoniae*	Total (%)
KPC-3	1	-	1 (1.54%)
NDM-1	-	1	1 (1.54%)
OXA-48	11	48	59 (90.77%)
VIM-1	-	4	4 (6.15%)
Total	12	53	65 (100%)

ESBL: Extended-spectrum beta-lactamases; CP: carbapenemase

Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of patients admitted during the research period and 6.6% of ESBL-E intestinal carriers. Of those 54 patients, all except one also showed a positive rectal swab, 90.74% of those (49 patients) with the same specie causing the infection, and 9.26% (5 patients) with a different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures) were urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

A total of 56 microorganisms were isolated in the 55 positive clinical cultures, as one of them was positive for two ESBL-E. The most frequently isolated microorganism was once again *E. coli* (67.86%), followed by ESBL and CP-*K. pneumoniae* (23.21%), ESBL-*K. pneumoniae* (7.14%); *K. oxytoca* was isolated in 1 culture (1.79%).

DISCUSSION

In our study, the prevalence of ESBL-E carriers at admission was 7.69%, ranging between 6.52% and 9.02% depending on the ward. The prevalence of ESBL-E carriers in healthy individuals as well as in ambulatory and hospitalized patients has been researched in a number of studies. In all of them, *E. coli* is always the most frequently isolated microorganism, similarly to our study (77.70%) [11-19]. In a meta-analysis published in 2016 which analyzed prevalence studies in healthy persons, and included 28,909 individuals from 66 studies, the mean global prevalence of colonization was 14%, with great variability among regions [19]. It was higher in Asia, with 46% and Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe, 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean prevalence was 2%, although it was admitted that there were very few studies for this region [20].

Our prevalence of intestinal carriers at admission is virtually the same to that found by a Dutch study recently published, which was 7.9% in patients coming from their homes and 8.6% in patients coming from long-term care facilities, a distinction not made in our research [21]. Studies in three different areas in Spain (Madrid, Barcelona and Zaragoza) show that the prevalence of carriers has increased in the last years, reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers among patients admitted to Emergency Units was 7.4%, also very similar to our figure [22].

In our facility, 10.44% of ESBL microorganisms were simultaneous carbapenemase producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the 65 carbapenemases typed (73.86% of total CP), the vast majority of them, 90.77% belonged to the OXA-48 type. This fact is especially important in the case of *K. pneumoniae* with 43.10% of them being ESBL and CP producers (90.57% OXA-48). ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed at hospital admission (69.27% of them urinary tract infections). We did not find a similar

study to compare our data with but we think this finding must be deeply analyzed. We found one case of KPC-3 (ESBL+CP *E. coli*) and one case of NDM-1 (ESBL+CP *K. pneumoniae*).

Male gender has been identified as a risk factor for the intestinal colonization by ESBL-E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized patients were men, but they were also the majority of the total number of hospitalized patients, the difference not being statistically significant [11]. Age is another risk factor identified in the bibliography; in our study, the mean age of colonized patients was higher than the mean age of hospitalized patients (69.27 years *vs* 64.91 years), being the difference statistically significant in this case (p = 0.0087) [23, 24].

The prevalence of carriers at admission was higher in the Gastroenterology ward, despite being younger than the mean, with a difference statistically significant as compared to the rest of included wards. In other published studies, liver disease has been identified as a risk factor for intestinal colonization by ESBL-E, being the prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in patients with chronic liver disease one of the possible explanations [25, 26]. Another risk factor for ESBL-E carriage recently described in the literature is proton pump inhibitors (PPI) use, and these type of patients are often receiving PPIs and other medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide an explanation as risk factors for every patient were not recorded.

Unfortunately total characterization was not feasible in all isolates due to budget issues so we decided to analyze a random selection. We were able to determine 24.67% of total ESBL producing isolates; that low percentage is a limitation of our study and the results could differ if all the ESBLs had been analyzed but they are compatible with the epidemiology described in the literature. The main enzyme group was CTX-M, the most common according to the literature, followed by SHV, CTX-M-15 group prevailing with 52.88% [8, 12-14, 19, 21, 22, 24].

In the last years, ESBL-E infections have become an increasing concern; in the United States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur per year [29]. Infections by these bacteria are associated to higher mortality rates and higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However, few studies have associated the fact of being an intestinal carrier of ESBL-E with the development of infections caused by these bacteria. A recent cohort study performed in patients with haematological malignancies found a 3.5-fold greater risk of developing bacteraemia by ESBL-E among colonized patients when compared to non-colonized patients; despite of the fact that mortality was similar in both groups, colonization was associated to longer hospital stays, shorter survival period and higher costs [31]. On the contrary, another similar study did not find correlation between ESBL-E colonization and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were diagnosed at admission and almost 70% were urinary tract infections. That means that 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of the colonized patients. Only in one patient with ESBL-E infection at admission no ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections were found in colonized patients, the total prevalence of infection is very low, and only in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract infection). In two cases patients died during hospital admission, although their infection had been fully resolved and death was caused by an underlying oncological disease.

This study, one of the most prolonged in time and with the largest number of patients, confirms once again the extension of ESBL-E intestinal colonization in the community showing, however, a low prevalence of infection. It is necessary to continue with the epidemiological surveillance of these microorganisms, in order to acquire a better knowledge of the implications of being an intestinal carrier of ESBL-E. The high percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply studied.

WORD COUNT

2,411

AUTHOR CONTRIBUTIONS

Conception and design of study: M Bonten, R Cantón, P Gastemeier, F Maechler.

Data collection: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa.

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DATA SHARING STATEMENT

Extra data is available by emailing: cristina.diazagero@salud.madrid.org

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Reporting checklist for cross sectional study.

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Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
	#12b	Describe any methods used to examine subgroups and interactions	6
	#12c	Explain how missing data were addressed	NA
	#12d	If applicable, describe analytical methods taking account of sampling strategy	NA
	#12e	Describe any sensitivity analyses	NA
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	7
	#13b	Give reasons for non-participation at each stage	7
	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7

	#14b	Indicate number of participants with missing data for each variable of interest	7
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
	#16b	Report category boundaries when continuous variables were categorized	7
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	10
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11-12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11-12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3