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

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Complete List of Authors:	DIAZ AGERO PEREZ, CRISTINA; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; INSTITUTO RAMON Y CAJAL DE INVESTIGACIÓN SANITARIRA (IRYCIS), LOPEZ FRESNEÑA, NIEVES; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Pública; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Rincon Carlavilla, Angela; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Pública; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Hernandez Garcia, Marta ; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) Ruiz-Garbajosa, Patricia; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) ARANAZ ANDRÉS , JESÚS MARIA; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; IRYCIS, INSTITUTO RAMÓN Y CAJAL DE INVESTIGACIÓN SANITARIA Maechler, F; Charite Universitarsmedizin Berlin, Gastmeier, Petra; Charite, Infection Control Bonten, Marc; University Medical Center Utrecht, Department of Medical Microbiology Canton, Rafael; Hospital Universitario Ramón y Cajal, Madrid, Spain, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI)
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# SCHOLARONE<sup>™</sup> Manuscripts

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2 3	1	TITLE:
4 5	2	Local prevalence of extended-spectrum beta-lactamase (ESBL) producing
6 7	3	Enterobacteriaceae intestinal carriers at admission in a Spanish University
8 9	4	Hospital and co-expression of ESBL and OXA-48 carbapenemase in Klebsiella
10 11	5	pneumoniae
12 13	6	
14 15	7	Authors:
16 17	8	C. Díaz-Agero Pérez <sup>1</sup> , N. López-Fresneña <sup>1</sup> , A.L. Rincón-Carlavilla <sup>1</sup> , M. Hernández-
18 19	9	García <sup>2, 3</sup> , P. Ruiz-Garbajosa <sup>2,3</sup> , J.M. Aranaz-Andrés <sup>1</sup> , F. Maechler <sup>4</sup> , P. Gastmeier <sup>4</sup> , M.
20 21	10	Bonten <sup>5</sup> , R Cantón <sup>2,3</sup>
22 23	11	
24 25	12	<sup>1</sup> Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal
26 27	13	and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
28 29	14	<sup>2</sup> Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y
30 31	15	Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
32 33	16	<sup>3</sup> Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain.
34 35	17	<sup>4</sup> Charité –University Medicine Berlin, Germany.
36 37	18	<sup>5</sup> University Medical Center Utrecht (UMC Utrecht), Utrecht, The Netherlands.
38 39	19	
40 41	20	Corresponding author:
42 43	21	Cristina Díaz-Agero Pérez
44 45	22	Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal.
46 47	23	Carretera de Colmenar Km 9,1. Madrid 28034. Spain
48 49	24	email address: cristina.diazagero@salud.madrid.org)
50 51	25	TI.: 0034913368372 / 0034913368604
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29	ABSTRACT
30	<b>Objective:</b> to assess the prevalence of ESBL-producing <i>Enterobacteriaceae</i> (ESBL-E)
31	fecal carriers at admission in a University Hospital in Spain.
32	Design: prevalence survey.
33	Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a
34	University tertiary hospital in Madrid (Spain).
35	Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-
36	2016 with a rectal swab taken at admission or as soon as possible within the first 48
37	hours.
38	Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers
39	and prevalence of ESBL-E infections at admission.
40	Results: the ESBL-E carriers prevalence on admission was 7.70% (CI 95% 7.19-8.22
41	Most of the isolates were Escherichia coli (77.51%), followed by Klebsiella pneumonia
42	(20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
43	carbapenemase (CP) producers, 1.83% in the case of <i>E. coli</i> and 42.86% among <i>K</i> .
44	pneumoniae isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
45	91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented ar
46	active infection by ESBL-E at admission.
47	Conclusions: The prevalence found in our study is very similar to that found in the
48	literature. However, we found a high percentage of simultaneous ESBL and CP
49	producers, particularly in Klebsiella pneumoniae. Despite the high prevalence of
50	colonized patients, the ESBL-infection rate on admission was very low.
51	Key words: Extended-spectrum beta-lactamase producing Enterobacteriacea
52	carbapenemase producing Enterobacteriaceae, surveillance, prevalence.
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57	ARTICLE SUMMARY
58	Strengths and limitations of this study
59	This study is one of the most prolonged in time and with the largest number
60	patients assessing colonization with multidrug resistant microorganism
61	including adult participants of variable age groups and gender from a univers
62	hospital providing specialized assistance to 8.51% of the population of Mad
63	(Spain)
64	• The large number of patients included (10,643) gives strength to the results.
65	Genes codifying ESBL and CP were characterized by PCR and sequencing
66	Unfortunately total characterization was not feasible in all isolates, only 24.73
67	of total ESBL producing isolates and 65.91% of total CP producing isolates.
68	
69	FUNDING STATEMENT
70	• The project falls within the R-GNOSIS study (Resistance of Gram-Negat
71	Organisms: Studying Intervention Strategies), within the Work Package
72	Patient isolation strategies for ESBL carriers in medical and surgical hosp
73	wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAG
74	N°282512).
75	• MH-G is supported with a contract from Instituto de Salud Carlos III of Sp
76	(iP-FIS program, ref. IFI14/00022).
77	
78	POTENTIAL CONFLICTS OF INTEREST
79	All authors declare that they have no conflict of interest.
80	
81	

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**TEXT** 

#### 83 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.

101 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 103 active surveillance screening program (R-GNOSIS project).

#### 105 METHODS

#### 106 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

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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.

#### 117 Patients

Between March 3<sup>rd</sup> 2014 and April 3<sup>rd</sup> 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.

#### 121 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.

#### 127 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*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>) and CP (*bla*<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*NDM, *bla*<sub>OXA-48</sub>) were characterized by PCR and sequencing.

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 **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.

### 145 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
159				•

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

## **TABLE 2. Age and gender of the included patients.**

Ward	Ger	lder	Age (years)		
Ward	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)	

163 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

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(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 .(10.4 anemase (CP), elinical records include. (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.

Т

184		TABLE 3. E	SBL-producing	Enterobacteriace	ae carriers at admi	ission.	
	Hospital admission	Ger	nder	Age (	years)	Prevalence (%) CI 95%	
	wards	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)	-
185				· • · · ·			]
186 ESBL: e 187 188	extended-spectrum beta-				tile range ; CI: confi		
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Ab Devious of the start published as 10.1136/bmjopen-2018-024879 on 1 March 2019. Downloaded from http://mjopen.bmj.com/ on June 11, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright,/mg/hg/hg/hg/hg/hg/hg/hg/hg/hg/hg/hg/hg/hg							

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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.

## 216 TABLE 4. Distribution of ESBL strains isolated and typed in rectal swabs at

## 217 hospital admission

				Microorgan	ism		
Enzyme	ESBL	ESBL	ESBL	ESBL	ESBL +CP	ESBL +CP	
	E. coli	K. pneum.	E. cloacae	C. freundii	E. coli	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	-	-	-	6 (2.87%)
CTX-M-32	2	-	-/	-	-	-	2 (0.96%)
CTX-M-55	1	-	-	<u> </u>	-	-	1 (0.48%)
SHV	5	1	-	· Z	-	-	6 (2.87%)
SHV-2	-	1	-	6	-	-	1 (0.48%)
SHV-12	7	8	-	1	-	5	21 (10.05%
SHV-28	-	5	-	-	-	1	6 (2.87%)
SHV-31	-	1	-	-	9	-	1 (0.48%)
Total	100	56	1	1	5	46	209 (100%
218	<u> </u>			I		ľ	
219 ES	BL: Extend	led-spectrum b	peta-lactamas	es; CP: carba	penemase.		
220							
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## TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal

## 229 swabs at hospital admission

	Microorganism				
Enzyme	ESBL +CP <i>E. coli</i>	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	5	3	3 (5.17%)		
Total	9	49	58 (100%)		

231 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%).

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247 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

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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 Page 15 of 23

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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.

- 326 WORD COUNT
- 327 2,256

- 329 AUTHOR CONTRIBUTIONS
- **Conception and design of study:** M Bonten, R Cantón, P Gastemeier, F Maechler.

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#### Reporting checklist for cross sectional study. 2 3 4 5 Based on the STROBE cross sectional guidelines. 6 7 8 Instructions to authors 9 10 Complete this checklist by entering the page numbers from your manuscript where readers will find 11 12 each of the items listed below. 13 14 Your article may not currently address all the items on the checklist. Please modify your text to 15 16 include the missing information. If you are certain that an item does not apply, please write "n/a" and 17 provide a short explanation. 18 19 Upload your completed checklist as an extra file when you submit to a journal. 20 21 22 In your methods section, say that you used the STROBE cross sectional reporting guidelines, and 23 cite them as: 24 25 von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening 26 27 the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for 28 reporting observational studies. 29 30 31 32 Reporting Item 33 34 35 Title #1a Indicate the study's design with a commonly used term in the 36 title or the abstract 37 38 #1b Provide in the abstract an informative and balanced summary 39 Abstract 40 of what was done and what was found 41 42 Background / #2 Explain the scientific background and rationale for the 43 44 rationale investigation being reported 45 46 Objectives #3 State specific objectives, including any prespecified 47 48 hypotheses 49 50 Study design #4 Present key elements of study design early in the paper 51 52 Setting #5 Describe the setting, locations, and relevant dates, including 53 54 periods of recruitment, exposure, follow-up, and data collection 55 56 Eligibility criteria #6a Give the eligibility criteria, and the sources and methods of 57 58 selection of participants. 59 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml 60

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24 25 26 27	Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	1 March 2019. I Enseignen or uses relatec 6
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	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	as 10.1136/bmjopen-2018-024879 on 1 March Protected by copyright, including for uses
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20 21 22 23		#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	879 on 1 Ma Iuding for u
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	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	bmjopen.bmj.com/ on June 11, 2025 at y Al training, and similar technologies. 12 1
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## 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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Complete List of Authors:	DIAZ AGERO PEREZ, CRISTINA; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; INSTITUTO RAMON Y CAJAL DE INVESTIGACION SANITARIA (IRYCIS) LOPEZ FRESNEÑA, NIEVES; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Pública; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Rincon Carlavilla, Angela; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Hernandez Garcia, Marta ; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) Ruiz-Garbajosa, Patricia; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) ARANAZ ANDRÉS , JESÚS MARIA; Hospital Universitario Ramon y Cajal, Servicio RAMÓN Y CAJAL DE INVESTIGACIÓN SANITARIA Maechler, F; Charite Universitarismedizin Berlin, Gastmeier, Petra; Charite, Infection Control Bonten, Marc; University Medical Center Utrecht, Department of Medical Microbiology Canton, Rafael; Hospital Universitario Ramón y Cajal, Madrid, Spain, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI)
<b>Primary Subject Heading</b> :	Epidemiology
Secondary Subject Heading:	Public health
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Carbapenemase producing Enterobacteriaceae, Surveillance, Prevalence

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1	TITLE:
2	Local prevalence of extended-spectrum beta-lactamase (ESBL) producing
3	Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL
4	and OXA-48 carbapenemase in <i>Klebsiella pneumoniae:</i> a prevalence survey in a
5	Spanish University Hospital
6	
7	Authors:
8	C. Díaz-Agero Pérez <sup>1</sup> , N. López-Fresneña <sup>1</sup> , A.L. Rincón-Carlavilla <sup>1</sup> , M. Hernández-
9	García <sup>2, 3</sup> , P. Ruiz-Garbajosa <sup>2,3</sup> , J.M. Aranaz-Andrés <sup>1</sup> , F. Maechler <sup>4</sup> , P. Gastmeier <sup>4</sup> , M.
10	Bonten <sup>5</sup> , R Cantón <sup>2,3</sup>
11	
12	<sup>1</sup> Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal
13	and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
14	<sup>2</sup> Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y
15	Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
16	<sup>3</sup> Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain.
17	<sup>4</sup> Charité –University Medicine Berlin, Germany.
18	<sup>5</sup> University Medical Center Utrecht (UMC Utrecht), Utrecht, The Netherlands.
19	
20	Corresponding author:

2		
3 4	21	Cristina Díaz-Agero Pérez
5 6		
7	22	Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal.
8 9		
10 11	23	Carretera de Colmenar Km 9,1. Madrid 28034. Spain
12		
13 14	24	email address: cristina.diazagero@salud.madrid.org)
14 15		
16 17	25	TI.: 0034913368372 / 0034913368604
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26 27	29	ABSTRACT
28		
29	30	<b>Objective:</b> to assess the prevalence of ESBL-producing <i>Enterobacteriaceae</i> (ESBL-E)
30 31		
32	31	fecal carriers at admission in a University Hospital in Spain.
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35	32	Design: prevalence survey.
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38	33	Setting: Pnoumology, Castroonterology, Urology and Nourosurgery units at a
39 40	55	Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a
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42	34	University tertiary hospital in Madrid (Spain).
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45	35	Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-
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48	36	2016 with a rectal swab taken at admission or as soon as possible within the first 48
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50 51	37	hours.
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53 54	38	Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers and
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56 57	39	prevalence of ESBL-E infections at admission.
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2 3 4 5	40	Results: the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19).
6 7 8	41	Most of the isolates were Escherichia coli (77.51%), followed by Klebsiella pneumoniae
9 10 11	42	(20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
12 13 14 15	43	carbapenemase (CP) producers, 1.83% in the case of <i>E. coli</i> and 42.86% among <i>K.</i>
16 17 18	44	<i>pneumoniae</i> isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
19 20 21	45	91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an
22 23 24	46	active infection by ESBL-E at admission.
25 26 27 28	47	Conclusions: The prevalence found in our study is very similar to that found in the
29 30 31	48	literature. However, we found a high percentage of simultaneous ESBL and CP
32 33 34	49	producers, particularly in Klebsiella pneumoniae. Despite the high prevalence of
35 36 37	50	colonized patients, the ESBL-infection rate on admission was very low.
38 39	51	Key words: Extended-spectrum beta-lactamase producing Enterobacteriaceae,
40 41 42	52	carbapenemase producing Enterobacteriaceae, surveillance, prevalence.
43	53	
44 45	54	
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51	57	ARTICLE SUMMARY
52 53 54	58	Strengths and limitations of this study
55 56	59	• This study is one of the most prolonged in time and with the largest number of
57 58	60	patients assessing colonization with multidrug resistant microorganisms,
59 60	61	including adult participants of variable age groups and gender from a university

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1 2		
2 3 4	62	hospital providing specialized assistance to 8.51% of the population of Madrid
5 6	63	(Spain).
7 8	64	• The large number of patients included (10,643) gives strength to the results.
9 10	65	• Genes codifying ESBL and CP were characterized by PCR and sequencing.
11 12	66	Unfortunately total characterization was not feasible in all isolates, only 24.67%
13 14	67	of total ESBL producing isolates and 73.86% of total CP producing isolates.
15 16	68	
17 18 19	69	FUNDING STATEMENT
20 21	70	• The project falls within the R-GNOSIS study (Resistance of Gram-Negative
22 23	71	Organisms: Studying Intervention Strategies), within the Work Package 5
24 25	72	Patient isolation strategies for ESBL carriers in medical and surgical hospital
26 27	73	wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-
28 29	74	N°282512).
30 31	75	• MH-G is supported with a contract from Instituto de Salud Carlos III of Spain
32 33	76	(iP-FIS program, ref. IFI14/00022).
34 35 26	77	
36 37 38	78	POTENTIAL CONFLICTS OF INTEREST
39 40	79	All authors declare that they have no conflict of interest.
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#### **TEXT**

## 84 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.

102 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 104 active surveillance screening program (R-GNOSIS project).

106 METHODS

## 107 Study design and settings

108 The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms:109 Studying Intervention Strategies), within the Work Package 5 Patient isolation

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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.

118 Patients

Between March 3<sup>rd</sup> 2014 and April 3<sup>rd</sup> 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.

122 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.

128 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<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M</sub>) and CP (bla<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*NDM, *bla*<sub>OXA-48</sub>) were characterized by PCR and sequencing.

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#### Ethics 138

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> The study was carried out in accordance with the Declaration of Helsinki and Good 139 140 Clinical Practice Guidelines (ICH-GCP-Guidelines, CMPM/ICH/135/95) of the 141 European Medicines Agency.

A waiver of written informed consent of individual patients in the participating wards 142 was requested. This waiver was granted by the Ethics Committee of Clinical 143 144 Research (Comité Ético de Investigación Clínica del Hospital Universitario Ramón y 145 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 146 investigational drugs, devices, or procedures were involved and verbal consent was 147 148 considered sufficient.

The study included all standard safeguards for ensuring the confidentiality of patient 149 information and specifications stipulated in the Personal Data Protection Act 150 151 15/1999, of 13 December were followed.

152

#### 153 Statistical analyses

A descriptive analysis of the variables collected was conducted, the gualitative 154 variables were expressed as percentages and the quantitative variables as measures 155 156 of central tendency (mean and median) and dispersion (standard deviation). Pearson's 157 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®) 158 software. 159

#### 160 RESULTS

161 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 162 first 48 hours of admission. Table 1. 163

# 165 TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and

## 166 Neurosurgery wards and patients included in the study.

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

168 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)

171 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

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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.

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Hospital admission		SBL-producing /			
-				years)	ត្ត៍ ភ្លាឝ្តិrevalence (%) Cl 95%
wards	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)	n 2019 seign
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)	1.23 (6.25-8.22)
Urology	234 (80.69)	56 (19.31)	69.82 (14.04)	72 (21)	a e d de d de f de f f
Neurosurgery	44 (66.67)	22 (33.33)	62.45 (17.26)	66.67 (25.84)	Image: Second state       Marcevalence (%) Cl 95%         Image: Second state       9.02 (7.96-10.08)         Image: Second state       9.02 (7.96-10.08)         Image: Second state       7.23 (6.25-8.22)         Image: Superied state       7.32 (6.49-8.14)         Image: Second state       6.52 (4.95-8.09)         Image: Second state       7.69 (7 18-8 19)
Total	559 (68.34)	259 (31.66)	69.27 (15.68)	72 (25)	Ding, ·
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> 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

## 226 hospital admission

					Microorgani	ism		
) I	Enzyme	ESBL	ESBL	ESBL	ESBL	ESBL +CP	ESBL +CP	
2 3 4		E. coli	K. pneum.	E. cloacae	C. freundii	E. coli	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	-	Ch.	-	-	-	6 (2.88%)
	CTX-M-32	2	-	-~	-	-	-	2 (0.96%)
	CTX-M-55	1	-	-	6 -	-	-	1 (0.48%)
	SHV	1	1	-	Έ.	-	-	2 (0.96%)
	SHV-2	1	1	-	0	-	-	2 (0.96%)
	SHV-12	10	8	-	1	-	5	24 (11.54%)
	SHV-28	-	5	-	-	-	1	6 (2.88%)
	SHV-31	-	1	-	-	5	-	1 (0.48%)
	Total	100	55	1	1	2	48	208 (100%)
	227							
	228 ES	BL: Extend	ed-spectrum t	peta-lactamas	es <i>;</i> CP: carba	penemase.		
	229 230							
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## 237 TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal

## 238 swabs at hospital admission

		Microor	ganism
Enzyme	ESBL +CP	ESBL +CP	
	E. coli	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%)

## 240 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**

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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

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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].

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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.

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2 3 4	340	WORD COUNT
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9	343	AUTHOR CONTRIBUTIONS
10 11	344	Conception and design of study: M Bonten, R Cantón, P Gastemeier, F Maechler.
12	345	
13 14	346	Data collection: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M
15 16	347	Hernández-García, P Ruiz-Garbajosa.
17	348	Analysis and/or interpretation of data: C Díaz-Agero Pérez, N López-Fresneña, AL
18 19	349	Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa, JM Aranaz-Andrés, R
20	350	Cantón.
21 22	351	
23 24	352	Drafting the manuscript: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-
25	353	Carlavilla, M Hernández-García.
26 27	354	
28	355	Revising the manuscript critically for important intellectual content: R Cantón, P
29 30	356	Ruiz-Garbajosa, JM Aranaz-Andrés, M Bonten, P Gastemeier, F Maechler.
31 32	357	
33	358	Approval of the version of the manuscript to be published: C Díaz-Agero Pérez, N
34 35	359	López-Fresneña, AL Rincón-Carlavilla, M. Hernández-García, P. Ruiz-Garbajosa, JM
36	360	Aranaz-Andrés, F Maechler, P Gastmeier, M Bonten, R Cantón.
37 38	361	
39 40	362	DATA SHARING STATEMENT
41 42	363	Extra data is available by emailing: cristina.diazagero@salud.madrid.org
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46 47		
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51	367	Previous presentation: preliminary results from this study were presented at ECCMID
52 53		
54 55	368	2016 on April 11, 2016, Amsterdam, the Netherlands.
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57 58	369 370	
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# Reporting checklist for cross sectional study.

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			Page
		Reporting Item	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 2 4 5
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
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34 35 36 37		#12d	If applicable, describe analytical methods taking account of sampling strategy	BES) . mining, Al NA
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49 50 51		#13b	Give reasons for non-participation at each stage	7's.
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#### 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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Complete List of Authors:	DIAZ AGERO PEREZ, CRISTINA; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; INSTITUTO RAMON Y CAJAL DE INVESTIGACION SANITARIA (IRYCIS) LOPEZ FRESNEÑA, NIEVES; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Pública; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Rincon Carlavilla, Angela; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) Hernandez Garcia, Marta ; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) Ruiz-Garbajosa, Patricia; Hospital Universitario Ramon y Cajal, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI) ARANAZ ANDRÉS , JESÚS MARIA; Hospital Universitario Ramon y Cajal, Servicio de Medicina Preventiva y Salud Publica; IRYCIS, INSTITUTO RAMÓN Y CAJAL DE INVESTIGACIÓN SANITARIA Maechler, F; Charite Universitatsmedizin Berlin, Gastmeier, Petra; Charite, Infection Control Bonten, Marc; University Medical Center Utrecht, Department of Medical Microbiology Canton, Rafael; Hospital Universitario Ramón y Cajal, Madrid, Spain, Servicio de Microbiologia; Red Española de Investigación en Patología Infecciosa (REIPI)
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1	TITLE:
2	Local prevalence of extended-spectrum beta-lactamase (ESBL) producing
3	Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL
4	and OXA-48 carbapenemase in <i>Klebsiella pneumoniae:</i> a prevalence survey in a
5	Spanish University Hospital
6	
7	Authors:
8	C. Díaz-Agero Pérez <sup>1</sup> , N. López-Fresneña <sup>1</sup> , A.L. Rincón-Carlavilla <sup>1</sup> , M. Hernández-
9	García <sup>2, 3</sup> , P. Ruiz-Garbajosa <sup>2,3</sup> , J.M. Aranaz-Andrés <sup>1</sup> , F. Maechler <sup>4</sup> , P. Gastmeier <sup>4</sup> , M.
10	Bonten <sup>5</sup> , R Cantón <sup>2,3</sup>
11	
12	<sup>1</sup> Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal
13	and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
14	<sup>2</sup> Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y
15	Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.
16	<sup>3</sup> Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain.
17	<sup>4</sup> Charité –University Medicine Berlin, Germany.
18	<sup>5</sup> University Medical Center Utrecht (UMC Utrecht), Utrecht, The Netherlands.
19	
20	Corresponding author:

2		
3 4	21	Cristina Díaz-Agero Pérez
5 6		
7	22	Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Ramón y Cajal.
8 9		
10 11	23	Carretera de Colmenar Km 9,1. Madrid 28034. Spain
12		
13 14	24	email address: cristina.diazagero@salud.madrid.org)
14 15		
16 17	25	TI.: 0034913368372 / 0034913368604
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26 27	29	ABSTRACT
28		
29	30	<b>Objective:</b> to assess the prevalence of ESBL-producing <i>Enterobacteriaceae</i> (ESBL-E)
30 31		
32	31	fecal carriers at admission in a University Hospital in Spain.
33 34		
35	32	Design: prevalence survey.
36 37		
38	33	Setting: Pnoumology, Castroonterology, Urology and Nourosurgery units at a
39 40	55	Setting: Pneumology, Gastroenterology, Urology and Neurosurgery units at a
40		
42	34	University tertiary hospital in Madrid (Spain).
43 44		
45	35	Participants: 10,643 patients aged 18 and older admitted from March-2014 to April-
46 47		
48	36	2016 with a rectal swab taken at admission or as soon as possible within the first 48
49		
50 51	37	hours.
52	-	
53 54	38	Primary and secondary outcome measures: prevalence of ESBL-E fecal carriers and
55		
56 57	39	prevalence of ESBL-E infections at admission.
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2 3 4 5	40	Results: the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19).
6 7 8	41	Most of the isolates were Escherichia coli (77.51%), followed by Klebsiella pneumoniae
9 10 11	42	(20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
12 13 14 15	43	carbapenemase (CP) producers, 1.83% in the case of <i>E. coli</i> and 42.86% among <i>K.</i>
16 17 18	44	<i>pneumoniae</i> isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
19 20 21	45	91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an
22 23 24	46	active infection by ESBL-E at admission.
25 26 27 28	47	Conclusions: The prevalence found in our study is very similar to that found in the
29 30 31	48	literature. However, we found a high percentage of simultaneous ESBL and CP
32 33 34	49	producers, particularly in Klebsiella pneumoniae. Despite the high prevalence of
35 36 37	50	colonized patients, the ESBL-infection rate on admission was very low.
38 39	51	Key words: Extended-spectrum beta-lactamase producing Enterobacteriaceae,
40 41 42	52	carbapenemase producing Enterobacteriaceae, surveillance, prevalence.
43	53	
44 45	54	
46 47	55	
48 49 50	56	
51	57	ARTICLE SUMMARY
52 53 54	58	Strengths and limitations of this study
55 56	59	• This study is one of the most prolonged in time and with the largest number of
57 58	60	patients assessing colonization with multidrug resistant microorganisms,
59 60	61	including adult participants of variable age groups and gender from a university

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1 2		
2 3 4	62	hospital providing specialized assistance to 8.51% of the population of Madrid
5 6	63	(Spain).
7 8	64	• The large number of patients included (10,643) gives strength to the results.
9 10	65	• Genes codifying ESBL and CP were characterized by PCR and sequencing.
11 12	66	Unfortunately total characterization was not feasible in all isolates, only 24.67%
13 14	67	of total ESBL producing isolates and 73.86% of total CP producing isolates.
15 16	68	
17 18 19	69	FUNDING STATEMENT
20 21	70	• The project falls within the R-GNOSIS study (Resistance of Gram-Negative
22 23	71	Organisms: Studying Intervention Strategies), within the Work Package 5
24 25	72	Patient isolation strategies for ESBL carriers in medical and surgical hospital
26 27	73	wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-
28 29	74	N°282512).
30 31	75	• MH-G is supported with a contract from Instituto de Salud Carlos III of Spain
32 33	76	(iP-FIS program, ref. IFI14/00022).
34 35 26	77	
36 37 38	78	POTENTIAL CONFLICTS OF INTEREST
39 40	79	All authors declare that they have no conflict of interest.
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#### **TEXT**

#### 84 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.

102 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 104 active surveillance screening program (R-GNOSIS project).

106 METHODS

#### 107 Study design and settings

108 The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms:109 Studying Intervention Strategies), within the Work Package 5 Patient isolation

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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.

118 Patients

Between March 3<sup>rd</sup> 2014 and April 3<sup>rd</sup> 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.

122 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.

<sup>3</sup> 129 **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,

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137 BioMérieux, France). Genes codifying ESBL (*bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>) and CP (*bla*<sub>VIM</sub>,

*bla*<sub>KPC</sub>, *bla*NDM, *bla*<sub>OXA-48</sub>) were characterized by PCR and sequencing.

139 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.

150 The study included all standard safeguards for ensuring the confidentiality of patient
151 information and specifications stipulated in the Personal Data Protection Act
152 15/1999, of 13 December were followed.

#### 154 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.

161 RESULTS

56 162 During the research period 12,590 admissions of 9,706 patients took place in the
57 163 participating wards. In 84.5% of admissions, a rectal swab could be obtained within the
59 60 164 first 48 hours of admission. Table 1.

## 165 TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and

#### 166 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	

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

#### **TABLE 2. Age and gender of the included patients.**

Ward	Ger	nder	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)	

171 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

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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.

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Hospital admission		SBL-producing /			
-				years)	ត្ត៍ ភ្លាឝ្តិ៍revalence (%) Cl 95%
wards	Men (%)	Women (%)	Mean (S.D.)	Median (I.R.)	n 2019 seign
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)	1.23 (6.25-8.22)
Urology	234 (80.69)	56 (19.31)	69.82 (14.04)	72 (21)	a e d de d de f de f f
Neurosurgery	44 (66.67)	22 (33.33)	62.45 (17.26)	66.67 (25.84)	Image: Second state       Marcevalence (%) Cl 95%         Image: Second state       9.02 (7.96-10.08)         Image: Second state       9.02 (7.96-10.08)         Image: Second state       7.23 (6.25-8.22)         Image: Superied state       7.32 (6.49-8.14)         Image: Second state       6.52 (4.95-8.09)         Image: Second state       7.69 (7 18-8 19)
Total	559 (68.34)	259 (31.66)	69.27 (15.68)	72 (25)	Ding, ·
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> 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

## 226 hospital admission

					Microorgani	ism		
) I	Enzyme	ESBL	ESBL	ESBL	ESBL	ESBL +CP	ESBL +CP	
2 3 4		E. coli	K. pneum.	E. cloacae	C. freundii	E. coli	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	-	Ch.	-	-	-	6 (2.88%)
	CTX-M-32	2	-	-~	-	-	-	2 (0.96%)
	CTX-M-55	1	-	-	6 -	-	-	1 (0.48%)
	SHV	1	1	-	Έ.	-	-	2 (0.96%)
	SHV-2	1	1	-	0	-	-	2 (0.96%)
	SHV-12	10	8	-	1	-	5	24 (11.54%)
	SHV-28	-	5	-	-	-	1	6 (2.88%)
	SHV-31	-	1	-	-	5	-	1 (0.48%)
	Total	100	55	1	1	2	48	208 (100%)
	227							
	228 ES	BL: Extend	ed-spectrum t	peta-lactamas	es <i>;</i> CP: carba	penemase.		
	229 230							
	230 231 232							
	232							

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## 237 TABLE 5. Distribution of carbapenemase strains isolated and typed in rectal

## 238 swabs at hospital admission

		Microor	ganism
Enzyme	ESBL +CP	ESBL +CP	
	E. coli	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%)

## 240 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**

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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

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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]. 

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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.

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3 4	341	WORD COUNT
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7 8	343	AUTHOR CONTRIBUTIONS
9	344	Conception and design of study: M Bonten, R Cantón, P Gastemeier, F Maechler.
10 11	345	Data collection: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-Carlavilla, M
12 13	346	Hernández-García, P Ruiz-Garbajosa.
14	347	Analysis and/or interpretation of data: C Díaz-Agero Pérez, N López-Fresneña, AL
15 16	348	Rincón-Carlavilla, M Hernández-García, P Ruiz-Garbajosa, JM Aranaz-Andrés, R
17	349	Cantón.
18 19	350	Drafting the manuscript: C Díaz-Agero Pérez, N López-Fresneña, AL Rincón-
20	351	Carlavilla, M Hernández-García.
21 22	352	Revising the manuscript critically for important intellectual content: R Cantón, P
23 24	353	Ruiz-Garbajosa, JM Aranaz-Andrés, M Bonten, P Gastemeier, F Maechler.
24 25	354	Approval of the version of the manuscript to be published: C Díaz-Agero Pérez, N
26 27	355	López-Fresneña, AL Rincón-Carlavilla, M. Hernández-García, P. Ruiz-Garbajosa, JM
28	356	Aranaz-Andrés, F Maechler, P Gastmeier, M Bonten, R Cantón.
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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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	Reporting Item	Pageo Number
#1a	Indicate the study's design with a commonly used term in the title or the abstract	mining, Ai training, 29, Ai training, 20, Ai training,
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#4	Present key elements of study design early in the paper	5
#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5
	#1b #2 #3 #4 #5 #6a	<ul> <li>#1a Indicate the study's design with a commonly used term in the title or the abstract</li> <li>#1b Provide in the abstract an informative and balanced summary of what was done and what was found</li> <li>#2 Explain the scientific background and rationale for the investigation being reported</li> <li>#3 State specific objectives, including any prespecified hypotheses</li> <li>#4 Present key elements of study design early in the paper</li> <li>#5 Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</li> <li>#6a Give the eligibility criteria, and the sources and methods of</li> </ul>

1 2 3 4 5		#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	BMJ Open: first
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19 20 21 22 23	Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	10.1136/bmjopen-2018-024879 on 1 March Ens Protected by copyright, including for uses ഗ്ര
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40 41 42 43 44 45 46 47 48	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.	mjopen.bmj.com/ on June 11, 2025 at Agence Al training, and similar technologies. N
49 50		#13b	Give reasons for non-participation at each stage	25 at A Jies. 7
51 52		#13c	Consider use of a flow diagram	
53 54 55 56 57 58 59 60	Descriptive data	<b>#14a</b> For pe	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. eer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	Bibliographique de I 7

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	Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	BMJ Open: first published as 7 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	as 10.1136/bmjopen-2018-024879 on 1 March Protected by copyright, including for uses
		#16b	Report category boundaries when continuous variables were categorized	n-2018-024 yright, inc
		#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	879 on 1 Ma Iuding for u N
	Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	arch 2019. I Enseigner Ises relatec
	Key results	#18	Summarise key results with reference to study objectives	Downlo nent Su 1 to tex 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.	baded from http uperieur (ABES tt and data min 1
	Interpretation	#20	Give a cautious overall interpretation considering objectives, 1 <sup>2</sup> limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	o://bmjopen.bm ) . Ing, Al training
	Generalisability	#21	Discuss the generalisability (external validity) of the study 1 <sup>-</sup> results	-12and simila
	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	bmjopen.bmj.com/ on June 11, 2025 a 3, Al training, and similar technologies 1-1 1-1
	Interpretation       #20       Cive a calculous overall interpretation considering objectives, in the present studies of analyses, results from similar studies, and other relevant evidence.       Interpretation of the study interpretation considering objectives, in the present evidence.         Generalisability       #21       Discuss the generalisability (external validity) of the study results       11-12a         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       36         The STROBE checklist is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 15. June 2018 using <a href="http://www.goodreports.org/">http://www.goodreports.org/</a> , a tool made by the <a href="http://www.goodreports.org/">EQUATOR Network</a> in collaboration with <a href="http://www.goodreports.org/">Penelope.ai</a>			
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