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

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Manuscripts

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3 1 **TITLE:**

4 2 **Local prevalence of extended-spectrum beta-lactamase (ESBL) producing**
5 3 ***Enterobacteriaceae* intestinal carriers at admission in a Spanish University**
6 4 **Hospital and co-expression of ESBL and OXA-48 carbapenemase in *Klebsiella***
7 5 ***pneumoniae***
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3 29 **ABSTRACT**

4 30 **Objective:** to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E)
5
6 31 fecal carriers at admission in a University Hospital in Spain.

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8 32 **Design:** prevalence survey.

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10 33 **Setting:** Pneumology, Gastroenterology, Urology and Neurosurgery units at a
11
12 34 University tertiary hospital in Madrid (Spain).

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14 35 **Participants:** 10,643 patients aged 18 and older admitted from March-2014 to April-
15
16 36 2016 with a rectal swab taken at admission or as soon as possible within the first 48
17
18 37 hours.

19
20 38 **Primary and secondary outcome measures:** prevalence of ESBL-E fecal carriers
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22 39 and prevalence of ESBL-E infections at admission.

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24 40 **Results:** the ESBL-E carriers prevalence on admission was 7.70% (CI 95% 7.19-8.22).
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26 41 Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae*
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28 42 (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
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30 43 carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K.*
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32 44 *pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
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34 45 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an
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36 46 active infection by ESBL-E at admission.

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38 47 **Conclusions:** The prevalence found in our study is very similar to that found in the
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40 48 literature. However, we found a high percentage of simultaneous ESBL and CP
41
42 49 producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of
43
44 50 colonized patients, the ESBL-infection rate on admission was very low.

45
46 51 **Key words:** Extended-spectrum beta-lactamase producing *Enterobacteriaceae*,
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48 52 carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.

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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.

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3 82 **TEXT**

4
5 83 **BACKGROUND**

6
7 84 The emergence of antimicrobial resistance represents a global challenge for healthcare
8
9 85 due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are
10
11 86 the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late
12
13 87 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in
14
15 88 *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among
16
17 89 countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the
18
19 90 Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization,
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21 91 prolonged hospitalization, ICU admission and previous use of cephalosporins and
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23 92 aminoglycosides were leading risk factors [3, 4].

24
25 93 The situation today is very different since their prevalence has increased dramatically
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27 94 in the community, especially in urinary tract infections, where these enzymes are more
28
29 95 frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the
30
31 96 inadequate empirical treatment, delaying the efficient antimicrobial treatment for
32
33 97 example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours
34
35 98 instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the
36
37 99 prevalence of microbial resistance in our geographic area and the epidemiological
38
39 100 characteristics in order to establish the scope of the problem and analyze its evolution.
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41 101 The aim of this study was to assess the prevalence of ESBL-producing
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43 102 *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an
44
45 103 active surveillance screening program (R-GNOSIS project).

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48 105 **METHODS**

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50 106 **Study design and settings**

51
52 107 The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms:
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54 108 Studying Intervention Strategies), within the Work Package 5 Patient isolation

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

111 The University Hospital Ramón y Cajal is a public referral center, located in the North
112 of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent
113 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179
114 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41
115 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds)
116 wards took part in the study.

117 **Patients**

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

121 **Patient involvement**

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

127 **Laboratory analysis**

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

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3 137 **Ethics**

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5 138 The study was carried out in accordance with the Declaration of Helsinki and Good
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7 139 Clinical Practice Guidelines (ICH-GCP-Guidelines, CMPM/ICH/135/95) of the
8
9 140 European Medicines Agency. It was granted authorization by the Ethics Committee of
10
11 141 Clinical Research and waiver of the requirements to obtain informed consent from
12
13 142 patients, being verbal consent considered sufficient (Ref. 251/13).

14
15 143 Specifications stipulated in the Personal Data Protection Act 15/1999, of 13 December
16
17 144 were followed.

18
19 145 **Statistical analyses**

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21 146 A descriptive analysis of the variables collected was conducted, the qualitative
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23 147 variables were expressed as percentages and the quantitative variables as measures
24
25 148 of central tendency (mean and median) and dispersion (standard deviation). Pearson's
26
27 149 Chi-squared test was used to compare proportions and the Student's T-test to compare
28
29 150 means. All statistics analysis was performed using SPSS Statistics v19 (IBM®)
30
31 151 software.

32
33 152 **RESULTS**

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35 153 During the research period 12,590 admissions of 9,706 patients took place in the
36
37 154 participating wards. In 84.5% of admissions, a rectal swab could be obtained within the
38
39 155 first 48 hours of admission. Table 1.

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43 157 **TABLE 1. Patients admitted to Gastroenterology, Pneumology, Urology and**
44
45 158 **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
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160 Gender and mean age of included patients are shown in Table 2.

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162 **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)

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

164

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

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

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

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3 177 (Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery),
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5 178 the difference was not statistically significant.
6
7 179 A total of 845 multiresistant *Enterobacteriaceae* were isolated in 820 patients, as 25
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9 180 patients were colonized by more than one microorganism at the time of admission
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11 181 (0.23%). Eighty-eight (10.41%) of the isolated *Enterobacteriaceae* were simultaneous
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13 182 ESBL and carbapenemase (CP) producers, 33.47% of these patients were known
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15 183 carriers, i.e., their clinical records included a previous positive culture for ESBL-E.
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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)

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

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3 189 The most frequently isolated ESBL-producer microorganism at admission was *E. coli*
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5 190 (77.51%; -n=655), followed by *K. pneumoniae* (20.71%, n=175), being only 1.78%
6
7 191 other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C.*
8
9 192 *amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%;
10
11 193 *Acinetobacter* spp 0.12%). Among ESBL-*E. coli* isolates, 1.83% were simultaneous
12
13 194 ESBL and CP producers (n=12). Among ESBL-*K. pneumoniae* isolates, 42.86% were
14
15 195 simultaneous ESBL and CP producers (n=75). Only one patient was colonized by a
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17 196 different ESBL and CP producer, *K. oxytoca*.

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19 197 The typing of 209 beta-lactamases (24.73% of total ESBL) and 58 carbapenemases
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21 198 was possible (65.91% of total CP). Most of ESBL (83.25%) belonged to the CTX-M
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23 199 group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining
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25 200 16.75% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the
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27 201 typed CP, 91.38% were OXA-48 type (Table 5). In the case of 4 patients colonized
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29 202 simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K.*
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31 203 *pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae*
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33 204 respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of
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35 205 them and CTX-M-14 in 1, and OXA-48 in the case of CP.

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

Enzyme	Microorganism						Total (%)
	ESBL <i>E. coli</i>	ESBL <i>K. pneum.</i>	ESBL <i>E. cloacae</i>	ESBL <i>C. freundii</i>	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneum.</i>	
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%)

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

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

Enzyme	Microorganism		
	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneumoniae</i>	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%)

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

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233 Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of
 234 patients admitted during the research period and 6.59% of ESBL-E intestinal carriers.

235 Of those 54 patients, all except one also showed a positive rectal swab, 90.74% of
 236 those (49 patients) with the same specie causing the infection, and 9.26% (5 patients)
 237 with a different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures)
 238 were urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a
 239 urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site
 240 infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1
 241 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

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

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3 247 **DISCUSSION**

4 248 In our study, the prevalence of ESBL-E carriers at admission was 7.7%, ranging
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6 249 between 6.62% and 9.05% depending on the ward. The prevalence of ESBL-E carriers
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8 250 in healthy individuals as well as in ambulatory and hospitalized patients has been
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10 251 researched in a number of studies. In all of them, *E. coli* is always the most frequently
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12 252 isolated microorganism, similarly to our study (77.51%) [11-19]. In a meta-analysis
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14 253 published in 2016 which analyzed prevalence studies in healthy persons, and included
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16 254 28,909 individuals from 66 studies, the mean global prevalence of colonization was
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18 255 14%, with great variability among regions [19]. It was higher in Asia, with 46% and
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20 256 Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe,
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22 257 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean
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24 258 prevalence was 2%, although it was admitted that there were very few studies for this
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26 259 region [20].

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28 260 Our prevalence of intestinal carriers at admission is virtually the same to that found by
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30 261 a Dutch study recently published, which was 7.9% in patients coming from their homes
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32 262 and 8.6% in patients coming from long-term care facilities, a distinction not made in our
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34 263 research [21]. Studies in three different areas in Spain (Madrid, Barcelona and
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36 264 Zaragoza) show that the prevalence of carriers has increased in the last years,
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38 265 reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study
39
40 266 findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers
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42 267 among patients admitted to Emergency Units was 7.4%, also very similar to our figure
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44 268 [22].

45
46 269 In our facility, 10.41% of ESBL microorganisms were simultaneous carbapenemase
47
48 270 producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the
49
50 271 58 carbapenemases typed (65.91% of total CP), the vast majority of them, 91.38%
51
52 272 belonged to the OXA-48 type. This fact is especially important in the case of *K.*
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54 273 *pneumoniae* with 42.86% of them being ESBL and CP producers (91.84% OXA-48).
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56 274 ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed

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3 275 at hospital admission (69.27% of them urinary tract infections). We did not find a similar
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5 276 study to compare our data with but we think this finding must be deeply analyzed.

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7 277 Male gender has been identified as a risk factor for the intestinal colonization by ESBL-
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9 278 E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized
10
11 279 patients were men, but they were also the majority of the total number of hospitalized
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13 280 patients, the difference not being statistically significant [11]. Age is another risk factor
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15 281 identified in the bibliography; in our study, the mean age of colonized patients was
16
17 282 higher than the mean age of hospitalized patients (69.29 years vs 64.91 years), being
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19 283 the difference statistically significant in this case ($p = 0.0087$) [23, 24].

20
21 284 The prevalence of carriers at admission was higher in the Gastroenterology ward,
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23 285 despite being younger than the mean, with a difference statistically significant as
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25 286 compared to the rest of included wards. In other published studies, liver disease has
26
27 287 been identified as a risk factor for intestinal colonization by ESBL-E, being the
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29 288 prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in
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31 289 patients with chronic liver disease one of the possible explanations [25, 26]. Another
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33 290 risk factor for ESBL-E carriage recently described in the literature is proton pump
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35 291 inhibitors (PPI) use, and these type of patients are often receiving PPIs and other
36
37 292 medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide
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39 293 an explanation as risk factors for every patient were not recorded.

40
41 294 Beta-lactamase characterization was not feasible in all isolates, only 24.73% of total
42
43 295 ESBL producing isolates. The main enzyme group was CTX-M, the most common
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45 296 according to the literature, followed by SHV, CTX-M-15 group prevailing with more than
46
47 297 52% [8, 12-14, 19, 21, 22, 24].

48
49 298 In the last years, ESBL-E infections have become an increasing concern; in the United
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51 299 States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur
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53 300 per year [29]. Infections by these bacteria are associated to higher mortality rates and
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55 301 higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However,
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57 302 few studies have associated the fact of being an intestinal carrier of ESBL-E with the

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3 303 development of infections caused by these bacteria. A recent cohort study performed in
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5 304 patients with haematological malignancies found a 3.5-fold greater risk of developing
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7 305 bacteraemia by ESBL-E among colonized patients when compared to non-colonized
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9 306 patients; despite of the fact that mortality was similar in both groups, colonization was
10
11 307 associated to longer hospital stays, shorter survival period and higher costs [31]. On
12
13 308 the contrary, another similar study did not find correlation between ESBL-E colonization
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15 309 and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were
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17 310 diagnosed at admission and almost 70% were urinary tract infections. That means that
18
19 311 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of
20
21 312 the colonized patients. Only in one patient with ESBL-E infection at admission no
22
23 313 ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections
24
25 314 were found in colonized patients, the total prevalence of infection is very low, and only
26
27 315 in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract
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29 316 infection). In two cases patients died during hospital admission, although their infection
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31 317 had been fully resolved and death was caused by an underlying oncological disease.
32
33 318 This study, one of the most prolonged in time and with the largest number of patients,
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35 319 confirms once again the extension of ESBL-E intestinal colonization in the community
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37 320 showing, however, a low prevalence of infection. It is necessary to continue with the
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39 321 epidemiological surveillance of these microorganisms, in order to acquire a better
40
41 322 knowledge of the implications of being an intestinal carrier of ESBL-E. The high
42
43 323 percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply
44
45 324 studied.

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326 WORD COUNT

327 2,256

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28 348 **DATA SHARING STATEMENT**

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43 357 **REFERENCES**

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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.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche 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

1		#7	Clearly define all outcomes, exposures, predictors, potential	6
2			confounders, and effect modifiers. Give diagnostic criteria, if	
3			applicable	
4				
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6	Data sources /	#8	For each variable of interest give sources of data and details of	6
7	measurement		methods of assessment (measurement). Describe	
8			comparability of assessment methods if there is more than one	
9			group. Give information separately for for exposed and	
10			unexposed groups if applicable.	
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14	Bias	#9	Describe any efforts to address potential sources of bias	6
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17	Study size	#10	Explain how the study size was arrived at	7
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19	Quantitative	#11	Explain how quantitative variables were handled in the	6
20	variables		analyses. If applicable, describe which groupings were chosen,	
21			and why	
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24	Statistical	#12a	Describe all statistical methods, including those used to control	6
25	methods		for confounding	
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28		#12b	Describe any methods used to examine subgroups and	6
29			interactions	
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32		#12c	Explain how missing data were addressed	NA
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34		#12d	If applicable, describe analytical methods taking account of	NA
35			sampling strategy	
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38		#12e	Describe any sensitivity analyses	NA
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41	Participants	#13a	Report numbers of individuals at each stage of study—eg	7
42			numbers potentially eligible, examined for eligibility, confirmed	
43			eligible, included in the study, completing follow-up, and	
44			analysed. Give information separately for for exposed and	
45			unexposed groups if applicable.	
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49		#13b	Give reasons for non-participation at each stage	7
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51		#13c	Consider use of a flow diagram	NA
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54	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7
55			clinical, social) and information on exposures and potential	
56			confounders. Give information separately for exposed and	
57			unexposed groups if applicable.	
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1		#14b	Indicate number of participants with missing data for each	7
2			variable of interest	
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5	Outcome data	#15	Report numbers of outcome events or summary measures.	7
6			Give information separately for exposed and unexposed	
7			groups if applicable.	
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10	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-	7
11			adjusted estimates and their precision (eg, 95% confidence	
12			interval). Make clear which confounders were adjusted for and	
13			why they were included	
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17		#16b	Report category boundaries when continuous variables were	7
18			categorized	
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21		#16c	If relevant, consider translating estimates of relative risk into	NA
22			absolute risk for a meaningful time period	
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24	Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and	8
25			interactions, and sensitivity analyses	
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28	Key results	#18	Summarise key results with reference to study objectives	9
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31	Limitations	#19	Discuss limitations of the study, taking into account sources of	10
32			potential bias or imprecision. Discuss both direction and	
33			magnitude of any potential bias.	
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36	Interpretation	#20	Give a cautious overall interpretation considering objectives,	11-12
37			limitations, multiplicity of analyses, results from similar studies,	
38			and other relevant evidence.	
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41	Generalisability	#21	Discuss the generalisability (external validity) of the study	11-12
42			results	
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45	Funding	#22	Give the source of funding and the role of the funders for the	3
46			present study and, if applicable, for the original study on which	
47			the present article is based	
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52 CC-BY. This checklist was completed on 15. June 2018 using <http://www.goodreports.org/>, a tool
53 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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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

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3 1 **TITLE:**

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5 2 **Local prevalence of extended-spectrum beta-lactamase (ESBL) producing**
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7 3 ***Enterobacteriaceae* intestinal carriers at admission and co-expression of ESBL**
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9 4 **and OXA-48 carbapenemase in *Klebsiella pneumoniae*: a prevalence survey in a**
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11 5 **Spanish University Hospital**

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29 **ABSTRACT**

30 **Objective:** to assess the prevalence of ESBL-producing *Enterobacteriaceae* (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 and
39 prevalence of ESBL-E infections at admission.

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4 40 **Results:** the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19).
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7 41 Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae*
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10 42 (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
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13 43 carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K.*
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16 44 *pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
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19 45 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an
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22 46 active infection by ESBL-E at admission.
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25 47 **Conclusions:** The prevalence found in our study is very similar to that found in the
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28 48 literature. However, we found a high percentage of simultaneous ESBL and CP
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31 49 producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of
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34 50 colonized patients, the ESBL-infection rate on admission was very low.
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38 51 **Key words:** Extended-spectrum beta-lactamase producing *Enterobacteriaceae*,
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40 52 carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.
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55 57 **ARTICLE SUMMARY**

56 58 **Strengths and limitations of this study**

- 57 59 • This study is one of the most prolonged in time and with the largest number of
58 60 patients assessing colonization with multidrug resistant microorganisms,
59 61 including adult participants of variable age groups and gender from a university

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3 62 hospital providing specialized assistance to 8.51% of the population of Madrid
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5 63 (Spain).

- 6
7 64 • The large number of patients included (10,643) gives strength to the results.
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9 65 • Genes codifying ESBL and CP were characterized by PCR and sequencing.
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11 66 Unfortunately total characterization was not feasible in all isolates, only 24.67%
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13 67 of total ESBL producing isolates and 73.86% of total CP producing isolates.
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17 18 69 **FUNDING STATEMENT**

- 19
20 70 • The project falls within the R-GNOSIS study (Resistance of Gram-Negative
21
22 71 Organisms: Studying Intervention Strategies), within the Work Package 5
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24 72 Patient isolation strategies for ESBL carriers in medical and surgical hospital
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26 73 wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-
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31
32 76 (iP-FIS program, ref. IFI14/00022).
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36 37 78 **POTENTIAL CONFLICTS OF INTEREST**

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39 79 All authors declare that they have no conflict of interest.
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3 824 83 **TEXT**5
6 84 **BACKGROUND**

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8 85 The emergence of antimicrobial resistance represents a global challenge for healthcare
9
10 86 due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are
11
12 87 the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late
13
14 88 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in
15
16 89 *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among
17
18 90 countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the
19
20 91 Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization,
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22 92 prolonged hospitalization, ICU admission and previous use of cephalosporins and
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24 93 aminoglycosides were leading risk factors [3, 4].

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26
27 94 The situation today is very different since their prevalence has increased dramatically
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29 95 in the community, especially in urinary tract infections, where these enzymes are more
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31 96 frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the
32
33 97 inadequate empirical treatment, delaying the efficient antimicrobial treatment for
34
35 98 example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours
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37 99 instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the
38
39 100 prevalence of microbial resistance in our geographic area and the epidemiological
40
41 101 characteristics in order to establish the scope of the problem and analyze its evolution.

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43 102 The aim of this study was to assess the prevalence of ESBL-producing
44
45 103 *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an
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47 104 active surveillance screening program (R-GNOSIS project).
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51 10552 106 **METHODS**53
54 107 **Study design and settings**

55
56 108 The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms:
57
58 109 Studying Intervention Strategies), within the Work Package 5 Patient isolation
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60

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

112 The University Hospital Ramón y Cajal is a public referral center, located in the North
113 of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent
114 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179
115 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41
116 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds)
117 wards took part in the study.

118 **Patients**

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

122 **Patient involvement**

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

128 **Laboratory analysis**

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

138 **Ethics**

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

142 A waiver of written informed consent of individual patients in the participating wards
143 was requested. This waiver was granted by the Ethics Committee of Clinical
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.
146 251-13), since the study did not expose patients to any novel risk, and no
147 investigational drugs, devices, or procedures were involved and verbal consent was
148 considered sufficient.

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

152

153 **Statistical analyses**

154 A descriptive analysis of the variables collected was conducted, the qualitative
155 variables were expressed as percentages and the quantitative variables as measures
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
158 means. All statistics analysis was performed using SPSS Statistics v19 (IBM®)
159 software.

160 **RESULTS**

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

164

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

167

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

169

170 **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.

172

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

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

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3 179 patients (69.27 -S.D.15.68 vs 64.91 -S.D. 16.79-), the difference being statistically
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5 180 significant ($p = 0.0087$).

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7 181 The difference in prevalence of colonization at admission among the surveyed wards
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9 182 was statistically significant ($p = 0.001$). The highest prevalence was found in the
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11 183 Gastroenterology ward, with 9.02%, the difference being significant with the rest of
12
13 184 wards ($p = 0.01$). When comparing the prevalence between medical wards
14
15 185 (Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery),
16
17 186 the difference was not statistically significant.

18
19 187 A total of 843 multiresistant *Enterobacteriaceae* were isolated in 818 patients, as 25
20
21 188 patients were colonized by more than one microorganism at the time of admission
22
23 189 (0.23%). Eighty-eight (10.44%) of the isolated *Enterobacteriaceae* were simultaneous
24
25 190 ESBL and carbapenemase (CP) producers, 33.99% of these patients were known
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27 191 carriers, i.e., their clinical records included a previous positive culture for ESBL-E.
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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)

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

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3 197 The most frequently isolated ESBL-producer microorganism at admission was *E. coli*
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5 198 (77.70%; -n=655), followed by *K. pneumoniae* (20.64%, n=174), being only 1.66%
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7 199 other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C.*
8
9 200 *amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%). Among
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11 201 ESBL-*E. coli* isolates, 1.83% were simultaneous ESBL and CP producers (n=12).
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13 202 Among ESBL-*K. pneumoniae* isolates, 43.10% were simultaneous ESBL and CP
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15 203 producers (n=75). Only one patient was colonized by a different ESBL and CP
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17 204 producer, *K. oxytoca*.

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20 205 The typing of 208 beta-lactamases (24.67% of total ESBL) and 65 carbapenemases
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22 206 was possible (73.86% of total CP). Most of ESBL (83.17%) belonged to the CTX-M
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24 207 group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining
25
26 208 16.83% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the
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28 209 typed CP, 90.77% were OXA-48 type (Table 5). In the case of 4 patients colonized
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30 210 simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K.*
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32 211 *pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae*
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34 212 respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of
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36 213 them and CTX-M-14 in 1, and OXA-48 in the case of CP.

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

Enzyme	Microorganism						Total (%)
	ESBL <i>E. coli</i>	ESBL <i>K. pneum.</i>	ESBL <i>E. cloacae</i>	ESBL <i>C. freundii</i>	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneum.</i>	
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%)

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

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

Enzyme	Microorganism		
	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneumoniae</i>	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%)

239

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

241

242 Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of
 243 patients admitted during the research period and 6.6% of ESBL-E intestinal carriers. Of
 244 those 54 patients, all except one also showed a positive rectal swab, 90.74% of those
 245 (49 patients) with the same specie causing the infection, and 9.26% (5 patients) with a
 246 different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures) were
 247 urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a
 248 urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site
 249 infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1
 250 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

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

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256 DISCUSSION

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3 257 In our study, the prevalence of ESBL-E carriers at admission was 7.69%, ranging
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5 258 between 6.52% and 9.02% depending on the ward. The prevalence of ESBL-E carriers
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7 259 in healthy individuals as well as in ambulatory and hospitalized patients has been
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9 260 researched in a number of studies. In all of them, *E. coli* is always the most frequently
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11 261 isolated microorganism, similarly to our study (77.70%) [11-19]. In a meta-analysis
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13 262 published in 2016 which analyzed prevalence studies in healthy persons, and included
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15 263 28,909 individuals from 66 studies, the mean global prevalence of colonization was
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17 264 14%, with great variability among regions [19]. It was higher in Asia, with 46% and
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19 265 Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe,
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21 266 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean
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23 267 prevalence was 2%, although it was admitted that there were very few studies for this
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25 268 region [20].

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28 269 Our prevalence of intestinal carriers at admission is virtually the same to that found by
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30 270 a Dutch study recently published, which was 7.9% in patients coming from their homes
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32 271 and 8.6% in patients coming from long-term care facilities, a distinction not made in our
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34 272 research [21]. Studies in three different areas in Spain (Madrid, Barcelona and
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36 273 Zaragoza) show that the prevalence of carriers has increased in the last years,
37
38 274 reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study
39
40 275 findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers
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42 276 among patients admitted to Emergency Units was 7.4%, also very similar to our figure
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44 277 [22].

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47 278 In our facility, 10.44% of ESBL microorganisms were simultaneous carbapenemase
48
49 279 producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the
50
51 280 65 carbapenemases typed (73.86% of total CP), the vast majority of them, 90.77%
52
53 281 belonged to the OXA-48 type. This fact is especially important in the case of *K.*
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55 282 *pneumoniae* with 43.10% of them being ESBL and CP producers (90.57% OXA-48).
56
57 283 ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed
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3 284 at hospital admission (69.27% of them urinary tract infections). We did not find a similar
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5 285 study to compare our data with but we think this finding must be deeply analyzed.
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9 287 Male gender has been identified as a risk factor for the intestinal colonization by ESBL-
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11 288 E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized
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13 289 patients were men, but they were also the majority of the total number of hospitalized
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15 290 patients, the difference not being statistically significant [11]. Age is another risk factor
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17 291 identified in the bibliography; in our study, the mean age of colonized patients was
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19 292 higher than the mean age of hospitalized patients (69.27 years vs 64.91 years), being
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21 293 the difference statistically significant in this case ($p = 0.0087$) [23, 24].
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24 294 The prevalence of carriers at admission was higher in the Gastroenterology ward,
25
26 295 despite being younger than the mean, with a difference statistically significant as
27
28 296 compared to the rest of included wards. In other published studies, liver disease has
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30 297 been identified as a risk factor for intestinal colonization by ESBL-E, being the
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32 298 prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in
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34 299 patients with chronic liver disease one of the possible explanations [25, 26]. Another
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36 300 risk factor for ESBL-E carriage recently described in the literature is proton pump
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38 301 inhibitors (PPI) use, and these type of patients are often receiving PPIs and other
39
40 302 medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide
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42 303 an explanation as risk factors for every patient were not recorded.
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45 304 Unfortunately total characterization was not feasible in all isolates due to budget issues
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47 305 so we decided to analyze a random selection. We were able to determine 24.67% of
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49 306 total ESBL producing isolates; that low percentage is a limitation of our study and the
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51 307 results could differ if all the ESBLs had been analyzed but they are compatible with the
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53 308 epidemiology described in the literature. The main enzyme group was CTX-M, the most
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55 309 common according to the literature, followed by SHV, CTX-M-15 group prevailing with
56
57 310 52.88% [8, 12-14, 19, 21, 22, 24].
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3 312 In the last years, ESBL-E infections have become an increasing concern; in the United
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5 313 States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur
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7 314 per year [29]. Infections by these bacteria are associated to higher mortality rates and
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9 315 higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However,
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11 316 few studies have associated the fact of being an intestinal carrier of ESBL-E with the
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13 317 development of infections caused by these bacteria. A recent cohort study performed in
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15 318 patients with haematological malignancies found a 3.5-fold greater risk of developing
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17 319 bacteraemia by ESBL-E among colonized patients when compared to non-colonized
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19 320 patients; despite of the fact that mortality was similar in both groups, colonization was
20
21 321 associated to longer hospital stays, shorter survival period and higher costs [31]. On
22
23 322 the contrary, another similar study did not find correlation between ESBL-E colonization
24
25 323 and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were
26
27 324 diagnosed at admission and almost 70% were urinary tract infections. That means that
28
29 325 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of
30
31 326 the colonized patients. Only in one patient with ESBL-E infection at admission no
32
33 327 ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections
34
35 328 were found in colonized patients, the total prevalence of infection is very low, and only
36
37 329 in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract
38
39 330 infection). In two cases patients died during hospital admission, although their infection
40
41 331 had been fully resolved and death was caused by an underlying oncological disease.
42
43 332 This study, one of the most prolonged in time and with the largest number of patients,
44
45 333 confirms once again the extension of ESBL-E intestinal colonization in the community
46
47 334 showing, however, a low prevalence of infection. It is necessary to continue with the
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49 335 epidemiological surveillance of these microorganisms, in order to acquire a better
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51 336 knowledge of the implications of being an intestinal carrier of ESBL-E. The high
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53 337 percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply
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55 338 studied.
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3 340 **WORD COUNT**
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5 341 2,450
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9 343 **AUTHOR CONTRIBUTIONS**

10 344 **Conception and design of study:** M Bonten, R Cantón, P Gastemeier, F Maechler.
11
12 345

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39 362 **DATA SHARING STATEMENT**
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41 363 Extra data is available by emailing: cristina.diazagero@salud.madrid.org
42 364
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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	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

1		#7	Clearly define all outcomes, exposures, predictors, potential	6
2			confounders, and effect modifiers. Give diagnostic criteria, if	
3			applicable	
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6	Data sources /	#8	For each variable of interest give sources of data and details of	6
7	measurement		methods of assessment (measurement). Describe	
8			comparability of assessment methods if there is more than one	
9			group. Give information separately for for exposed and	
10			unexposed groups if applicable.	
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14	Bias	#9	Describe any efforts to address potential sources of bias	6
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17	Study size	#10	Explain how the study size was arrived at	7
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19	Quantitative	#11	Explain how quantitative variables were handled in the	6
20	variables		analyses. If applicable, describe which groupings were chosen,	
21			and why	
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24	Statistical	#12a	Describe all statistical methods, including those used to control	6
25	methods		for confounding	
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28		#12b	Describe any methods used to examine subgroups and	6
29			interactions	
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32		#12c	Explain how missing data were addressed	NA
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34		#12d	If applicable, describe analytical methods taking account of	NA
35			sampling strategy	
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38		#12e	Describe any sensitivity analyses	NA
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41	Participants	#13a	Report numbers of individuals at each stage of study—eg	7
42			numbers potentially eligible, examined for eligibility, confirmed	
43			eligible, included in the study, completing follow-up, and	
44			analysed. Give information separately for for exposed and	
45			unexposed groups if applicable.	
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49		#13b	Give reasons for non-participation at each stage	7
50				
51		#13c	Consider use of a flow diagram	NA
52				
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54	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7
55			clinical, social) and information on exposures and potential	
56			confounders. Give information separately for exposed and	
57			unexposed groups if applicable.	
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1		#14b	Indicate number of participants with missing data for each	7
2			variable of interest	
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5	Outcome data	#15	Report numbers of outcome events or summary measures.	7
6			Give information separately for exposed and unexposed	
7			groups if applicable.	
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10	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-	7
11			adjusted estimates and their precision (eg, 95% confidence	
12			interval). Make clear which confounders were adjusted for and	
13			why they were included	
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17		#16b	Report category boundaries when continuous variables were	7
18			categorized	
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21		#16c	If relevant, consider translating estimates of relative risk into	NA
22			absolute risk for a meaningful time period	
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24	Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and	8
25			interactions, and sensitivity analyses	
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28	Key results	#18	Summarise key results with reference to study objectives	9
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31	Limitations	#19	Discuss limitations of the study, taking into account sources of	10
32			potential bias or imprecision. Discuss both direction and	
33			magnitude of any potential bias.	
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36	Interpretation	#20	Give a cautious overall interpretation considering objectives,	11-12
37			limitations, multiplicity of analyses, results from similar studies,	
38			and other relevant evidence.	
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41	Generalisability	#21	Discuss the generalisability (external validity) of the study	11-12
42			results	
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45	Funding	#22	Give the source of funding and the role of the funders for the	3
46			present study and, if applicable, for the original study on which	
47			the present article is based	
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53 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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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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Secondary Subject Heading:	Public health
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Carbapenemase producing Enterobacteriaceae, Surveillance, Prevalence

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3 1 **TITLE:**

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5 2 **Local prevalence of extended-spectrum beta-lactamase (ESBL) producing**
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7 3 ***Enterobacteriaceae* intestinal carriers at admission and co-expression of ESBL**
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9 4 **and OXA-48 carbapenemase in *Klebsiella pneumoniae*: a prevalence survey in a**
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11 5 **Spanish University Hospital**

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27 **ABSTRACT**

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29 **Objective:** to assess the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E)
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31 fecal carriers at admission in a University Hospital in Spain.
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35 **Design:** prevalence survey.
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39 **Setting:** Pneumology, Gastroenterology, Urology and Neurosurgery units at a
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41 University tertiary hospital in Madrid (Spain).
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45 **Participants:** 10,643 patients aged 18 and older admitted from March-2014 to April-
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47 2016 with a rectal swab taken at admission or as soon as possible within the first 48
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49 hours.
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53 **Primary and secondary outcome measures:** prevalence of ESBL-E fecal carriers and
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55 prevalence of ESBL-E infections at admission.
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4 40 **Results:** the ESBL-E carriers prevalence on admission was 7.69% (CI 95% 7.18-8.19).
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7 41 Most of the isolates were *Escherichia coli* (77.51%), followed by *Klebsiella pneumoniae*
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10 42 (20.71%). Eighty-eight (10.41%) of ESBL-E were simultaneous ESBL and
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13 43 carbapenemase (CP) producers, 1.83% in the case of *E. coli* and 42.86% among *K.*
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16 44 *pneumoniae* isolates. Of the ESBL typed, 52.15% belonged to the CTX-M-15 type and
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19 45 91.38% of the carbapenemases were OXA-48 type. Only 0.43% patients presented an
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22 46 active infection by ESBL-E at admission.
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25 47 **Conclusions:** The prevalence found in our study is very similar to that found in the
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28 48 literature. However, we found a high percentage of simultaneous ESBL and CP
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31 49 producers, particularly in *Klebsiella pneumoniae*. Despite the high prevalence of
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34 50 colonized patients, the ESBL-infection rate on admission was very low.
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38 51 **Key words:** Extended-spectrum beta-lactamase producing *Enterobacteriaceae*,
39 52 carbapenemase producing *Enterobacteriaceae*, surveillance, prevalence.
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53 57 **ARTICLE SUMMARY**

54 58 **Strengths and limitations of this study**

- 55 59 • This study is one of the most prolonged in time and with the largest number of
56 60 patients assessing colonization with multidrug resistant microorganisms,
57 61 including adult participants of variable age groups and gender from a university

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3 62 hospital providing specialized assistance to 8.51% of the population of Madrid
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5 63 (Spain).

- 6
7 64 • The large number of patients included (10,643) gives strength to the results.
8
9 65 • Genes codifying ESBL and CP were characterized by PCR and sequencing.
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11 66 Unfortunately total characterization was not feasible in all isolates, only 24.67%
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13 67 of total ESBL producing isolates and 73.86% of total CP producing isolates.
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17 18 69 **FUNDING STATEMENT**

- 19
20 70 • The project falls within the R-GNOSIS study (Resistance of Gram-Negative
21
22 71 Organisms: Studying Intervention Strategies), within the Work Package 5
23
24 72 Patient isolation strategies for ESBL carriers in medical and surgical hospital
25
26 73 wards, funded by the European Union (FP7-HEALTH-2011-SINGLE STAGE-
27
28 74 N°282512).
29
30 75 • MH-G is supported with a contract from Instituto de Salud Carlos III of Spain
31
32 76 (iP-FIS program, ref. IFI14/00022).
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36 37 78 **POTENTIAL CONFLICTS OF INTEREST**

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39 79 All authors declare that they have no conflict of interest.
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3 824 83 **TEXT**5
6 84 **BACKGROUND**

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8 85 The emergence of antimicrobial resistance represents a global challenge for healthcare
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10 86 due to the limited treatment options. Extended-spectrum beta-lactamases (ESBL) are
11
12 87 the main mechanisms of acquired resistance in Gram-negative bacteria. Until the late
13
14 88 90s most ESBLs were isolated in nosocomial outbreaks, their prevalence was higher in
15
16 89 *Klebsiella pneumoniae* than *Escherichia coli*, and there was significant variation among
17
18 90 countries, hospitals and wards [1, 2]. They were isolated in higher frequency in the
19
20 91 Intensive Care Units (ICU) and recent surgery, catheterization, urinary catheterization,
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22 92 prolonged hospitalization, ICU admission and previous use of cephalosporins and
23
24 93 aminoglycosides were leading risk factors [3, 4].

25
26
27 94 The situation today is very different since their prevalence has increased dramatically
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29 95 in the community, especially in urinary tract infections, where these enzymes are more
30
31 96 frequently isolated in *E. coli* [5-8]. The main clinical relevance of ESBL seems to be the
32
33 97 inadequate empirical treatment, delaying the efficient antimicrobial treatment for
34
35 98 example up to six times in the case of *E. coli* and *K. pneumoniae* ESBL (i.e., 72 hours
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37 99 instead of 11 hours for susceptible strains) [9, 10]. It is necessary to know the
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39 100 prevalence of microbial resistance in our geographic area and the epidemiological
40
41 101 characteristics in order to establish the scope of the problem and analyze its evolution.

42
43 102 The aim of this study was to assess the prevalence of ESBL-producing
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45 103 *Enterobacteriaceae* (ESBL-E) fecal carriers at admission in hospital wards during an
46
47 104 active surveillance screening program (R-GNOSIS project).
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51 10552 106 **METHODS**53
54 107 **Study design and settings**

55
56 108 The project falls within the R-GNOSIS study (Resistance of Gram-Negative Organisms:
57
58 109 Studying Intervention Strategies), within the Work Package 5 Patient isolation
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110 strategies for ESBL carriers in medical and surgical hospital wards, funded by the EU
111 (FP7-HEALTH-2011-SINGLE STAGE-N°282512).

112 The University Hospital Ramón y Cajal is a public referral center, located in the North
113 of Madrid (Spain). It provides specialized assistance to 558,373 citizens, who represent
114 8.51% of the population of Madrid. With 1,118 beds, it accounted for 31,179
115 admissions in year 2014; 31,253 in 2015, and 31,847 in 2016. The Pneumology (41
116 beds), Gastroenterology (40 beds), Urology (41 beds) and Neurosurgery (20 beds)
117 wards took part in the study.

118 **Patients**

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

122 **Patient involvement**

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

129 **Laboratory analysis**

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

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3 137 BioMérieux, France). Genes codifying ESBL (*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}) and CP (*bla*_{VIM},
4
5 138 *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}) were characterized by PCR and sequencing.

7 139 **Ethics**

9 140 The study was carried out in accordance with the Declaration of Helsinki and Good
10
11 141 Clinical Practice Guidelines (ICH-GCP-Guidelines, CPM/ICH/135/95) of the
12
13 142 European Medicines Agency.

14
15 143 The Ethics Committee of Clinical Research (Comité Ético de Investigación Clínica
16
17 144 del Hospital Universitario Ramón y Cajal, Madrid, Spain) formally reviewed and
18
19 145 approved the study protocol on October 2013 (Ref. 251-13). A waiver of written
20
21 146 informed consent of individual patients in the participating wards was requested and
22
23 147 granted by the Committee as well as by the Medical Direction, since the study did
24
25 148 not expose patients to any novel risk, and no investigational drugs, devices, or
26
27 149 procedures were involved and verbal consent was considered sufficient.

28
29 150 The study included all standard safeguards for ensuring the confidentiality of patient
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31 151 information and specifications stipulated in the Personal Data Protection Act
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33 152 15/1999, of 13 December were followed.

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38 154 **Statistical analyses**

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40 155 A descriptive analysis of the variables collected was conducted, the qualitative
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42 156 variables were expressed as percentages and the quantitative variables as measures
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44 157 of central tendency (mean and median) and dispersion (standard deviation). Pearson's
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46 158 Chi-squared test was used to compare proportions and the Student's T-test to compare
47
48 159 means. All statistics analysis was performed using SPSS Statistics v19 (IBM®)
49
50 160 software.

51 161 **RESULTS**

52
53 162 During the research period 12,590 admissions of 9,706 patients took place in the
54
55 163 participating wards. In 84.5% of admissions, a rectal swab could be obtained within the
56
57 164 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

167

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

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170 **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.

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

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

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3 179 patients (69.27 -S.D.15.68 vs 64.91 -S.D. 16.79-), the difference being statistically
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5 180 significant ($p = 0.0087$).

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7 181 The difference in prevalence of colonization at admission among the surveyed wards
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9 182 was statistically significant ($p = 0.001$). The highest prevalence was found in the
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11 183 Gastroenterology ward, with 9.02%, the difference being significant with the rest of
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13 184 wards ($p = 0.01$). When comparing the prevalence between medical wards
14
15 185 (Pneumology and Gastroenterology) and surgical wards (Urology and Neurosurgery),
16
17 186 the difference was not statistically significant.

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19 187 A total of 843 multiresistant *Enterobacteriaceae* were isolated in 818 patients, as 25
20
21 188 patients were colonized by more than one microorganism at the time of admission
22
23 189 (0.23%). Eighty-eight (10.44%) of the isolated *Enterobacteriaceae* were simultaneous
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25 190 ESBL and carbapenemase (CP) producers, 33.99% of these patients were known
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27 191 carriers, i.e., their clinical records included a previous positive culture for ESBL-E.
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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)

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

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3 197 The most frequently isolated ESBL-producer microorganism at admission was *E. coli*
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5 198 (77.70%; -n=655), followed by *K. pneumoniae* (20.64%, n=174), being only 1.66%
6
7 199 other species (*E. cloacae* 0.59%; *C. freundii* 0.36%; *E. aerogenes* 0.24%; *C.*
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9 200 *amalonaticus* 0.12%; *C. koseri* 0.12%; *E. asburiae* 0.12%; *K. oxytoca* 0.12%). Among
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11 201 ESBL-*E. coli* isolates, 1.83% were simultaneous ESBL and CP producers (n=12).
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13 202 Among ESBL-*K. pneumoniae* isolates, 43.10% were simultaneous ESBL and CP
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15 203 producers (n=75). Only one patient was colonized by a different ESBL and CP
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17 204 producer, *K. oxytoca*.

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20 205 The typing of 208 beta-lactamases (24.67% of total ESBL) and 65 carbapenemases
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22 206 was possible (73.86% of total CP). Most of ESBL (83.17%) belonged to the CTX-M
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24 207 group, CTX-M-15 being the most numerous, followed by CTX-M-14. The remaining
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26 208 16.83% belonged to the SHV group, SHV-12 being the most frequent (Table 4). For the
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28 209 typed CP, 90.77% were OXA-48 type (Table 5). In the case of 4 patients colonized
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30 210 simultaneously by 2 different ESBL-E (in 2 patients ESBL-*E. coli* and ESBL-*K.*
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32 211 *pneumoniae* and in the other ESBL+CP-*E. coli* and ESBL+CP-*K. pneumoniae*
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34 212 respectively), both microorganisms carried the same enzyme type, CTX-M-15 in 3 of
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36 213 them and CTX-M-14 in 1, and OXA-48 in the case of CP.

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

Enzyme	Microorganism						Total (%)
	ESBL <i>E. coli</i>	ESBL <i>K. pneum.</i>	ESBL <i>E. cloacae</i>	ESBL <i>C. freundii</i>	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneum.</i>	
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%)

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

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

Enzyme	Microorganism		
	ESBL +CP <i>E. coli</i>	ESBL +CP <i>K. pneumoniae</i>	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%)

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

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242 Fifty-four patients presented an active infection by ESBL-E at admission, i.e., 0.43% of
 243 patients admitted during the research period and 6.6% of ESBL-E intestinal carriers. Of
 244 those 54 patients, all except one also showed a positive rectal swab, 90.74% of those
 245 (49 patients) with the same specie causing the infection, and 9.26% (5 patients) with a
 246 different ESBL-E. Out of the diagnosed infections, 69.09% (38 urine cultures) were
 247 urinary tract infections, 14.55% bacteraemia (n=8; 1 of them secondary to a
 248 urinary tract infection), two community acquired pneumonias (3.64%), 2 surgical site
 249 infections (3.64%), 2 abscesses (3.64%), 1 lower respiratory infection (1.82%), 1
 250 gastrostomy insertion site infection (1.82%), and 1 Fournier's gangrene (1.82%).

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

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256 DISCUSSION

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3 257 In our study, the prevalence of ESBL-E carriers at admission was 7.69%, ranging
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5 258 between 6.52% and 9.02% depending on the ward. The prevalence of ESBL-E carriers
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7 259 in healthy individuals as well as in ambulatory and hospitalized patients has been
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9 260 researched in a number of studies. In all of them, *E. coli* is always the most frequently
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11 261 isolated microorganism, similarly to our study (77.70%) [11-19]. In a meta-analysis
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13 262 published in 2016 which analyzed prevalence studies in healthy persons, and included
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15 263 28,909 individuals from 66 studies, the mean global prevalence of colonization was
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17 264 14%, with great variability among regions [19]. It was higher in Asia, with 46% and
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19 265 Africa with 22%; in Europe the mean prevalence was 4%, with 3% in Central Europe,
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21 266 4% in Northern Europe and 6% in Southern Europe. Finally, in America, the mean
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23 267 prevalence was 2%, although it was admitted that there were very few studies for this
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25 268 region [20].

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28 269 Our prevalence of intestinal carriers at admission is virtually the same to that found by
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30 270 a Dutch study recently published, which was 7.9% in patients coming from their homes
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32 271 and 8.6% in patients coming from long-term care facilities, a distinction not made in our
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34 272 research [21]. Studies in three different areas in Spain (Madrid, Barcelona and
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36 273 Zaragoza) show that the prevalence of carriers has increased in the last years,
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38 274 reaching rates ranging from 5.5% and 8.1% in 2002 and 2004, similarly to our study
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40 275 findings [11, 13, 16]. In another study performed in Seville, the prevalence of carriers
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42 276 among patients admitted to Emergency Units was 7.4%, also very similar to our figure
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44 277 [22].

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47 278 In our facility, 10.44% of ESBL microorganisms were simultaneous carbapenemase
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49 279 producers, being 85.22% *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the
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51 280 65 carbapenemases typed (73.86% of total CP), the vast majority of them, 90.77%
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53 281 belonged to the OXA-48 type. This fact is especially important in the case of *K.*
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55 282 *pneumoniae* with 43.10% of them being ESBL and CP producers (90.57% OXA-48).
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57 283 ESBL and CP *K. pneumoniae* was responsible for 23.21% of the infections diagnosed
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59 284 at hospital admission (69.27% of them urinary tract infections). We did not find a similar

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3 285 study to compare our data with but we think this finding must be deeply analyzed. We
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5 286 found one case of KPC-3 (ESBL+CP *E. coli*) and one case of NDM-1 (ESBL+CP *K.*
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7 287 *pneumoniae*).

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9 288 Male gender has been identified as a risk factor for the intestinal colonization by ESBL-
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11 289 E [7, 20, 21, 23, 24]. In our study, as in Valverde et al., the majority of colonized
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13 290 patients were men, but they were also the majority of the total number of hospitalized
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15 291 patients, the difference not being statistically significant [11]. Age is another risk factor
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17 292 identified in the bibliography; in our study, the mean age of colonized patients was
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19 293 higher than the mean age of hospitalized patients (69.27 years vs 64.91 years), being
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21 294 the difference statistically significant in this case ($p = 0.0087$) [23, 24].

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24 295 The prevalence of carriers at admission was higher in the Gastroenterology ward,
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26 296 despite being younger than the mean, with a difference statistically significant as
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28 297 compared to the rest of included wards. In other published studies, liver disease has
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30 298 been identified as a risk factor for intestinal colonization by ESBL-E, being the
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32 299 prophylactic use of fluoroquinolones to prevent spontaneous bacterial peritonitis in
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34 300 patients with chronic liver disease one of the possible explanations [25, 26]. Another
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36 301 risk factor for ESBL-E carriage recently described in the literature is proton pump
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38 302 inhibitors (PPI) use, and these type of patients are often receiving PPIs and other
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40 303 medication for gastroesophageal reflux disease [27, 28]. In our case, we cannot provide
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42 304 an explanation as risk factors for every patient were not recorded.

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45 305 Unfortunately total characterization was not feasible in all isolates due to budget issues
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47 306 so we decided to analyze a random selection. We were able to determine 24.67% of
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49 307 total ESBL producing isolates; that low percentage is a limitation of our study and the
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51 308 results could differ if all the ESBLs had been analyzed but they are compatible with the
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53 309 epidemiology described in the literature. The main enzyme group was CTX-M, the most
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55 310 common according to the literature, followed by SHV, CTX-M-15 group prevailing with
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57 311 52.88% [8, 12-14, 19, 21, 22, 24].

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3 313 In the last years, ESBL-E infections have become an increasing concern; in the United
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5 314 States for example 140,000 hospital-acquired ESBL-E infections are estimated to occur
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7 315 per year [29]. Infections by these bacteria are associated to higher mortality rates and
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9 316 higher hospital costs compared to antibiotic-sensitive microorganisms [30]. However,
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11 317 few studies have associated the fact of being an intestinal carrier of ESBL-E with the
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13 318 development of infections caused by these bacteria. A recent cohort study performed in
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15 319 patients with haematological malignancies found a 3.5-fold greater risk of developing
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17 320 bacteraemia by ESBL-E among colonized patients when compared to non-colonized
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19 321 patients; despite of the fact that mortality was similar in both groups, colonization was
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21 322 associated to longer hospital stays, shorter survival period and higher costs [31]. On
22
23 323 the contrary, another similar study did not find correlation between ESBL-E colonization
24
25 324 and infection in neutropenic patients [32]. In our study 55 ESBL-E infections were
26
27 325 diagnosed at admission and almost 70% were urinary tract infections. That means that
28
29 326 0.43% of patients were admitted with an ESBL-E infection, which represents 6.59% of
30
31 327 the colonized patients. Only in one patient with ESBL-E infection at admission no
32
33 328 ESBL-E was isolated in the rectal swabs. Even though the vast majority of infections
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35 329 were found in colonized patients, the total prevalence of infection is very low, and only
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37 330 in 8 cases it consisted of bacteraemia (1 of those secondary to a urinary tract
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39 331 infection). In two cases patients died during hospital admission, although their infection
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41 332 had been fully resolved and death was caused by an underlying oncological disease.
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43 333 This study, one of the most prolonged in time and with the largest number of patients,
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45 334 confirms once again the extension of ESBL-E intestinal colonization in the community
46
47 335 showing, however, a low prevalence of infection. It is necessary to continue with the
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49 336 epidemiological surveillance of these microorganisms, in order to acquire a better
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51 337 knowledge of the implications of being an intestinal carrier of ESBL-E. The high
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53 338 percentage of ESBL and CP *K. pneumoniae* producers must also be more deeply
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55 339 studied.
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3 341 **WORD COUNT**

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5 342 2,411

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7 343 **AUTHOR CONTRIBUTIONS**

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22 357

23 358 **DATA SHARING STATEMENT**

24 359 Extra data is available by emailing: cristina.diazagero@salud.madrid.org

25 360

26 361

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30 365

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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.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche 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

1		#7	Clearly define all outcomes, exposures, predictors, potential	6
2			confounders, and effect modifiers. Give diagnostic criteria, if	
3			applicable	
4				
5				
6	Data sources /	#8	For each variable of interest give sources of data and details of	6
7	measurement		methods of assessment (measurement). Describe	
8			comparability of assessment methods if there is more than one	
9			group. Give information separately for for exposed and	
10			unexposed groups if applicable.	
11				
12				
13				
14	Bias	#9	Describe any efforts to address potential sources of bias	6
15				
16				
17	Study size	#10	Explain how the study size was arrived at	7
18				
19	Quantitative	#11	Explain how quantitative variables were handled in the	6
20	variables		analyses. If applicable, describe which groupings were chosen,	
21			and why	
22				
23				
24	Statistical	#12a	Describe all statistical methods, including those used to control	6
25	methods		for confounding	
26				
27				
28		#12b	Describe any methods used to examine subgroups and	6
29			interactions	
30				
31				
32		#12c	Explain how missing data were addressed	NA
33				
34		#12d	If applicable, describe analytical methods taking account of	NA
35			sampling strategy	
36				
37				
38		#12e	Describe any sensitivity analyses	NA
39				
40				
41	Participants	#13a	Report numbers of individuals at each stage of study—eg	7
42			numbers potentially eligible, examined for eligibility, confirmed	
43			eligible, included in the study, completing follow-up, and	
44			analysed. Give information separately for for exposed and	
45			unexposed groups if applicable.	
46				
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49		#13b	Give reasons for non-participation at each stage	7
50				
51		#13c	Consider use of a flow diagram	NA
52				
53				
54	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7
55			clinical, social) and information on exposures and potential	
56			confounders. Give information separately for exposed and	
57			unexposed groups if applicable.	
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1		#14b	Indicate number of participants with missing data for each	7
2			variable of interest	
3				
4				
5	Outcome data	#15	Report numbers of outcome events or summary measures.	7
6			Give information separately for exposed and unexposed	
7			groups if applicable.	
8				
9				
10	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-	7
11			adjusted estimates and their precision (eg, 95% confidence	
12			interval). Make clear which confounders were adjusted for and	
13			why they were included	
14				
15				
16				
17		#16b	Report category boundaries when continuous variables were	7
18			categorized	
19				
20				
21		#16c	If relevant, consider translating estimates of relative risk into	NA
22			absolute risk for a meaningful time period	
23				
24	Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and	8
25			interactions, and sensitivity analyses	
26				
27				
28	Key results	#18	Summarise key results with reference to study objectives	9
29				
30				
31	Limitations	#19	Discuss limitations of the study, taking into account sources of	10
32			potential bias or imprecision. Discuss both direction and	
33			magnitude of any potential bias.	
34				
35				
36	Interpretation	#20	Give a cautious overall interpretation considering objectives,	11-12
37			limitations, multiplicity of analyses, results from similar studies,	
38			and other relevant evidence.	
39				
40				
41	Generalisability	#21	Discuss the generalisability (external validity) of the study	11-12
42			results	
43				
44				
45	Funding	#22	Give the source of funding and the role of the funders for the	3
46			present study and, if applicable, for the original study on which	
47			the present article is based	
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