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

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## ABSTRACT

**Objective** To assess the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) faecal carriers at admission in a University Hospital in Spain.

**Design** Prevalence survey.

**Setting** Pneumology, gastroenterology, urology and neurosurgery units at a university tertiary hospital in Madrid (Spain).

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

**Primary and secondary outcome measures** Prevalence of ESBL-E faecal carriers and prevalence of ESBL-E infections at admission.

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

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

## Strengths and limitations of this study

- This study is one of the most prolonged in time, with the largest number of patients, assessing colonisation by multidrug resistant micro-organisms. It includes adult participants of variable age groups and gender from a university hospital providing specialised 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 extended-spectrum beta-lactamase (ESBL) and carbapenemase (CP) were characterised by polimerase chain reaction (PCR) and sequencing. Unfortunately total characterisation was not feasible in all isolates but only in 24.67% of total ESBL producing isolates and 73.86% of total CP producing isolates.

## BACKGROUND

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

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

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

## METHODS

### Study design and settings

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

The University Hospital Ramón y Cajal is a public referral centre located in the North of Madrid (Spain). It provides specialised assistance to 558 373 citizens who represent 8.51% of the population of Madrid. With 1118 beds, it accounted for 31 179 admissions in the year 2014, 31 253 in 2015 and 31 847 in 2016. The pneumology (41 beds), gastroenterology (40 beds), urology (41 beds) and neurosurgery (20 beds) wards took part in the study.

### Patients

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

### Patient involvement

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

### Laboratory analysis

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

### Ethics

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

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

### Statistical analyses

A descriptive analysis of the variables collected was conducted; qualitative variables were expressed as percentages and quantitative variables as measures of central tendency (mean and median) and dispersion (SD and IQR). Pearson's $\chi^2$ test was used to compare proportions and the Student's T-test was used to compare means. All statistical analyses were performed using SPSS Statistics V.19 (IBM) software.

## RESULTS

During the research period 12 590 admissions of 9706 patients took place in the participating wards. In 84.5% of admissions, a rectal swab could be obtained within the first 48 hours of admission. table 1.

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

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

Majority of patients colonised with ESBL-E were men, just like the majority of hospital patients, the difference not being statistically significant. The mean age of colonised patients was higher than the mean age of the

**Table 1** Patients admitted to gastroenterology, pneumology, urology and neurosurgery wards and swabs taken

| Ward | Admissions (n) | Swab at admission (n) | % |
|---|---|---|---|
| Gastroenterology | 3380 | 2916 | 86.27 |
| Pneumology | 3240 | 2752 | 84.94 |
| Urology | 4685 | 3963 | 84.59 |
| Neurosurgery | 1285 | 1012 | 78.75 |
| **Total** | 12 590 | 10 643 | **84.55** |

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Table 2 Gender and age of the included patients

| Ward | Gender | | Age (years) | |
|---|---|---|---|---|
| | Men (%) | Women (%) | Mean (SD) | Median (IQR) |
| Gastroenterology | 1732 (59.39) | 1184 (40.61) | 66.53 (16.59) | 69 (26.75) |
| Pneumology | 1625 (59.05) | 1127 (40.95) | 70.72 (15.28) | 74 (19) |
| Urology | 3009 (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** | **6899 (64.82)** | **3744 (35.18)** | **64.91 (16.79)** | **67 (25)** |

total number of hospitalised patients (69.27 SD 15.68 vs 64.91, SD 16.79), the difference being statistically significant (p=0.0087).

The difference in prevalence of colonisation at admission among the surveyed wards was statistically significant (p=0.001). The highest prevalence was found in the gastroenterology ward with 9.02%, the difference being significant when compared with the rest of wards (p=0.01). When comparing the prevalence between medical wards (pneumology and gastroenterology) and surgical wards (urology and neurosurgery), the difference was not statistically significant.

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

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

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

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

A total of 56 micro-organisms were isolated in the 55 positive clinical cultures as one of them was positive for two ESBL-E. The most frequently isolated micro-organism was, once again, *E. coli* (67.86%), followed by ESBL and

Table 3 ESBL-producing *Enterobacteriaceae* carriers at admission

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

ESBL, extended-spectrum beta-lactamases.

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

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

CP, carbapenemase; ESBL, extended-spectrum beta-lactamases.

CP *K. pneumoniae* (23.21%), ESBL *K. pneumoniae* (7.14%); *K. oxytoca* was isolated in one culture (1.79%).

## DISCUSSION

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

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

| | Micro-organism | | |
|---|---|---|---|
| Enzyme | ESBL+CP *Escherichia coli* | ESBL+CP *Klebsiella pneumoniae* | Total (%) |
| KPC-3 | 1 | – | 1 (1.54) |
| NDM-1 | – | 1 | 1 (1.54) |
| OXA-48 | 11 | 48 | 59 (90.77) |
| VIM-1 | – | 4 | 4 (6.15) |
| Total | 12 | 53 | 65 (100) |

CP, carbapenemase; ESBL, extended-spectrum beta-lactamases.

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

In our facility, 10.44% of ESBL micro-organisms were simultaneous CP producers, with 85.22% being *K. pneumoniae*, 13.64% *E. coli* and 1.14 *K. oxytoca*. Of the 65 CP typed (73.86% of total CP), the vast majority of them (90.77%) belonged to the OXA-48 type. This fact is especially important in the case of *K. pneumoniae*, with 43.10% being ESBL and CP producers (90.57% OXA-48). ESBL and CP *K. pneumoniae* were responsible for 23.21% of the infections diagnosed at hospital admission (69.27% of them urinary tract infections). We did not find a similar study to compare our data with but we think this finding must be deeply analysed. We found one case of KPC-3 (ESBL+CP *E. coli*) and one case of NDM-1 (ESBL+CP *K. pneumoniae*).



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

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

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

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

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

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**Competing interests** None declared.

**Patient consent for publication** Not required.

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

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** Extra data are available by emailing: cristina.diazagero@salud.madrid.org.

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