

## Original

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# Antimicrobial susceptibility trends and evolution of isolates with extended spectrum $\beta$ -lactamases among Gram-negative organisms recovered during the SMART study in Spain (2011-2015)

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## ABSTRACT

**Introduction.** The SMART (*Study for Monitoring Antimicrobial Resistance Trends*) surveillance study monitors antimicrobial susceptibility and extended spectrum  $\beta$ -lactamases (ESBLs) in Gram-negative bacilli recovered from intra-abdominal infections (IAI).

**Material and methods.** Antimicrobial susceptibility of 5,343 isolates from IAI recovered in 11 centres during the 2011-2015 SMART-Spain program was analysed by standard microdilution (EUCAST criteria) and compared with that from 2002-2010. ESBLs were phenotypically detected.

**Results.** *Escherichia coli*, the most common isolate, significantly decreased in community acquired IAI (60.9% 2002-2010 vs. 56.1% 2011-2015,  $P=0.0003$ ). It was followed in prevalence by *Klebsiella pneumoniae* that increased both in the community (8.9% vs. 10.8%,  $P=0.016$ ) and nosocomial (9.2% vs. 10.8%,  $P=0.029$ ) IAI and *P. aeruginosa*, which significantly increased in community acquired IAI (5.6% vs. 8.0%,  $P=0.0003$ ). ESBLs were more prevalent in *K. pneumoniae* (16.3%) than in *E. coli* (9.5%) of nosocomial

origin and were more frequently isolated from elderly patients (>60 years). Considering all Enterobacteriaceae, ertapenem (92.3-100%) and amikacin (95.5%-100%) were the most active antimicrobials. Ertapenem activity, unlike amoxicillin-clavulanate or piperacillin-tazobactam, remained virtually unchanged in ESBL (100%) and non-ESBL (98.8%) *E. coli* producers. Its activity decreased in ESBL-*K. pneumoniae* (74.7%) but was higher than that of amoxicillin-clavulanate (14.0%) and piperacillin-tazobactam (24.0%). Interestingly, ertapenem susceptibility was maintained in >60% of ESBL isolates that were resistant to amoxicillin-clavulanate, piperacillin-tazobactam or fluoroquinolones.

**Conclusions.** SMART-Spain results support current guidelines which include ertapenem as empiric treatment in mild-moderate community-acquired IAI, particularly with ESBL producers. These recommendations will need to be updated with the recently introduction of new antimicrobials.

**Key words:** surveillance study, intra-abdominal infections, carbapenems, extended spectrum  $\beta$ -lactamases

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## Evolución de la sensibilidad y de los aislados productores de $\beta$ -lactamasas de espectro extendido en microorganismos gramnegativos en el estudio SMART en España (2011-2015)

### RESUMEN

**Introducción.** El estudio SMART (*Study for Monitoring Antimicrobial Resistance Trends*) monitoriza la sensibilidad antimicrobiana y las  $\beta$ -lactamasas de espectro extendido (BLEE) en bacilos gramnegativos obtenidos de infecciones intraabdominales (IIA).

**Material y Métodos.** Se ha analizado la sensibilidad antimicrobiana (microdilución estándar, criterios EUCAST) y las BLEE (detección fenotípica) de 5.343 aislados de IIA en 11 centros del programa SMART-España durante 2011-2015 en comparación con 2002-2010.

**Resultados.** *Escherichia coli*, el microorganismo más prevalente, disminuyó significativamente en las IIA de origen comunitario (60,9% 2002-2010 vs. 56,1% 2011-2015,  $P=0,0003$ ). Fue seguido en prevalencia por *Klebsiella pneumoniae* que aumentó tanto en IIA comunitaria (8,9% vs. 10,8%,  $P=0,016$ ) como nosocomial (9,2% vs. 10,8%,  $P=0,029$ ) y por *P. aeruginosa* que aumentó en la IIA comunitaria (5,6% vs. 8,0%,  $P=0,0003$ ). Las BLEE fueron más prevalentes en la IIA nosocomial por *K. pneumoniae* (16,3%) que por *E. coli* (9,5%), siendo más frecuentes en pacientes de mayor edad (>60 años). Considerando todas las Enterobacteriaceae, ertapenem (92,3-100%) y amikacina (95,5%-100%) fueron los antimicrobianos más activos. La sensibilidad a ertapenem, al contrario que a amoxicilina-clavulánico o piperacilina-tazobactam, se mantuvo sin cambios en *E. coli* con (98,8%) y sin BLEE (100%). Su sensibilidad disminuyó en BLEE-*K. pneumoniae* (74,7%) pero fue mayor que la de amoxicilina-clavulánico (14,0%) o piperacilina-tazobactam (24,0%). Es de resaltar que esta actividad se mantuvo >60% en los aislados con BLEE resistentes a amoxicilina-clavulánico, piperacilina-tazobactam o fluoroquinolonas.

**Conclusiones.** El estudio SMART-España sustenta las guías actuales que incluyen al ertapenem como tratamiento empírico en la IIA leve-moderada comunitaria, en particular con BLEE. Estas recomendaciones precisaran actualizarse con la reciente introducción de nuevos antimicrobianos.

**Palabras clave:** estudio de vigilancia epidemiológica, infección intraabdominal, carbapenems,  $\beta$ -lactamasas de espectro extendido

### INTRODUCTION

Intra-abdominal infections (IAI) are recognized as one of the most common adverse events in the healthcare settings and range in severity from appendicitis to serious peritonitis [1,4]. Antimicrobial treatment failure and increased morbidity and mortality of these infections are frequently recognized due to the absence of both an early diagnosis and increasing prevalence of antimicrobial resistance, particularly in high-

risk patients [3,4,6]. These infections are often polymicrobial, including a wide variety of Gram-negative aerobic and facultative bacilli, such as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and, to a lesser extent, *Pseudomonas aeruginosa* and other non-fermenting Gram-negative bacilli [2,3,7,8]. As a result, empiric treatment with broad-spectrum antibiotics is recommended [4,6,8-10]. The antimicrobial agents currently used include the carbapenems and combinations of penicillins with  $\beta$ -lactamase inhibitors depending on the origin of the infection, and extended-spectrum cephalosporins and fluoroquinolones, usually in combination with metronidazole. Recently, new cephalosporin- $\beta$ -lactamase inhibitor combinations, including ceftolozane-tazobactam and ceftazidime-avibactam have been approved for the IAI indication [4,11]. These new agents circumvent antimicrobial resistance mechanisms commonly present in organisms associated with IAIs which include extended-spectrum  $\beta$ -lactamases (ESBLs), plasmidic AmpC, AmpC hyperproduction and/or carbapenemases in Enterobacteriaceae and/or *P. aeruginosa* and are candidates to spare carbapenems, particularly those of class II (imipenem, meropenem and doripenem) [4,12].

On the other hand, and to address the problem of antimicrobial resistance in IAIs in line with antimicrobial stewardship programs, the implementation of epidemiological surveillance programs and the use of data collected in the design of therapeutic guidelines have been also proposed as first steps in the management of IAIs [8]. The SMART study (*Study for Monitoring Antimicrobial Resistance Trends*) is an ongoing global surveillance program started in 2002 involving over 180 hospitals from all over the world. It monitors the *in vitro* susceptibility to antimicrobials of aerobic and facultative anaerobic Gram-negative bacilli isolated from IAI, focusing on those producing ESBL. In addition, since 2009 it has enlarged its focus in urinary tract infection [13].

In this article we perform a sub-analysis of the SMART study and evaluate the susceptibility patterns of antimicrobials against aerobic and facultative anaerobic Gram-negative pathogens isolated from IAI in the period 2011-2015 in 11 Spanish hospitals, with particular focus on ESBL producers. These data are also compared with those previously published covering the 2002-2010 period [14].

### MATERIAL AND METHODS

**Microorganisms and participating sites.** Isolates were recovered from abdominal samples from patients with diagnosis of an IAI. Each participating centre collected up to 100 non-selected consecutive isolates of aerobic and facultative anaerobic Gram-negative pathogens. To avoid duplicates, one strain per species and patient was included. During the 5 years of the study (2011 to 2015) a total of 11 hospitals participated (H. Basurto, Bilbao; H. Universitario Marqués de Valdecilla, Santander; H. Universitario Bellvitge, Hospitalet de Llobregat, Barcelona; H. Son Espases, Mallorca; H. Clínico Universitario Lozano Blesa, Zaragoza; H. Universitario

y Politécnico La Fe, Valencia; H. Universitario Ramón y Cajal, Madrid; H. Universitario Gregorio Marañón, Madrid; H. Clínico San Carlos, Madrid; H. Universitario Virgen del Rocío, Sevilla; H. Universitario Virgen Macarena, Sevilla). Figure 1 shows the distribution of participating centres per year.

A total of 5,343 isolates were collected from the Spanish centres and the most frequent intra-abdominal sample was peritoneal fluid (39%), followed by intra-abdominal abscesses (32%) and gall bladder (16%), and, to a lesser extent and in decreasing order, specimens from the liver, small bowel, appendix, pancreas, stomach, colon, rectum, and other minor sources. Most of the isolates were obtained during surgery procedures and others from paracentesis and percutaneous aspiration of intra-abdominal abscesses. Isolates from blood, urine, abdominal drainages, superficial wounds, and perirectal abscesses were excluded. The isolates were identified by species at each hospital and sent to a central laboratory (International Health Management Associates, Schaumburg, IL, US) to confirm identification and establish the antimicrobial susceptibility to different antimicrobials of choice in IALs. The source of the sample, patient age and the results were incorporated in a centralized database. Following the conventional criteria of the *Centers for Disease Control and Prevention* (CDC) the organisms were rated as community-acquired when they were obtained in samples within 48 hours after hospitalization and as nosocomial-acquired when obtained in samples recovered after 48 hours of hospital stay [15].

**Antimicrobial susceptibility.** Antimicrobial susceptibility testing results were obtained at the central laboratory using

the standard ISO broth microdilution method [16]. Dried MicroScan (Beckman, West Sacramento, CA, US) microdilution panels were used. The antimicrobials analyzed in this study were: piperacillin-tazobactam, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, ertapenem, amikacin, ciprofloxacin and levofloxacin. In addition, susceptibility to amoxicillin-clavulanate was measured with a gradient test (Etest®, bioMérieux, Lyon, France). For interpreting antibiotic susceptibility, the breakpoints proposed by the EUCAST in the year 2016 were used [17]. For amoxicillin-clavulanate, the amoxicillin value from this combination was used as a reference for the application of CLSI breakpoints [18].

The quality controls strains used were *E. coli* ATCC 25922, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 (positive ESBL control) and *P. aeruginosa* ATCC 27853. *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* isolates were classified as ESBL producers if there was at least an 8-fold reduction of the MICs for ceftazidime and/or cefotaxime tested in combination with clavulanate compared with their MICs when tested alone, according to the CLSI and EUCAST specifications [18,19].

**Statistical analysis.** The frequency comparison (incidence between hospital and community isolates) was performed using the chi-squared test ( $\chi^2$ ) taking  $P < 0.05$  as statistically significant.

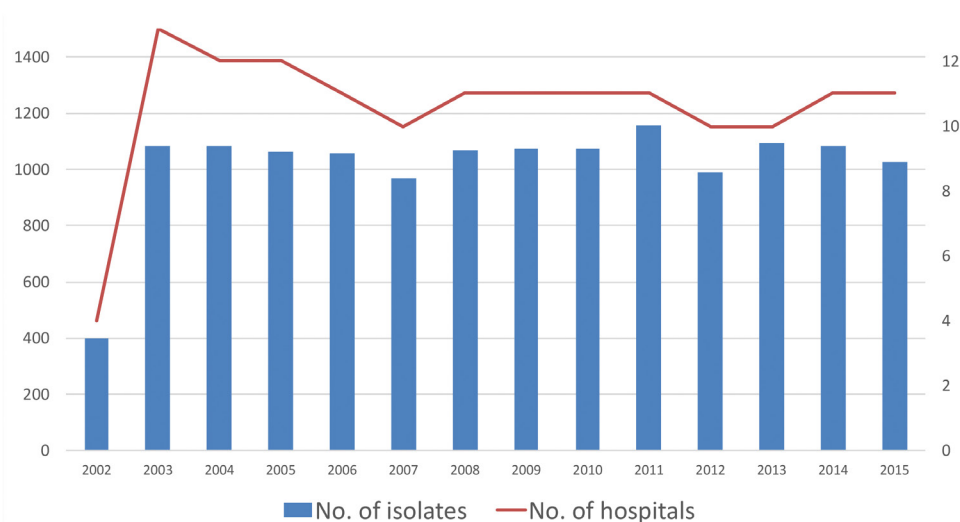
## RESULTS

The number of isolates and centres participating by year and compared with our previous study are presented in figure 1. Overall, 5,295 isolates were finally included in the study as 48 isolates (0.9%) were not available for further studies or information was not complete in the SMART database. The analysis of all microorganisms and the entire follow-up period showed that enterobacterial isolates (4,844) accounted for 91.4% of the isolates, with *E. coli* as the most common species (58.9%), followed by *Klebsiella* spp. (17.6%) and *E. cloacae* (6.0%). The most common non-fermenting Gram-negative bacilli were *P. aeruginosa* (6.6% of the total isolates). When the origin of the isolates was considered, 53.7% were of nosocomial origin and 46.3% community-acquired. In few isolates (0.9%) this information was not specified in the case report forms.

Table 1 shows the distribution of the 11 most commonly isolated microorganisms differentiating between community and nosocomial origin. There was a significant difference ( $P < 0.01$ ) in the percentage of *E. coli* isolates causing community (56.1%) and nosocomial (52.1%) IALs but not in *K. pneumoniae* (10.8% in both cases).

**Table 1** Distribution of the most common Gram-negative organisms collected in intra-abdominal infections in Spain in the SMART study (2011-2015).

Organisms	No. of isolates	Community acquired		Nosocomial acquired	
		No.	%	No.	%
<i>Escherichia coli</i>	2,857	1,375	56.1	1,482	52.1
<i>Klebsiella pneumoniae</i>	570	264	10.8	306	10.8
<i>Klebsiella oxytoca</i>	283	138	5.6	145	5.1
<i>Proteus mirabilis</i>	222	79	3.2	143	5.0
<i>Proteus vulgaris</i>	32	13	0.5	19	0.7
<i>Enterobacter cloacae</i>	293	109	4.5	184	6.5
<i>Enterobacter aerogenes</i>	85	37	1.5	48	1.7
<i>Citrobacter freundii</i>	108	41	1.7	67	2.4
<i>Morganella morganii</i>	133	36	1.5	97	3.4
<i>Serratia marcescens</i>	59	27	1.1	32	1.1
Other Enterobacteriaceae	202	103	4.2	99	3.5
<i>Pseudomonas aeruginosa</i>	353	197	8.0	156	5.5
Other Gram-negative bacilli	98	30	1.2	68	2.4
TOTAL	5,295	2,449	46.3	2,846	53.7



**Figure 1** Number of hospitals participating and microorganisms recovered per year in the SMART study in Spain from 2002 to 2015

**Table 2** Frequency of Enterobacteriaceae with extended-spectrum  $\beta$ -lactamases (ESBLs) by origin of acquisition of infection in the SMART study in Spain comparing 2002–2010 and 2011–2015 periods.

Acquisition of infection	<i>Escherichia coli</i>		<i>Klebsiella pneumoniae</i>		<i>Klebsiella oxytoca</i>		<i>Proteus mirabilis</i>	
	2002–2010	2011–2015	2002–2010	2011–2015	2002–2010	2011–2015	2002–2010	2011–2015
<48 h	6.2	8.0	5.3	9.5	1.3	1.4	0.7	1.3
>48 h	10.0	9.5	10.3	16.3	6.5	4.1	2.2	2.1

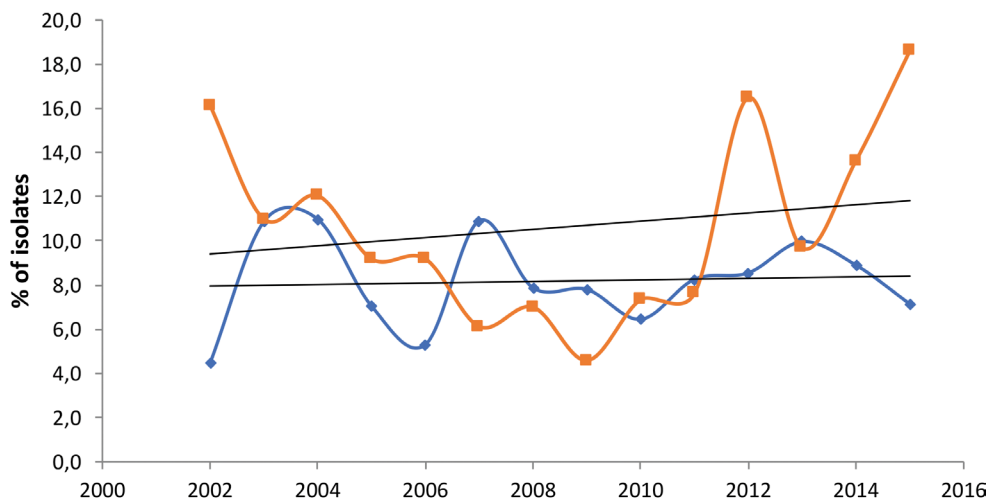
Contrary to what was expected, *P. aeruginosa* represented 8.0% and 5.5% of isolates from community and nosocomial origin, respectively. Unlike this finding, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Morganella morganii* and *Serratia marcescens* (all of them with chromosomal inducible AmpC  $\beta$ -lactamases) were also more common in IAIs of hospital origin (table 1).

When comparing this data (2011–2015) with that previously reported (2002–2010) [14], *E. coli* was also the most prevalent isolate but significantly decreased ( $P=0.0003$ ) in community acquired IAI (60.9% 2002–2010 vs. 56.1% 2011–2015). However, *P. aeruginosa* significantly increased ( $P=0.0003$ ) in community acquired IAI (5.6% 2002–2010 vs. 8.0% 2011–2015). For *K. pneumoniae*, figures revealed an increased prevalence both in the community (8.9% 2002–2010 vs. 10.8% 2011–2015,  $P=0.016$ ) and nosocomial (9.2% 2002–2010 vs. 10.8% 2011–2015,  $P=0.029$ ) IAI.

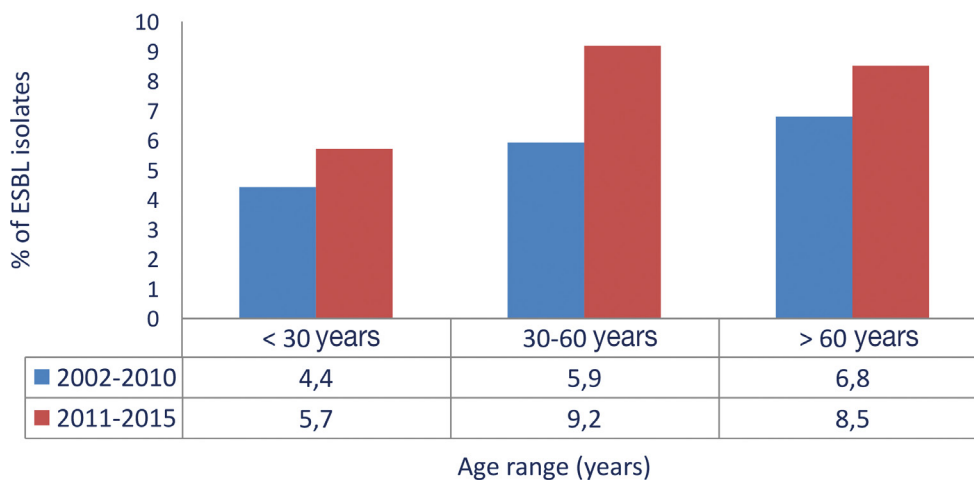
Considering all the enterobacterial isolates tested for ESBL ( $n=3,932$ ; 2,857 *E. coli*, 853 *Klebsiella* spp. and 222 *Proteus mirabilis*), 338 (8.6%) were producers of these enzymes in the studied period (2011–2015). ESBLs were more prevalent in *K. pneumoniae* (16.3%) and *E. coli* (9.5%) isolates of nosocomial origin, followed by *K. pneumoniae* (9.5%) community-acquired

isolates. With the exception of *E. coli* of nosocomial origin, all these figures increased when compared with the previous study (2002–2011) [14] (table 2). The incidence over time of ESBL-producing *E. coli* and *K. pneumoniae* isolates is shown in figure 2, indicating a relative stability in *E. coli* and an overall increase in *K. pneumoniae*. In addition, an age-associated increase was observed in ESBL-producing isolates, reaching a frequency of more than 8% in patients over 60 years of age (figure 3). The increase of ESBL prevalence was observed in all patients irrespective of age when compared with the 2002–2010 period [14].

The antibiotic susceptibility comparative profile of the most common organisms in IAIs isolated in 2011–2015 is shown in table 3. The compounds most active against Enterobacteriaceae were amikacin (susceptibility rates between 95.5 and 100%), ertapenem (92.3–100%) and imipenem (59.7–100%). Those which performed worst were the fluoroquinolones with, in general, lower rates of susceptibility. In the case of *E. coli* nearly 30% of the isolates were resistant to ciprofloxacin and levofloxacin. Enterobacterial isolates producing chromosomal inducible AmpC  $\beta$ -lactamases (*E. cloacae*, *E. aerogenes*, *C. freundii*, *M. morganii* and *S. marcescens*), known to be intrinsically



**Figure 2** Percentage of *Escherichia coli* and *Klebsiella pneumoniae* isolates with extended spectrum  $\beta$ -lactamases recovered per year in the SMART study in Spain from 2002 to 2015



**Figure 3** Frequency of Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis*) with extended spectrum  $\beta$ -lactamases according to age of the patients in the SMART study in Spain comparing 2002-2010 and 2011-2015 periods

resistant to the amoxicillin-clavulanate combination [20], were excluded from the analysis of resistance to this antibiotic. This ranged between 72.8% in *E. coli* and 92.0% in *Proteus vulgaris*. Piperacillin-tazobactam susceptibility of all the Enterobacteriaceae ranged from 72.9% in *E. aerogenes* to 98.7% in *P. mirabilis*. Piperacillin-tazobactam, ceftazidime, cefepime, imipenem and amikacin maintained their activity against *P. aeruginosa* in a range from 81.6 to 89.3% of the isolates.

When only ESBL-producing *E. coli* and *K. pneumoniae*

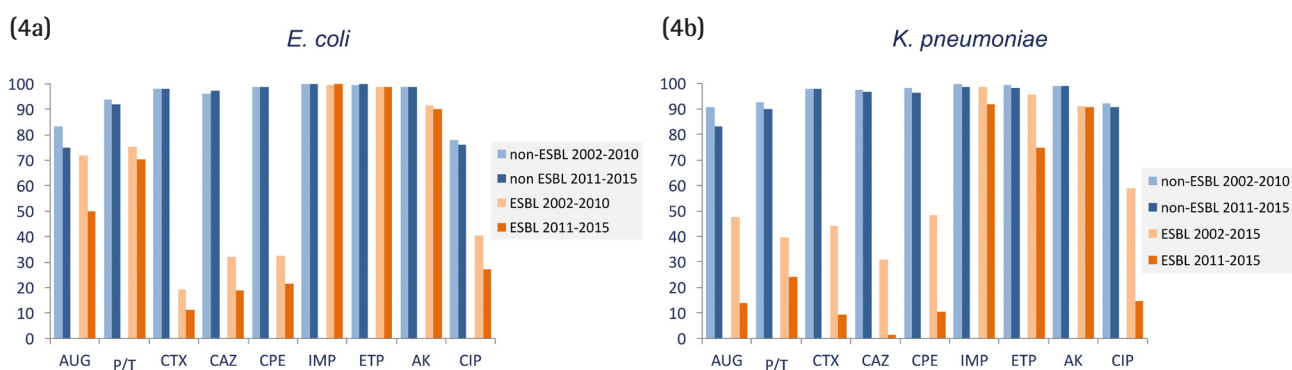
were taken into consideration and compared to non-ESBL-producers over time, imipenem and ertapenem activity remained virtually unchanged in *E. coli* irrespective of the ESBL production status (figure 4a) and was slightly affected in *K. pneumoniae* isolates (figure 4b). As expected, other  $\beta$ -lactam antibiotics were highly affected by the ESBL production and the reduction of susceptibility rates was higher in the 2011-2015 period. The decrease of susceptibility to all these antibiotics was higher in *K. pneumoniae* than in *E. coli*. It must be noted that ESBL-producing *E. coli* susceptibility rates

**Table 3** Activity of different antimicrobials used in intra-abdominal infections against the most common microorganisms collected in Spain in the SMART study (2011–2015).

Microorganism	Percentage of susceptible isolates <sup>a</sup>									
	AUG <sup>a</sup>	P/T	CTX	CAZ	CPE	IMI	ETP	AK	CIP	LVX
<i>Escherichia coli</i>	72.8	90.0	90.5	90.4	92.2	99.8	99.9	98.1	71.7	73.4
<i>Klebsiella pneumoniae</i>	73.2	81.3	86.2	84.1	85.2	97.7	95.1	97.9	80.6	85.7
<i>Klebsiella oxytoca</i>	87.1	93.0	92.0	97.6	97.2	100.0	99.7	99.7	97.2	98.3
<i>Proteus mirabilis</i>	90.1	98.7	95.6	94.7	96.9	72.9	100.0	98.7	70.7	82.2
<i>Proteus vulgaris</i>	92.0	100.0	57.6	78.8	100.0	81.8	100.0	97.0	100.0	97.0
<i>Enterobacter cloacae</i>	- <sup>b</sup>	82.2	71.1	73.8	85.9	98.7	92.3	99.3	95.6	96.3
<i>Enterobacter aerogenes</i>	- <sup>b</sup>	72.9	61.2	60.0	92.9	98.8	96.5	100.0	89.4	92.9
<i>Citrobacter freundii</i>	- <sup>b</sup>	79.1	70.0	68.2	85.5	98.2	98.2	98.2	90.0	92.7
<i>Morganella morganii</i>	- <sup>b</sup>	96.3	75.4	64.2	97.8	59.7	100.0	95.5	71.6	85.8
<i>Serratia marcescens</i>	- <sup>b</sup>	96.6	94.9	96.6	96.6	96.6	100.0	100.0	89.8	94.9
Other Enterobacteriaceae	21.1	91.5	70.8	78.3	99.1	79.3	93.4	100.0	93.4	95.3
<i>Pseudomonas aeruginosa</i>	- <sup>b</sup>	84.2	- <sup>b</sup>	86.4	84.8	81.6	- <sup>b</sup>	89.3	77.7	74.0

<sup>a</sup>EUCAST criteria except AUG in which CLSI criteria were considered; <sup>b</sup>This antimicrobial is not considered adequate against the microorganism tested.

AUG: amoxicillin-clavulanate; P/T: piperacillin-tazobactam; CTX: cefotaxime; CAZ: ceftazidime; CPE: cefepime; IMI: imipenem; ETP: ertapenem; AK: amikacin; CP: ciprofloxacin; LVX: levofloxacin.



**Figure 4** Activity of antimicrobials used in intra-abdominal infections against ESBL producing and non-producing *Escherichia coli* (4a) and *Klebsiella pneumoniae* (4b) isolates in the SMART study in Spain comparing 2002–2010 and 2011–2015 periods

for amoxicillin-clavulanate were 50%, and even lower in ESBL-producing *K. pneumoniae* in which susceptibility values of 14% were not achieved. The corresponding values for piperacillin-tazobactam were 70.5% for *E. coli* and 24.0% for *K. pneumoniae*.

When ertapenem activity was specifically analyzed, it was scarcely modified in ESBL and non-ESBL *E. coli* producers that were resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam and levofloxacin. In all cases, susceptibility values were above 99% (table 4). In *K. pneumoniae*, ertapenem susceptibility was maintained in a range of 75.0% to 86.4% in

non-ESBL producers and 59.6% to 72.5% in ESBL producers.

## DISCUSSION

Surveillance of antimicrobial resistance trends has been highlighted as a tool for fighting antimicrobial resistance and is an important tool in antimicrobial stewardship programs [21]. Local and international efforts in surveillance are being conducted and different governmental studies such as those promoted by the European Centre for Diseases Prevention and Control (ECDC) [22], the Centre for Diseases Control and

**Table 4** Activity of ertapenem in ESBL and non-ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolates resistant to amoxicillin/clavulanate, piperacillin/tazobactam, and levofloxacin collected in the SMART study (2011–2015).

Microorganisms	ESBL	Antimicrobial	% of resistant isolates	Ertapenem		
				Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
<i>Escherichia coli</i>	Negative	AMC	11.4	297 (99.7)	1 (0.3)	
			20.3	50 (100)		
	Positive	P/T	6.5	169 (99.4)	1 (0.6)	
			22.0	51 (99.4)	1 (1.9)	2 (3.7)
	Negative	LVX	21.8	571 (99.8)	1 (0.2)	
			69.5	170 (99.4)		1 (0.6)
<i>Klebsiella pneumoniae</i>	Negative	AUG	8.8	38 (86.4)	2 (4.5)	4 (9.1)
			49.3	24 (64.9)	2 (5.4)	11 (29.7)
	Positive	P/T	7.2	27 (75.0)	6 (16.7)	3 (8.3)
			62.7	28 (59.6)	2 (4.3)	17 (36.2)
	Negative	LVX	4.8	19 (79.2)	1 (4.2)	4 (16.7)
			6.8	37 (72.5)	1 (2.0)	13 (25.5)

ETP: ertapenem; AUG: amoxicillin/clavulanate; P/T: piperacillin-tazobactam; LVX: levofloxacin.

Prevention (CDC) [23] and the World Health Organization [24] monitor susceptibility and resistance over time. These programs are complemented with those sponsored by pharmaceutical companies following specific microorganisms and antimicrobials (normally those marketed by the sponsor) and are focused on certain infections or resistance problems. Data from these sponsored surveillance studies are also useful when confronting antimicrobial use for monitoring their potential ecological impact on the development of resistance and for the implementation of antimicrobial guidelines in accordance with local epidemiology [4,25,26]. With the new antimicrobials and beyond the spectrum of activity, surveillance studies are used to define wild type populations, to understand the potential impact of resistance mechanisms on MIC distributions, and to perform PK/PD analysis [27]. All of them are essential in the procedure of setting clinical breakpoints, a role recognized by both the European Medicines Agency (EMA) and Food and Drugs Administration (FDA) not only in the process of commercialization of antimicrobials but also in the post-marketing period [28,29].

The SMART study, an ongoing surveillance program that collects antimicrobial susceptibility testing data among hospitalized patients on a global scale since 2002, was initially designed to monitor ertapenem susceptibility in IAI and ESBL producing Enterobacteriaceae [13]. Their objectives have now been enlarged to also monitor respiratory and urinary tract infections and, more recently, data on acquired carbapenemase-producing isolates are also being analysed [30]. In a previous publication [14], we analysed data from IAI obtained from the SMART study in Spain since its

implementation in 2002 until 2010 with special focus on ertapenem susceptibility and also ESBLs. In the current study and with similar objectives we have enlarged this information in the 2011–2015 period. The SMART study provides a worldwide and local representative analysis of antimicrobial susceptibility profiles of microorganisms involved in IAI [13].

In line with recent publications [31], *E. coli* and *K. pneumoniae* are the most prevalent microorganisms in IAI in Spain, followed by *Enterobacter* spp. and *P. aeruginosa*. However, when comparing the current analysed period (2011–2015) with the previous one (2002–2010) [14], we found some differences when classifying the isolates as of nosocomial or community origin. The most important results were an increase of *K. pneumoniae* isolates both in the community and in nosocomial isolates and also in *P. aeruginosa* isolates among community isolates. These results might reflect a changing epidemiology due to a potential increase of patients with sociosanitary conditions or with previous antimicrobial therapy and/or hospital admission, all risk factors associated with these pathogens [11,32]. However, we cannot demonstrate this hypothesis with the patient's data recorded in the SMART database.

As previously noted, surveillance studies also help to support empiric therapeutic recommendations in local guidelines. In Spain, the last IAI guidelines were published in 2009 [9] and in the USA [26] and at international level during 2017 [4]. These guidelines recommend carbapenems for empiric antimicrobial treatment of IAI; ertapenem or other carbapenems in patients at risk for infection with community-acquired ESBL-producing Enterobacteriaceae and meropenem,

imipenem or doripenem for in-patients, including the critically ill, with healthcare-associated infections and these at higher risk for infection with multi-drug resistant microorganisms. On the other hand, they do not recommend (or questioned) the use of amoxicillin-clavulanate or ampicillin-sulbactam. Piperacillin-tazobactam, as meropenem, imipenem or doripenem, is recommended only for higher-risk patients. In Spain, and waiting for new recommendations with the approval of new antimicrobials with IAI indications, such as ceftazidime-avibactam and ceftolozane-tazobactam, these recommendations can also be supported with the SMART susceptibility testing results and data of ESBL producing Enterobacteriaceae.

Carbapenems, as well as amikacin, present the highest susceptibility values with percentages higher than 98% and 95% in *E. coli* and *K. pneumoniae* respectively. This situation was similar to that recently published for IAI microorganisms included in the SMART database in the USA [31]. Moreover, the overall prevalence of ESBL producers, specifically monitored in the SMART study, demonstrated higher values in *K. pneumoniae* (13.1%) than in *E. coli* (8.6%) isolates with a clear trend to increase over time in the former species but not in the latter (8.4% and 8.7%, respectively, in 2002-2011 period) [14]. This is consistent with the figures published in the SMART reports from the USA and Asia [13,31] and in other surveillance studies, such as EARS-net, TEST or SENTRY, that depict an overall increase of ESBLs irrespective of the origin of *K. pneumoniae* isolates [22,33,34]. They show that ESBL-producing isolates are more prevalent in the Mediterranean countries than in Northern Europe and North America, but lower than in Asian countries. Local epidemiology of ESBL producers is also highlighted to influence IAI empiric therapy [4,26]. Moreover, as in the analysis of SMART data from 2002-2010 [14], we demonstrated in the 2011-2015 study period a higher frequency of ESBL producers in older patients and in those with longer hospitalizations. Both situations have been repeatedly highlighted as risk factors for acquisition and infection due to ESBL-producing Enterobacteriaceae [11,32,35].

On the other hand, in our study we could also define co-resistances in ESBL producing isolates, a relevant issue for the design of treatment protocols and for the selection of antimicrobials [36]. The activity of penicillins plus  $\beta$ -lactamase inhibitors (amoxicillin-clavulanate and piperacillin-tazobactam), and to a higher degree extended spectrum cephalosporins (cefotaxime, ceftazidime and cefepime) and fluoroquinolones (levofloxacin and ciprofloxacin) was importantly affected in ESBL producers. Resistance values for ciprofloxacin were extremely high in ESBL-positive *E. coli* (72.9%) and *K. pneumoniae* (85.3%) isolates, even higher than those found previously (59.5% and 41.2%, respectively) [14]. This trend is also observed in other parts of the world [13,31].

Interestingly, further analysis of MIC values showed that carbapenems, including ertapenem, maintained a good activity in ESBL-producing isolates that were resistant to amoxicillin-clavulanate, piperacillin-tazobactam or fluoroquinolones. This effect was higher in *E. coli* than in *K.*

*pneumoniae* (Table 4). This difference, more pronounced in the 2011-2015 than in the 2002-2010 period, might denote coproduction of carbapenemases in ESBL producers, a fact demonstrated when molecular characterization of  $\beta$ -lactamases was performed [30,37]. In Spain, coproduction of OXA-48-like or KPC carbapenemases with an ESBL is not an infrequent event and is linked to dissemination of specific clones [38,39].

The microbiological data of the SMART study in Spain support the current therapeutic guidelines in IAI which advocate ertapenem, a class I carbapenem remarkable for its long half-life and lower impact than class II carbapenems (imipenem, meropenem and doripenem) in the selection of resistant isolates [40], as the empiric treatment of choice for mild-moderate community-acquired infections, particularly for patients with risk factors for ESBL-producing or AmpC-hyperproducing Enterobacteriaceae and in patients not at risk of infection by *P. aeruginosa* [4,9,26]. In addition, the absence of a collateral effect or ecological impact on organisms with natural low susceptibility to ertapenem such as *P. aeruginosa* reinforces recommendation of class I carbapenems [40]. The selection of other carbapenems should depend on the type of patient, the possible origin of the infection and if *P. aeruginosa* infection is suspected. Nevertheless, the recent irruption of cephalosporins and  $\beta$ -lactamase inhibitors combinations, such as ceftazidime-avibactam and ceftolozane-tazobactam, in the therapeutic armamentarium and the need of sparing carbapenems, specifically of class II, due to the increasing prevalence of carbapenemases reinforces the need to update existing IAI guidelines [41].

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## CONFLICTS OF INTEREST

Rafael Cantón has collaborated in educational meetings sponsored by MSD, Pfizer and AstraZeneca. He has also had research grants from MSD and AstraZeneca. F. Javier Castillo has collaborated in educational meetings sponsored by MSD.

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