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Evolution of antimicrobial susceptibility patterns of aerobic and facultative gram-negative bacilli causing intra-abdominal infections: results from the SMART studies 2003-2007

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Study for Monitoring Antimicrobial Resistance Trends (SMART) is an ongoing global antimicrobial surveillance program focused on clinical isolates from intra-abdominal infections (IAI). The objective of this subanalysis was to assess the evolution of the antimicrobial susceptibility patterns among aerobic and facultative gram-negative bacilli (GNB) recovered over a 5-year period at our institution. We tested the *in vitro* activity of the antimicrobials, commonly used to treat IAI, against consecutive unique isolates from IAI using microdilution techniques according to the CLSI guidelines for MIC testing. All isolates were screened phenotypically for extended-spectrum beta-lactamase (ESBL) production. Isolates recovered within 48 h of hospitalization were considered community-acquired (CA). Over the study period a total of 572 aerobic and facultative gram-negative bacilli were recovered from 510 patients, of which 258 (45%) were CA. *Enterobacteriaceae* composed 91% of the total isolates. *Escherichia coli* was the most common isolated species (52%). Susceptibility rates of *Enterobacteriaceae* ranged from 96.5%-100% to ertapenem, 96.5%-100% to imipenem, 87.7%-94.3% to piperacillin-tazobactam, 85.1%-94.3% to cefotaxime, 89.5%-100% to cefepime, 76.3%-84.8% to ciprofloxacin, and 93.8%-100% to amikacin. ESBL were detected in 6.3% of *E. coli,* 5.7% of *Klebsiella* spp. and 2.7% of *Enterobacter* spp. ESBL producers generally had a more antibiotic-resistant profile than non-ESBL producers and 16% of them were CA. Susceptibility rates to ertapenem, imipenem, piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin and amikacin were, respectively, for *P. aeruginosa*: 28.2%, 58.9%, 82%, 84.6%, 76.9%, 71.8% and 82%; for *Acinetobacter baumannii*: 33.3%, 100%, 66.6%, 66.6%, 66.6%, 66.6% y 66.6%, and for *Stenotrophomonas maltophilia:* 0%, 0%, 0%, 28.6%, 0%, 42.9% and 14.3%. Over the 5 year-study period we have not observed significant increases in resistance of aerobic and facultative GNB causing IAI to commonly used beta-lactam antimicrobial drugs. A minority of ESBL-producing *Enterobacteriaceae* were CA. Carbapenems, including group I agents like ertapenem, were the most reliably active drugs in vitro against isolates producing IAI.

Key words:
Intra-abdominal infections. Extended-spectrum beta-lactamases (ESBL). Carbapenems. Antimicrobial resistance.

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Evolución de la sensibilidad a antimicrobianos de bacilos gramnegativos aerobios y facultativos causantes de infección intraabdominal: resultados de los estudios SMART 2003-2007

El Study for Monitoring Antimicrobial Resistance Trends (SMART) es un programa mundial de vigilancia de resistencia a antimicrobianos de microorganismos aislados de infecciones intraabdominales. El objetivo de este subanálisis fue estudiar la evolución de los patrones de sensibilidad a antimicrobianos de bacilos gramnegativos aerobios y facultativos aislados durante un período de 5 años en nuestra institución. Se determinaron las concentraciones mínimas inhibitorias (CMI) de los antimicrobianos más comúnmente usados para tratar infecciones intraabdominales por el método de microdilución en caldo siguiendo las recomendaciones del CLSI frente a aislados consecutivos procedentes de pacientes con estas infecciones. En todos los aislados se confirmó fenotípicamente la producción de betalactamasas de espectro extendido (BLEE). Se consideraron de adquisición comunitaria aquellos microorganismos aislados durante un máximo de 48 h de hospitalización. Durante el período de estudio se recogieron un total de 572 bacilos gram-negativos aerobios y facultativos correspondientes a 510 pacientes, de los cuales 258 (45%) fueron de adquisición comunitaria. El 91% de

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los aislados fueron enterobacterias y *Escherichia coli* fue la especie más frecuentemente aislada (52%). Los porcentajes de sensibilidad de las enterobacterias a lo largo de los 5 años oscilaron entre el 96,5-100% para ertapenem, el 96,5-100% para imipenem, el 87,7-94,3% para piperacilina-tazobactam, el 85,1-94,3% para cefotaxima, el 89,5-100% para cefepima, el 76,3-84,8% para ciprofloxacino y el 93,8-100% para amikacina. Se detectaron BLEE en el 6,3% de los aislados de *E. coli,* el 5,7% de *Klebsiella* spp. y 2,7% de *Enterobacter* spp. Los aislados productores de BLEE presentaron generalmente mayor multirresistencia a otros antibióticos que los no productores de BLEE y el 16% de ellos fueron de adquisición comunitaria. Los porcentajes de sensibilidad de *P. aeruginosa* a ertapenem, imipenem, piperacilina/tazobactam, ceftazidima, cefepima, ciprofloxacino y amikacina fueron, respectivamente: 28,2, 58,9, 82, 84,6, 76,9, 71,8 y 82%; para *Acinetobacter baumannii:* 33,3, 100, 66,6, 66,6, 66,6, 66,6 y 66,6% y para *Stenotrophomonas maltophilia:* 0, 0, 0, 28,6, 0, 42,9 y 14,3%. Durante los 5 años del estudio no se observaron aumentos significativos de resistencia de los bacilos gramnegativos aerobios y facultativos causantes de infecciones intraabdominales a los betalactámicos y solamente una minoría de las enterobacterias productoras de BLEE fueron de adquisición comunitaria. Los carbapenémicos, incluidos los agentes del grupo I como el ertapenem, fueron los antimicrobianos más activos *in vitro* frente a los aislados causantes de infecciones intraabdominales.

Palabras clave:
Infección intraabdominal. Betalactamasas de espectro extendido (BLEE). Carbapenémicos. Resistencia a antimicrobianos.

INTRODUCTION

There is great concern about the threat of increasing antimicrobial resistance in bacteria not only in hospitals but also in the community. In response to these concerns, national and international surveillance programmes have been developed to monitor resistance[1-5]. The Study for Monitoring Antimicrobial Resistance Trends (SMART) is an ongoing worldwide surveillance programme that was designed in 2002 to monitor longitudinally the *in vitro* antimicrobial susceptibility of aerobic and facultative gram-negative bacilli isolated exclusively from intra-abdominal sites from hospitalized and from outpatients. The SMART program provides actualized data to establish local and global resistance frequency, to detect potential resistance trends over time, and to provide information when empiric treatment has to be chosen against intra-abdominal infections[6].

In this subanalysis we assess the evolution of the antimicrobial susceptibility patterns among aerobic and facultative gram-negative bacilli isolated from intraabdominal infections and recovered over a period of 5 years (from 2003 to 2007) at our participating institution.

MATERIAL AND METHODS

Setting

Our institution is a public general reference hospital with 1,750 beds attending a population of 715,000 inhabitants in the South area of Madrid, Spain. The study was performed from 2003 to 2007.

Bacterial isolates

Over a period of six months of every year of the study we prospectively collected consecutive unique aerobic and facultative gram-negative bacilli isolated from intra-abdominal infections as follows: 2003 (121 isolates), 2004 (122 isolates), 2005 (113 isolates), 2006 (111 isolates), and 2007 (105 isolates).

Acceptable specimens included tissue, fluid or deep wound cultures obtained intraoperatively, and fluid from paracentesis or percutaneous aspiration of abscesses. By protocol, duplicate isolates (the same genus and species from the same patient) were excluded, regardless of the elapsed time between procurement of the specimens and differences in antimicrobial susceptibilities. Isolates obtained from abdominal drains or drainage bottles, stool, superficial wounds, or perirectal abscesses were excluded. Organisms were divided into those isolated within ≤48 h of hospitalization (community-acquired) and those isolated >48 h after hospitalization (nosocomially-acquired). Bacterial identification was performed using the automated system MicroScan (Dade MicroScan, Inc. Sacramento, CA, USA).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using broth microdilution according to guidelines for MIC testing from the Clinical and Laboratory Standards Institute (CLSI)[7]. Custom-made microtitre panels (Dade MicroScan, Inc. Sacramento, CA, USA) designed every year specifically for the study were used and included the following antimicrobials and range of concentrations (mg/l): piperacillin/tazobactam (2/4-64/4), cefotaxime (0.5-64), ceftazidime (0.5-64), cefepime (0.5-32), ertapenem (0.03-4), imipenem (0.06-8), ciprofloxacin (0.5-2), levofloxacin (0.5-4) and amikacin (4-32). Since the above panels did not include amoxicillin-clavulanic acid, susceptibility to this antimicrobial (range: 4/2-16/8) was determined using additional commercialized MicroScan panels type Neg Combo 2S. Susceptibility was based on CLSI breakpoints[7]. Reference strains *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas*

aeruginosa ATCC 27853 were used as quality control strains for each batch of MIC tests. Phenotypic identification of extended-spectrum beta-lactamase (ESBL) production in *E. coli* and *Klebsiella* spp. was confirmed following the CLSI guidelines[7]. Phenotypic confirmation of ESBL production in *Enterobacter* spp. was performed using a modification of the CLSI method. If the ceftazidime, cefotaxime or cefepime MIC was ≥2 mg/l, then the MIC of cefepime was compared with the MIC of cefepime plus clavulanic acid (10 µg). ESBL production was defined as a ≥8-fold decrease in the cefepime MIC when tested in combination with clavulanic acid compared with in the absence of clavulanic acid. *E. coli, Klebsiella* spp. and *Enterobacter* spp. confirmed phenotypically to produce ESBL were designated as resistant to cefotaxime, ceftazidime, and cefepime regardless of whether their MIC were below the CLSI breakpoint for susceptibility.

STATISTICAL ANALYSIS

In order to compare the susceptibility rates of the different groups, 95% confidence intervals were calculated for the difference by means of the Miettenen-Nurminen method[8]. The Mantel-Haenszel chi square test was used to analyze the linear trend of susceptibility rates over the 5 year-period (2003-2007) of the study[9]. A two-tailed p-value of ≤0.05 was considered significant.

RESULTS

Over the period of study (2003-2007), a total of 572 aerobic and facultative gram-negative bacilli were isolated from intra-abdominal infections in 510 patients at our institution. Among these, 258 (45%) were community-acquired. The distribution of the organisms isolated and the susceptibility results are shown in table 1. *Enterobacteriaceae* composed 91% of the total isolates. *E. coli* (52%) was the most common isolated species, followed by *Klebsiella* spp. (16%), *Proteus mirabilis* (6.3%) and *Enterobacter* spp. (6.3%). Among the non-fermenters, *P. aeruginosa* was the most common isolated species (6.8%). The carbapenems (imipenem and ertapenem) and amikacin were the antimicrobial agents most consistently active *in vitro* against the *Enterobacteriaceae,* whereas piperacillin/tazobactam, ceftazidime and amikacin were the most active antimicrobials against *P. aeruginosa.* The fluoroquinolones were the least reliably active agents against all isolates tested. Table 2 shows the evolution of the susceptibility of *Enterobacteriaceae* to the different antimicrobials over the study period. Susceptibility rates ranged from 96.5%-100% to ertapenem, 96.5%-100% to imipenem, 87.7%-94.3% to piperacillin-tazobactam, 85.1%-94.3% to cefotaxime, 89.5%-100% to cefepime, 76.3%-84.8% to ciprofloxacin, 79.2%-85.8% to levofloxacin and 93.8%-100% to amikacin. Overall, there were no significant increases in resistance of aerobic and facultative

Tabla 1 **Distribution of the microorganisms isolated and *in vitro* susceptibility rates (% susceptibility) over the 5-year study period**

Microorganisms	Nº isolates (%)	EPM	IPM	A/C	TZP	CFT	CAZ	FEP	CIP	LVX	AMK
E. coli	300 (52.4%)	99.6	100	68.6	92.3	93.6	93.6	93.6	74.3	74.6	98.6
K. pneumoniae	58 (10.1%)	100	98.2	100	100	94.8	94.8	94.8	96.5	100	98.2
P. aeruginosa	39 (6.8%)	28.2	58.9	0	82	0	84.6	76.9	71.8	74.3	82
P. mirabilis	36 (6.3%)	100	91.4	100	100	100	100	100	85.7	97.1	94.3
K. oxytoca	35 (6.1%)	100	100	91.4	91.4	91.4	91.4	91.4	91.4	100	100
E. cloacae	29 (5.1%)	100	100	0	79.3	75.8	75.8	96.5	100	100	100
C. freundii	18 (3.1%)	100	100	0	100	100	100	100	94.4	94.4	94.4
M. morganii	15 (2.6%)	100	93.4	0	92.9	78.6	78.6	100	78.6	100	100
P. vulgaris	10 (1.7%)	100	100	100	100	100	100	100	100	100	100
E. aerogenes	7 (1.2%)	71.4	71.4	0	28.6	28.6	28.6	85.7	57.1	85.7	100
S. maltophilia	7 (1.2%)	0	0	0	0	0	28.6	0	42.9	57.1	14.3
S. marcescens	3 (0.5%)	100	100	0	100	100	100	100	100	100	100
A. baumannii	3 (0.5%)	33.3	100	66.6	66.6	33.3	66.6	66.6	66.6	66.6	66.6
Other GNB	12 (2.1%)	91.7	91.7	66.7	100	100	100	100	83.3	83.3	100
Overall % susceptibility	572 (100%)	80.3	86.1	42.4	80.7	71.2	81.6	86.1	81.6	88.1	89.2

GNB: gram-negative bacilli includes (isolates): *Acinetobacter lwoffii* (1), *Aeromonas hydrophila* (1), *Citrobacter amalonaticus* (2), *Citrobacter koseri* (1), *Enterobacter sakazakii* (1), *Kluyvera ascorbata* (1), *Salmonella* spp. (1), *Serratia plymuthica* (1), *Proteus penneri* (1), *Providencia rettgeri* (1) and *Providencia stuartii* (1); EPM: ertapenem; IPM: imipenem; A/C: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CFT: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CIP: ciprofloxacin; LVX: levofloxacin; AMK: amikacin.

Tabla 2 **Evolution of the susceptibility of *Enterobacteriaceae* to the different antimicrobials over the study period**

Year*	EPM	IPM	A/C	TZP	CFT	CAZ	FEP	CIP	LVX	AMK
2003	99.1	98.2	75.0	88.4	92.8	92.8	100	80.3	83.0	99.1
2004	96.5	96.5	70.1	87.7	85.1	85.1	89.5	76.3	81.6	93.8
2005	97.2	97.2	90.6	94.3	94.3	94.3	97.2	79.2	79.2	96.1
2006	100	100	81.8	91.9	93.9	93.9	97.0	84.8	85.8	100
2007	99	97.9	79.2	93.8	91.7	91.7	95.8	84.4	84.4	99

*For each antimicrobial, there was not a significant linear trend of the susceptibility rates over the 5-year period (Mantel-Haenszel chi square test). EPM: ertapenem; IPM: imipenem; A/C: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CFT: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CIP: ciprofloxacin; LVX: levofloxacin; AMK: amikacin.

gram-negative bacilli to the antimicrobials evaluated over the five years of the study.

In table 3 are summarized the results of the *in vitro* activity of the antimicrobials tested against the most frequently isolated microorganisms. The MIC_{90} (mg/l) of ertapenem and imipenem against *E. coli* were 0.06 and 0.25 respectively, 0.06 and 1 against *Klebsiella* spp., and were respectively 1 and 2 against *Enterobacter* spp. The fluoroquinolones were the least active agents against *E. coli*, being the MIC_{90} (mg/l) of ciprofloxacin and levofloxacin >2 and >4, respectively. One *E. coli* isolate was resistant to ertapenem, and two *Enterobacter aerogenes* isolates were resistant to ertapenem and imipenem, however, no carbapenemase activity was detected in any of the isolates, as determined by kinetic analysis of imipenem hydrolysis (data not shown). The activity of imipenem against *P. aeruginosa* was poor, being the MIC_{90}>8 mg/l. Amikacin (MIC_{90} 32 mg/l), was the most active antimicrobial against this microorganism. The presence of ESBL among *Enterobacteriaceae* was phenotypically detected in 25 (4.4%) isolates, and was most frequent in *E. coli* (76%), followed by *Klebsiella* spp. (20%) and *Enterobacter* spp. (4%). The prevalence of ESBL producing strains was 6.3% (19/300) in *E. coli*, 5.6% (5/88) in *Klebsiella* spp. (5.4% *K. pneumoniae* and 6% *K. oxytoca*) and 2.7% (1/36) in *Enterobacter* spp. When susceptibilities of ESBL and non-ESBL producers were compared, there were, overall, no statistically significant differences of *E. coli*

Tabla 3 **Comparative *in vitro* activity of the antimicrobials against the most frequently isolated microorganisms over the 5-year study period**

	E. coli (n=300)		*Klebsiella* spp. (n=93)		*Enterobacter* spp. (n=37)		*Proteus* spp. (n=47)*		*Pseudomonas aeruginosa* (n=39)	
	MIC_{90} (mg/l)	Range (mg/l)	MIC_{90} (mg/l)	Range (mg/l)	MIC_{90} (mg/l)	Range (mg/l)	MIC_{90} (mg/l)	Range (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
EPM	0.06	≤0.03->4	0.06	≤0.03-0.25	1	≤0.03->4	0.06	≤0.03-0.5	>4	2->4
IPM	0.25	≤0.06-4	1	0.12-8	2	0.12->8	4	≤0.06-8	>8	0.5->8
A/C	≤8/4	≤4/2->16/8	8/4	≤4/2->16/8	>16/8	>16/8	8/4	≤4/2-8/4	>16/8	>16/8
TZP	16/8	≤2/4->64/4	8/4	≤2/4->64/4	>64/4	≤2/4->64/4	4/2	≤2/4-4/4	>64	≤2/4->64/4
CFT	1	≤0.5->64	1	≤0.5->64	>64	≤0.5->64	1	≤0.5->64	>64	16->64
CAZ	1	≤0.5->64	1	≤0.5->64	>64	≤0.5->64	1	≤0.5-4	64	1->64
FEP	1	≤0.5->32	1	≤0.5->32	4	≤0.5->32	1	≤0.5-1	32	≤0.5->32
CIP	>2	≤0.5->2	1	≤0.5->2	1	≤0.5->2	1	≤0.5->2	>2	≤0.5->2
LEV	>4	≤0.5->4	1	≤0.5->4	1	≤0.5-4	1	≤0.5-4	>4	≤0.5->4
AMK	16	≤4->32	16	≤4->32	8	≤4-16	16	≤4-32	32	≤4->32

*Includes: *P. mirabilis* (36 isolates), *P. vulgaris* (10 isolates) and *P. penneri* (1 isolate). n: number of isolates; EPM: ertapenem; IPM: imipenem; A/C: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CFT: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CIP: ciprofloxacin; LVX: levofloxacin; AMK: amikacin.

Tabla 4 *In vitro* **susceptibility (% susceptibility) of ESBL and non-ESBL producing** *E. coli, Klebsiella* **spp. and** *Enterobacter aerogenes*

Microorganisms	No. isolates	EPM	IPM	AUG	TZP	CIP	LVX	AMK
Escherichia coli								
non-ESBL	281	100	100	93.2	93.2	77.6	77.6	100
ESBL	19	94.7	100	57.9	78.9	73.7	73.7	78.9
difference		5.3	0	35.3	14.3	3.9	3.9	21.1
(95% CI)*		0.9-24.7	−13.5-16.8	16.1-57.2	1.2-36.8	−11.7-26.9	−11.7-26.9	8.4-43.3
Klebsiella pneumoniae								
non-ESBL	55	100	100	100	100	96.4	100	100
ESBL	3	100	100	33.3	66.6	100	100	66.6
difference		0	0	66.7	33.4	−3.6	0	33.3
(95% CI)		−6.5-65.2	−6.5-65.2	20.6-93.9	6.1-79.5	−11.4-55.0	−6.5-65.2	6.1-79.5
Klebsiella oxytoca								
non-ESBL	33	100	100	96.7	96.7	96.7	100	100
ESBL	2	100	100	0	0	0	100	100
difference		0	0	96.7	96.7	96.7	0	0
(95% CI)		−10.4-65.8	−10.4-65.8	30.1-99.5	30.1-99.5	30.1-99.5	−10.4-65.8	−10.4-65.8
E. aerogenes								
non-ESBL	6	66.6	66.6	0	33.3	66.6	100	100
ESBL	1	100	100	0	0	0	0	100
difference		−33.3	−33.3	0	33.3	66.6	100	0
(95% CI)		−72.1-59.1	−72.1-59.1	−79.4-39.0	−59.1-72.1	−30.7-91.2	29.1-100	−39.0-79.4

*The 95% confidence intervals (CI) for the difference calculated as the % susceptible rate for non-ESBL producers minus the % susceptible rate for ESBL producers were determined using the Miettinen-Nurminen method. EPM: ertapenem; IPM: imipenem; A/C: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CIP: ciprofloxacin; LVX: levofloxacin; AMK: amikacin.

and *Klebsiella* spp. in susceptibility to the carbapenems and to the fluoroquinolones, however, statistically significant differences were found for amoxicillin/clavulanic, piperacillin/tazobactam and amikacin (table 4).

Of the 572 organisms isolated, 45% (258) were community-acquired: 45.1% of the *Enterobacteriaceae*, 45.1% of the non-fermenting gram-negative bacilli, and 51.3% of the *P. aeruginosa* isolates (table 5). All isolates of *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* were nosocomially acquired. Community-acquired isolates were more susceptible to all antimicrobials tested than nosocomial isolates. For the *Enterobacteriaceae*, there were no significant differences in the percentages of susceptibility to carbapenems, amikacin and fluoroquinolones between both groups, but significant differences were observed for the betalactams, that presented less activity against nosocomial isolates. Only 16% (4/25) of the *Enterobacteriaceae* which produced ESBL were community-acquired, and corresponded to 4 *E. coli* isolates. All ESBL-producing *K. pneumoniae* and *E. aerogenes* were nosocomially-acquired. Over the 5-year study period we have not observed any significant changes in the number of ESBL-producing *Enterobacteriaceae* (fig. 1).

DISCUSSION

Intraabdominal infections include a variety of conditions including peritonitis, diverticulitis, appendicitis, intra-abdominal abscesses and intrahepatic infection. In general, these infections involve a mixture of aerobic and anaerobic intestinal flora, although the most frequently isolated microorganisms are the *Enterobacteriaceae*[10]. In this study, *Enterobacteriaceae* were by far the most common isolates and composed 91% of the total. During the last decade, the emergence of multidrug-resistant *Enterobacteriaceae* and other gram-negative bacilli have become a growing problem[11]. In the specific context of intra-abdominal infections the performance of global ongoing antimicrobial surveillance studies has revealed resistance trends over time[6,12,13]. In our study we did not observed significant increases in resistance of aerobic and facultative gram-negative bacilli to the antimicrobials evaluated over the five-year period. The

Tabla 5 In vitro **susceptibility (% susceptibility) of microorganisms isolated ≤ 48 h (community-acquired) *vs.* > 48 h (nosocomially acquired) after admission to the hospital**

Microorganisms	No. isolates	EPM	IPM	A/C	TZP	CFT	CAZ	FEP	CIP	LVX	AMK
Total	572										
(≤48 h)	258	92.2	97.7	80.2	94.2	88.8	96.9	98.1	84.9	86.4	98.4
(>48 h)	314	91.1	93.0	68.2	87.3	81.5	88.2	90.4	76.8	80.3	94.3
difference		1.1	4.7	12	6.9	7.3	8.7	7.7	8.1	6.1	4.1
95% IC*		-3.6-5.7	1.3-8.4	4.9-19.1	2.2-11.7	1.4-13.0	4.6-13.1	4.0-11.5	1.6-14.5	-0.01-12.2	1.2-7.5
Enterobacteriaceae	521										
(≤48 h)	235	99.6	100	87.2	94.9	97.0	97.0	98.7	85.1	86.4	99.1
(>48 h)	286	98.9	98.6	74.1	90.2	88.8	88.8	94.8	78.7	82.2	97.9
difference		0.7	1.4	13.1	4.7	8.2	8.2	3.9	6.4	4.2	1.2
95% IC		-1.4-2.7	-0.2-3.5	6.4-19.7	0.1-9.3	4.0-12.8	4.0-12.8	1.0-7.4	-0.3-13.0	-2.2-10.4	-1.2-3.8
Non-fermenters	51										
(≤48 h)	23	17.4	73.9	8.8	87.0	4.3	95.7	91.3	82.6	87.0	91.3
(>48 h)	28	10.7	35.7	7.1	57.1	7.1	57.1	46.4	57.1	60.7	57.1
difference		6.7	38.2	1.7	29.9	-2.8	38.6	44.9	25.5	26.3	34.2
95% IC		-13.3-28.5	10.6-60.2	-15.7-21.0	4.8-51.2	-19.3-15.0	16.5-57.9	20.3-64.3	-0.4-47.8	1.5-47.8	10.3-54.6

*The 95% confidence intervals (CI) for the difference calculated as the % susceptible rate for community-adquired isolates minus the % susceptible rate for nosocomially-adquired isolates were determined using the Miettinen-Nurminen method. EPM: ertapenem; IPM: imipenem; A/C: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CFT: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CIP: ciprofloxacin; LVX: levofloxacin; AMK: amikacin.

carbapenems (imipenem and ertapenem) and amikacin were the most consistently active *in vitro* antimicrobial agents against *Enterobacteriaceae*, whereas piperacillin/tazobactam, ceftazidime and amikacin were the most active antimicrobials against *P. aeruginosa.* Although clinical outcomes may not always reflect *in vitro* susceptibility results in intra-abdominal infections where surgical drainage has a major impact, results of surveillance data may help to guide the selection of empirical antimicrobial therapy for some patients, given that intra-operative cultures in general are not routinely obtained.

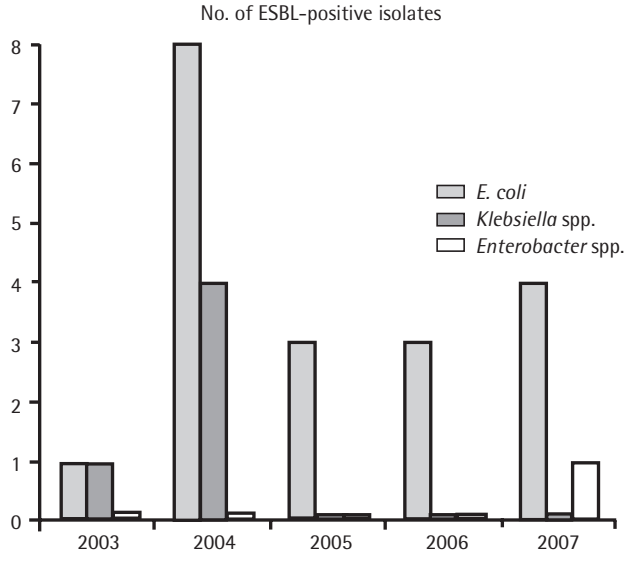

Figura 1 *ESBL producing* E. coli, Klebsiella *spp. and* Enterobacter *spp. over the 5 year study period.*

Our study shows that the beta-lactams presented good activity against *Enterobacteriaceae* being cefepime the most active beta-lactam, since it was active against species that hyperproduce AmpC beta-lactamases. However, the emergence of ESBL-producing *Enterobacteriaceae* compromises the use of all beta-lactams, including cefepime[14]. Moreover, ESBL are codified in plasmids that carry other genes that give resistance to other antimicrobial agents[15]. Although in our study, ESBL-producing *Enterobacteriaceae* represented only 4.4%, these results suggest that third- and fourth-generation cephalosporins may not be an ideal choice in the empirical therapy of intra-abdominal infections. In addition, these isolates were significantly more resistant to amikacin than ESBL non-producers and were not only confined to the hospital setting, but were also isolated from the community. Until recent years, the majority of the infections caused by ESBL-producing *E. coli* or *K. pneumoniae* were described as nosocomial[14]. However, recent data suggest that infections caused by ESBL-producing microorganisms are an emerging problem in community patients[16-20]

and a cause community-acquired intra-abdominal infections[13]. In this study, community-acquired isolates represented a minority (16%). In this situation, the carbapenems have to be consider the better treatment for severe infections and the empiric alternative of choice for infections with high suspicion of being caused by ESBL-producing or AmpC-derepressed *Enterobacteriaceae*[21-23]. As indicated above, the carbapenems showed the highest percentages of activity against the *Enterobacteriaceae.* Although resistance to these antimicrobials is still very rare (<0.005%) among *Enterobacteriaceae*[24-26], carbapenem resistance due to the production of carbepenemases is emerging in certain geographic areas[27-29], and it has also been described in Spain[30]. In our study, one *E. coli* isolate was resistant to ertapenem, and two *E. aerogenes* isolates were resistant to ertapenem and imipenem. This resistance was probably due to ESBL-production or AmpC-derepression combined with impermeability, as described previously[31], since no carbapenemase activity was detected in any of the isolates. In the case of *P. aeruginosa,* the third most frequenly isolated species in our study, the activity of imipenem was poor, and only 58.9% of the isolates were susceptible to this agent. Thirty percent of community-acquired *P. aeruginosa* isolates were resistant to imipenem. The use of group II carbapenemes (imipenem and meropenem) has been related to the emergence of bacterial species with resistance to these agents, like *Pseudomonas* spp. or *S. maltophilia,* and some authors have indicated that group I carbapenems, such as ertapenem, could be the best option to treat infections caused by ESBL-producing or Amp-C derepressed microorganisms, because of its low activity against non-fermenting colonizing bacteria compared to that of group II carbapenems[21,32].

The activity of the fluoroquinolones against *Enterobacteriaceae* ranged from 76.3% to 85.8% (table 2), however, against *E. coli,* which was the most common species recovered in our study (52%), the susceptibility rates to ciprofloxacin and levofloxacin were 74.3% and 74.6%, respectively. In addition, the fluoroquinolones showed poor activity against *P. aeruginosa* isolates, consequently, ciprofloxacin and levofloxacin should not remain among the first line choices for empirical therapy of complicated intra-abdominal infections in our clinical setting, and in general, they are not recommended[33,34]. In this study, amikacin showed high activity against aerobic and facultative gram-negative bacilli, however it is recommended to combine an aminoglycoside with a beta-lactam for the treatment of severe infections[23].

Approximately half of the isolates in this study were community-acquired, and these isolates were in general more susceptible to the antimicrobials tested than nosocomial isolates. Nosocomially-acquired *Enterobacteriaceae* were significantly more resistant to beta-lactams that community isolates, and nosocomially-acquired *P. aeruginosa* were significantly more resistant to all antimicrobial classes evaluated. Although the division of cultures in our study based solely on time of culture into those performed ≤48 h *versus* >48 h after hospitalization has its limitations, these results are consistent with the concept that isolates acquired in the hospital are generally more resistant than those acquired in the community.

All surveillance studies have their limitations[35]. In our study we only analyzed a consecutive group of samples every year and consequently not all the population was represented. However, as data accrue on an annual basis, this 5-year study has provided an analysis of longitudinal trends in antimicrobial resistance patterns among gram-negative bacilli isolated from intra-abdominal infections at our institution. Overall, there were no significant increases in resistance of the microorganisms to the antimicrobials evaluated over the period of study, however, the emergence of community and nosocomially-adquired ESBL-producing *Enterobacteriaceae* is a cause of concern. The carbapenems, including group I agents like ertapenem, were the most reliable active drugs in vitro against *Enterobacteriaceae*, whereas piperacillin/tazobactam and ceftazidime were the most active agents against *P. aeruginosa.*

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