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Urinary tract infection by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: evolution of antimicrobial resistance and therapeutic alternatives

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Abstract

Purpose. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are responsible for numerous nosocomial infections. The objective of this study was to determine the development of their susceptibility to ten antibiotics and the antibiotic consumption of patients with suspicion of urinary tract infection (UTI).

Methodology. A retrospective study was conducted on the susceptibility profiles of *A. baumannii* and *P. aeruginosa* isolates from 749 urine samples gathered between January 2013 and December 2016, and on the consumption of imipenem, meropenem and piperacillin-tazobactam between 2014 and 2016.

Results. Hospital patients were the source of 82 (91.1 %) of the 90 *A. baumannii* isolates detected and 555 (84.2 %) of the 659 *P. aeruginosa* isolates. Globally, the lowest percentage susceptibility values were found for fosfomycin, aztreonam and ciprofloxacin, while colistin continued to be the most active antibiotic *in vitro*. In 2016, the susceptibility of *A. baumannii* to carbapenem and piperacillin-tazobactam decreased to very low values, while the susceptibility of *P. aeruginosa* to carbapenem remained stable but its susceptibility to piperacillin-tazobactam decreased. There was a marked increase in the consumption of piperacillin-tazobactam.

Conclusion. In our setting, it is no longer possible to use carbapenems and piperacillin-tazobactam for empirical treatment of UTI due to *A. baumannii* or to use piperacillin-tazobactam for empirical treatment of UTI due to *P. aeruginosa*. Colistin was found to be the most active antibiotic *in vitro*. There was a marked increase in the consumption of piperacillin-tazobactam.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative, non-glucose-fermenting, strictly aerobic, immobile, catalase-positive and cytochrome oxidase-negative bacillus [1, 2]. It is widely distributed in nature and can colonize the human skin, respiratory and digestive systems [3]. *Pseudomonas aeruginosa* has the same characteristics as *A. baumannii* except that it is cytochrome oxidase-positive and motile. Although both microorganisms are infrequent opportunistic pathogens in our setting, they have become important aetiological agents in nosocomial infections over the past few decades and are associated with high morbidity and mortality rates, especially in fragile and immunosuppressed patients [4].

Multiple mechanisms are involved in the antibiotic resistance profile of these microorganisms, including the dysregulation of intrinsic resistance mechanisms, the acquisition of resistance factors from other bacteria, membrane permeability alterations and the appearance of efflux pumps. Given the clinical relevance of increased β-lactam resistance, largely to carbapenems, reports have been published on the presence of AmpC-type chromosomal cephalosporins, extended-spectrum β-lactamases (ESBLs), both chromosomal (OXA-51) and plasmid (OXA-23, OXA-24 and OXA-58) type D and type B (metallo-β-lactamases) carbapenemases in *A. baumannii*, alongside porin and efflux pump disorders, but type A carbapenemases have not been

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Keywords: *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; piperacillin/tazobactam; imipenem; meropenem; colistin.

Abbreviations: AK, Amikacin; AZT, Aztreonam; CAZ, Ceftazidime; CI, confidence interval; Cip, Ciprofloxacin; COL, Colistin; FEP, Cefepime; FOS, Fosfomycin; IP, Imipenem; MP, Meropenem; ORs, odds ratios; PTZ, Piperacillin-Tazobactam; UTI, urinary tract infection.

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reported [5]. *P. aeruginosa* possesses an AmpC-type chromosomal cephalosporin that confers resistance to penicillins and to 1st, 2nd and 3rd generation cephalosporins, with the exception of ceftazidime. It can also acquire type A, B or D carbapenemases and show alterations in OprD porin and active expulsion mechanisms such as MexAB-OprM, conferring carbapenem resistance [6–8].

Carbapenems continue to be the treatment of choice for *A. baumannii* and *P. aeruginosa* when these are carbapenem-susceptible. Aminoglycosides, especially amikacin and tobramycin, may be useful for treatment but are frequently combined with carbapenems [9]. The combination of sulbactam and carbapenems is also a treatment of choice for *A. baumannii*. Ciprofloxacin is not active against *A. baumannii* but shows good activity against *P. aeruginosa*, although some isolates are resistant. If the microorganism is resistant to both carbapenems and aminoglycosides, treatment possibilities are considerably reduced and colistin is generally administered as a last therapeutic option. Unfortunately, there has been an alarming worldwide increase in the resistance of *A. baumannii* to antibiotics, mainly attributable to the extensive use of wide-spectrum antibiotics, including latest-generation carbapenems and cephalosporins [10]. Thus, it is not uncommon to encounter multiresistant *A. baumannii* isolates that can be treated only with colistin, although resistance to this antibiotic has also been reported [11]. The involvement of this pathogen in infections affecting fragile patients has focused particular interest on this issue. The use of colistin is restricted to infections by these multi-resistant microorganisms, given the associated risk of nephrotoxicity. The correct initiation of empirical treatment in urinary tract infections (UTIs) can have a major impact on the acquisition of resistance by pathogens such as *A. baumannii* and *P. aeruginosa*.

The objective of this study was to analyse changes in the antibiotic susceptibility profile of *A. baumannii* and *P. aeruginosa* isolates in our setting over the four-year study period and to examine the consumption of imipenem (IP), meropenem (MP) and piperacillin-tazobactam (PTZ).

METHODS

A retrospective analysis was conducted on the antibiotic susceptibility of all 90 *A. baumannii* isolates and 659 *P. aeruginosa* isolates from consecutive urine samples with suspicion of UTI studied at the Microbiology Laboratory of the University Hospital Virgen de las Nieves, Granada, between January 2013 and December 2016, a centre in the southern Spanish region of Andalusia.

The isolates were obtained from mid-stream urine samples or samples from indwelling or intermittent catheters in patients attending primary care centres or admitted to the Virgen de las Nieves Hospital Complex, a reference third-level centre containing three hospitals (Specialty Hospital, Maternal and Child Hospital and Rehabilitation and Traumatology Hospital) and serving a population of 440 000. All samples were gathered in either wide-mouth sterile containers with screw caps or tubes with boric acid preservative (Vacutainer; Becton Dickinson, NJ). Samples were either processed within 24 h of their reception at the laboratory or were refrigerated when immediate processing was not possible. Processing of urine samples consisted of culture in chromogenic medium CHROMagar Orientation (Becton Dickinson, Franklin Lakes, NJ) and, for patients with renal disease, in Columbia blood agar (Becton Dickinson) with CO_2. A 1 µl calibrated loop (COPA, Brescia, Italy) was used to count rapid-growth uropathogens after incubation for 18–24 h at 37 °C. Samples were classified as negative (<10 000 UFC ml^{-1}), presumptive (10 000–100 000 UFC ml^{-1} 2 uropathogens or 1 uropathogen without leukocyturia), significant (bacteriuria with >100 000 UFC ml^{-1} of 1 or 2 uropathogens or 10 000–100 000 UFC ml^{-1} of 1 uropathogen with leukocyturia) or mixed (>10 000 UFC ml^{-1} of more than 2 uropathogens). For samples obtained from an intermittent catheter, the above values were reduced by 10 % for their classification tio be considered significant [12].

A MicroScan Walkaway automatized system (Siemens Healthcare Diagnostics, Barcelona, Spain) was used to identify microorganisms and evaluate susceptibility to the following antibiotics: amikacin, colistin, fosfomycin, ciprofloxacin, aztreonam, cefepime, ceftazidime, IP, MP and PTZ. In accordance with the manufacturer's instructions, the susceptibility of *A. baumannii* isolates to IP, MP and colistin was evaluated with the E-test (Liofilchem, Roseto degli Abruzzi, Italy). Only isolates identified as *A. baumannii* or *P. aeruginosa* were selected for study, and were classified as susceptible, intermediate or resistant for each antibiotic following CLSI 2016 recommendations. Duplicate positive urine cultures (i.e. of the same genus or species obtained sequentially from the same UTI episode) were excluded. Furthermore, if a patient contributed more than one urine sample, regardless of the origin (community or hospital), an interval of at least 30 days had to elapse before the second sample was considered a significant finding and included in the report. This procedure was adopted to avoid duplicate isolates from a single UTI episode.

In-hospital consumption of IP, MP and PTZ during the study period 2014–2016 was recorded as daily dose per 1000 stays (DDD/1000 stays).

SPSS version 17.0 (IBM, Chicago, IL) was used for statistical analysis. Categorical variables were expressed as distribution frequencies. Either the chi-square test for linear trend or Fisher's exact test was used to analyse the development of susceptibility in *A. baumannii* and *P. aeruginosa* when the number of cases was sufficient. Logistic regression analysis was used to study antibiotic susceptibility trends over time. Odds ratios (ORs) and 95 % confidence intervals were calculated to estimate the risk of antibiotic susceptibility in each year. The intermediate-resistant category was included in this analysis. Spearman's correlation test coefficient (with 95 % confidence interval) was used to determine the

Table 1. Susceptibility of *A. baumannii* to antibiotics

CAZ, ceftazidime (S MIC: <8; I MIC: 16; R MIC: >32); FEP, cefepime (S MIC: <8; I MIC: 16; R MIC: >32); AZT, aztreonam (S MIC: <8; IMIC: 16; RMIC: >32); PTZ, piperacillin-tazobactam (S MIC: <16/4; I MIC: 32/4-64/4; R MIC: >128/4); IP, imipenem (S MIC: <2; I MIC: 4; R MIC: >8); MP, meropenem (S MIC: <2; I MIC: 4; R MIC: >8); Cip, ciprofloxacin (S MIC: <1; I MIC: 2; R MIC: >4); AK, amikacin (S MIC: <16; I MIC: 32; R MIC: >64); COL, colistin (S MIC: <2; R MIC: >4).

Year	Interpretive categories	Results (%) (susceptible, intermediate or resistant strains/total strains)										
		CAZ	FEP	AZT	PTZ	IP	MP	CIP	AK	COL	FOS*	FOS†
2013	Susceptible	15 (3/20)	10 (2/20)	0 (0/20)	95.2 (20/21)	69.2 (18/26)	29.6 (8/27)	10 (2/20)	55 (11/20)	75 (17/20)	0 (0/21)	100 (21/21)
	Intermediate	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/21)	0 (0/26)	0 (0/27)	0 (0/20)	0 (0/20)	0 (0/75)		
	Resistant	85 (17/20)	90 (18/20)	100 (20/20)	4.8 (1/21)	30.8 (8/26)	70.4 (19/27)	90 (18/20)	45 (9/20)	25 (3/75)		
2014	Susceptible	3.8 (1/26)	7.7 (2/26)	0 (0/22)	96 (24/25)	57.1 (8/14)	26.7 (4/15)	3.8 (1/26)	9.1 (2/22)	100 (26/26)	4.7 (1/21)	95.3 (20/21)
	Intermediate	0 (0/26)	0 (0/26)	0 (0/22)	0 (0/25)	0 (0/14)	0 (0/15)	0 (0/26)	0 (0/22)	0 (0/26)		
	Resistant	96.2 (25/26)	92.3 (24/26)	100 (22/22)	4 (1/25)	42.9 (6/14)	73.3 (11/15)	96.2 (25/26)	90.9 (20/22)	0 (0/26)		
2015	Susceptible	0 (0/14)	0 (0/14)	0 (0/13)	87 (13/15)	26.7 (4/15)	0 (0/13)	0 (0/14)	7.7 (1/13)	100 (13/13)	7.1 (1/14)	92.9 (13/14)
	Intermediate	0 (0/14)	0 (0/14)	0 (0/13)	0 (0/15)	6.7 (1/15)	7.7 (1/13)	0 (0/14)	0 (0/13)	0 (0/13)		
	Resistant	100 (14/14)	100 (14/14)	100 (13/13)	13 (2/15)	66.7 (10/15)	92.3 (12/13)	100 (14/14)	92.3 (12/13)	0 (0/13)		
2016	Susceptible	10 (3/30)	10 (3/30)	0 (0/26)	8 (2/25)	15 (3/20)	6.7 (2/30)	6.7 (2/30)	15.4 (4/26)	96.1 (25/26)	0 (0/30)	100 (30/30)
	Intermediate	3.3 (1/30)	10 (3/30)	7.9 (2/26)	0 (0/25)	5 (1/20)	23.3 (7/30)	0 (0/30)	0 (0/26)	0 (0/26)		
	Resistant	86.7 (26/30)	80 (24/30)	92.1 (24/26)	92 (23/25)	80 (16/20)	70 (21/30)	93.3 (28/30)	84.6 (22/26)	3.9 (1/26)		
Total	Susceptible	7.8 (7/90)	7.8 (7/90)	0 (0/81)	68.6 (59/86)	44 (33/75)	16.5 (14/85)	5.5 (5/90)	22.2 (18/81)	95.3 (81/85)	2.3 (2/86)	97.7 (84/86)
	Intermediate	1.1 (1/90)	3.3 (3/90)	2.5 (2/81)	0 (0/86)	2.7 (2/75)	9.4 (8/85)	0 (0/90)	0 (0/81)	0 (0/85)		
	Resistant	91.1 (82/90)	88.9 (80/90)	97.5 (79/81)	31.4 (27/86)	53.3 (40/75)	74.1 (63/85)	94.4 (85/90)	77.8 (63/81)	4.7 (4/85)		

*FOS, fosfomycin MIC$\leq$16 mg l^{-1}.

†FOS, fosfomycin MIC$\geq$64 mg l^{-1}.

relationship between the consumption of IP, MP and PTZ and changes in their susceptibility in hospital-acquired isolates.

RESULTS

This study of urine samples included 90 *A. baumannii* isolates, of which 82 (91.1 %) were obtained from hospitalized adult patients, and 659 *P. aeruginosa* isolates, of which 555 (84.2 %) were from hospitalized adult patients. The percentage of samples obtained from indwelling or intermittent catheters was 60, 59, 65 and 64 %, respectively, in 2013, 2014, 2015 and 2016.

Table 1 shows the data obtained on the susceptibility of *A. baumannii* to the antibiotics under study. Globally, the lowest percentage susceptibility values were found for fosfomycin, aztreonam, ciprofloxacin, ceftazidime and cefepime, while colistin continued to be the most active antibiotic *in vitro*. Between 2013 and 2016, there was a statistically significant reduction in the susceptibility of *A. baumannii* to IP (P<0.001), MP (P=0.021) and PTZ (P<0.001). In the case of *A. baumannii* (Table 2), the OR for susceptibility to IP markedly diminished over the study period, reaching 0.078 (0.-018–0.346) in 2016, a 12-fold decrease in susceptibility with respect to 2013. The same was observed for MP, with an OR of 0.17 (0.032–0.088), a 5.8-fold decrease with respect to 2013; and for PTZ, with an OR of 0.004 (0–0.052), a decrease with respect to 2013.

Table 3 shows the data obtained on the antibiotic susceptibility of *P. aeruginosa*. Overall, fosfomycin, aztreonam, ciprofloxacin and amikacin showed the lowest activity against this pathogen, while colistin was again the most active. Nevertheless, the number of strains susceptible to amikacin increased over the years under study (P<0.001). The minimum inhibitory concentration (MIC) of fosfomycin decreased over the study period, reaching $\leq 16\,mg\,l^{-1}$ in almost 50 % of isolates in 2016 (P<0.001). Between 2013 and 2016, the susceptibility of *P. aeruginosa* to IP and MP did not significantly change (P=0.294 and P=0.663, respectively), while there was a significant reduction in the susceptibility to PTZ (P<0.001). For *P. aeruginosa* (Table 4), the OR for susceptibility to PTZ was 0.319 (0.173–0.588) in 2016, a 3-fold decrease in susceptibility with respect to 2013; however, there was no statistically significant decrease in susceptibility to MP or IP over this time period.

Table 5 shows the annual consumption of IP, MP and PTZ. In general, there was a marked increase in the consumption of PTZ over the study period that paralleled the reduction in susceptibility to this antibiotic of the two microorganisms studied. There was a slight reduction in IP consumption and a marked reduction in MP consumption over this period.

An inverse but non-significant correlation (−0.387, P=0.304) was found between the susceptibility of *A. baumannii* to IP, MP and PTZ and the in-hospital consumption of these drugs, expressed as DDD/1000 stays. An inverse but non-significant correlation (−0.267, P=0.488) was also found between the susceptibility of *P. aeruginosa* and the hospital administration of these drugs.

DISCUSSION

There has been a continuous increase worldwide in the resistance of Gram-negative microorganisms to antibiotics, including enterobacteriaceae, *A. baumannii* and *P. aeruginosa* [13–15], limiting the postential of adequate antibiotic treatment [7].

The inappropriate use of antibiotics against UTIs is a key factor in the acquisition of resistance mechanisms, attributable to the high prevalence of these infections and the frequent aetiological involvement of Gram-negative bacilli, the main microorganisms responsible for the acquisition of plasmid-encoded resistance [16, 17]. It should be borne in mind that UTIs can represent an important health risk if the main uropathogens cannot be adequately treated [18, 19].

A. baumannii has shown a high capacity to acquire resistance mechanisms and to spread these resistant phenotypes among the general population [15]. Multi-resistant *A. baumannii* is highly complicated to treat and has become a global threat over the past few years. The correct use of antibiotics is a key measure in controlling the acquisition of bacterial multi-resistances [12].

Carbapenem resistance has been observed in areas with a high prevalence of ESBL-producing microorganisms, for which carbapenems are the treatment of choice [20]. The past two decades have seen a marked increase in carbapenem consumption [21], mainly in developing countries, and

Table 2. Odds ratio and 95 % confidence interval (CI) for the susceptibility of *A. baumannii* to s antibiotics

Year	IP		MP		PTZ	
	OR [95 % CI]	p	OR [95 % CI]	p	OR [95 % CI]	P
2013	1	0.002	1	0.204	1	0.000
2014	0.593 [0.154–2.279]	0.446	0.864 [0.211–3.542]	0.839	1.2 [0.070–20.429]	0.900
2015	0.162 [0.039–0.666]	0.012	0	0.999	0.325 [0.027–3.959]	0.378
2016	0.078 [0.018–0.346]	0.001	0.170 [0.032–0.888]	0.036	0.004 [0.000–0.052]	0.000

PTZ, piperacillin-tazobactam; IP, imipenem; MP, meropenem.

Table 3. Susceptibility of *P. aeruginosa* to antibiotics

CAZ, ceftazidime (S MIC: <8; I MIC: 16; R MIC: >32); FEP, cefepime (S MIC: <8; I MIC: 16; R MIC: >32); AZT, aztreonam (S MIC: <8; I MIC: 16; R MIC: >32); PTZ, piperacillin-tazobactam (S MIC: <16/4; I MIC: 32/4-64/4; R MIC: >128/4); IP, imipenem (S MIC: <2; I MIC: 4; R MIC: >8); MP, meropenem (S MIC: <2; I MIC: 4; R MIC: >8); Cip, ciprofloxacin (S MIC: <1; I MIC: 2; R MIC: >4); AK, amikacin (S MIC: <16; I MIC: 32; R MIC: >64); COL, colistin (S MIC: <2; R MIC: >4).

Year	Interpretive categories	Results (%) (susceptible, intermediate or resistant strains/total strains										
		CAZ	FEP	AZT	PTZ	IP	MP	CIP	AK	COL	FOS*	FOS†
2013	Susceptible	89.2 (166/186)	83.9 (156/186)	2 (3/146)	90.3 (168/186)	82.3 (154/187)	81.1 (120/148)	63 (116/184)	62.1 (90/145)	78.2 (115/147)	13.4 (25/186)	86.6 (161/186)
	Intermediate	1.7 (3/186)	3.2 (6/186)	4.2 (6/146)	2.7 (5/186)	1.7 (3/187)	1.3 (2/148)	4.9 (9/184)	2 (3/145)	0 (0/147)		
	Resistant	9.1 (17/186)	12.9 (24/186)	93.8 (137/146)	7 (13/186)	16 (30/187)	17.6 (26/148)	32.1 (59/184)	35.9 (52/145)	21.8 (32/147)		
2014	Susceptible	88.5 (193/218)	76.6 (167/218)	0.5 (1/207)	88.5 (193/218)	85.1 (177/208)	84.5 (169/200)	56.9 (124/218)	49.2 (98/199)	93.5 (188/201)	25.2 (55/218)	74.8 (163/218)
	Intermediate	2.3 (5/218)	6.9 (15/218)	4.8 (10/207)	1.4 (3/218)	4.8 (10/208)	6.5 (13/200)	5.5 (12/218)	2.6 (5/199)	0 (0/201)		
	Resistant	9.2 (20/218)	16.5 (36/218)	94.7 (196/207)	10.1 (22/218)	10.1 (21/208)	9 (18/200)	37.6 (82/218)	48.2 (96/199)	1.5 (3/201)		
2015	Susceptible	90.7 (98/108)	75 (81/108)	1 (1/101)	88.9 (96/108)	78.7 (85/108)	75.9 (79/104)	57.4 (62/108)	61.9 (65/105)	99 (100/101)	42 (45/107)	57.9 (62/107)
	Intermediate	9.3 (10/108)	25 (27/108)	30.9 (31/101)	0 (0/108)	18.5 (20/108)	10.6 (11/104)	0.9 (1/108)	11.4 (12/105)	1 (1/101)		
	Resistant	0 (0/108)	0 (0/108)	68.1 (69/101)	11.1 (12/108)	2.8 (3/108)	13.5 (14/104)	41.7 (45/108)	26.7 (28/105)	0 (0/101)		
2016	Susceptible	79.1 (110/139)	89.9 (98/109)	1.7 (2/119)	74.8 (110/147)	89.1 (115/129)	81.8 (99/121)	58.1 (75/129)	76 (92/121)	99.1 (114/115)	48.4 (62/128)	51.6 (66/128)
	Intermediate	10.8 (15/139)	9.2 (10/109)	33.6 (40/119)	0.7 (1/147)	10.1 (13/129)	10.7 (13/121)	0.8 (1/129)	14.9 (18/121)	0.9 (1/115)		
	Resistant	10.1 (14/139)	0.9 (1/109)	64.7 (77/119)	24.5 (36/147)	0.8 (1/129)	7.7 (9/121)	41.1 (53/129)	9.1 (11/121)	0 (0/115)		
Total	Susceptible	87.1 (567/651)	80.7 (501/621)	1.2 (7/573)	86.0 (567/659)	84.0 (531/632)	81.5 (467/573)	59.0 (377/639)	60.5 (345/570)	91.7 (517/564)	29.3 (187/639)	70.7 (452/639)
	Intermediate	5.1 (33/651)	9.3 (58/621)	15.2 (87/573)	1.6 (9/659)	7.3 (46/632)	6.8 (39/573)	3.6 (23/639)	6.7 (38/570)	0.3 (2/564)		
	Resistant	7.8 (51/651)	10 (62/621)	83.6 (479/573)	12.6 (83/659)	8.7 (55/632)	11.7 (67/573)	37,4 (239/639)	32.8 (187/570)	8.0 (45/564)		

*FOS, osfomycin MIC $\leq$16 mg l^{-1}.

†FOS, fosfomycin MIC $\geq$64 mg l^{-1}.

Table 4. Odds ratio and 95 % confidence interval (CI) for the susceptibility of *P. aeruginosa* to selected antibiotics

Year	PTZ		MP		IP	
	OR IC 95 %	p	OR IC 95 %	p	OR IC 95 %	p
2013	1	0.000	1	0.348	1	0.154
2014	0.827 [0.436–1.569]	0.561	1.272 [0.725–2.231]	0.401	1.224 [0.716–2.091]	0.460
2015	0.857 [0.396–1.855]	0.696	0.737 [0.401–1.356]	0.327	0.792 [0.437–1.435]	0.442
2016	0.319 [0.173–0.588]	0.000	1.050 [0.566–1.949]	0.877	1.760 [0.901–3.440]	0.098

PTZ, piperacillin-tazobactam; IP, imipenem; MP, meropenem; AK, amikacin.

the accompanying rise in carbapenem-resistant Gram-negative microorganisms has become a worldwide healthcare challenge within a short time period.

It is therefore essential to monitor the *A. baumannii* susceptibility profile in the local setting and to control the administration of antibiotics for its treatment. The goal is to reverse the current situation and prevent the spread of these strains, especially in hospital departments with more vulnerable patients (e.g. intensive care units or haematology departments).

Exposure to certain antibiotics may lead to the acquisition of resistance mechanisms by potential pathogens that have already colonized patients, and their eradication poses a major challenge. Cephalosporins and quinolones have long been extensively administered as the empirical treatment of choice in ICUs, with few restrictions [22, 23], which may explain the frequent finding in critically ill patients of high resistance rates for microorganisms such as *A. baumannii* and *P. aeruginosa*. The present results indicate that ciprofloxacin is no longer an appropriate therapeutic option for *A. baumannii*, which showed a susceptibility below 10 % throughout the study period (0% in 2015), or for *P. aeruginosa,* which showed a susceptibility below 60 % from 2014. With respect to cephalosporins, the susceptibility of *A. baumannii* was always <20 % for ceftazidime and cefepime, and no susceptible isolates were recorded in 2015. We also highlight the low percentage of *P. aeruginosa* isolates that were susceptible to cephalosporins in 2016 (79.1 % for ceftazidime and 75.1 % for cefepime), with ceftazidime having previously been considered the anti-Pseudomonas cephalosporin of choice [24]. Accordingly, conventional treatments of *P. aeruginosa* can no longer be considered appropriate in our hospital, given that resistant rates exceed 20 %. Ceftolozane is a new cephalosporin with high anti-Pseudomonas activity that can be inactivated by β-lactamase enzymes but is formulated with tazobactam, improving the range of action against Gram-negative microorganisms, including multi-resistant strains. In December 2014, the USA Food and Drug Administration (FDA) approved its administration for the treatment of complicated UTIs [25, 26].

Table 5. Consumption (DDD/1000 patient-days) of imipenem, meropenem and piperacillin-tazobactam between 2014 and 2016

Year	Imipenem	Meropenem	Piperacillin-tazobactam
2014	17.41	80.77	35.42
2015	14.96	79.29	43.97
2016	15.08	71.31	49.09

According to the present findings, although the susceptibility of *P. aeruginosa* to colistin has been >99 % over the past few years, it was lower in 2013 and 2014. Polymyxin resistance in *P. aeruginosa* is mainly due to disorders in transmembrane transporters [27, 28]. A reduction in the susceptibility of *A. baumanii* to colistin was recorded in 2016 due to the appearance of a pan-resistant isolate. In 2011, the percentage of colistin-resistant *A. baumannii* was 3.3 % worldwide, but there were considerable differences among countries. Thus, the percentage of isolates of this microorganism that were polymyxin-resistant was reported in 2012 to be 6.4 % in Canada but 3.9 % in Europe, largely in isolates from Greece and Italy. We highlight the alarming presence in Greece of *A. baumannii* resistant to both colistin and carbapenems, as observed in 2016 in the present study. Polymyxin resistance to this microorganism is mainly due to mutations in *pmrA* and *pmrB* genes [27].

During 2015, *A. baumannii* was susceptible only to amikacin, IP, PTZ and colistin in our hospital, with the latter being active against all isolates. This high resistance rate resulted from an outbreak produced by a multi-resistant strain of *A. baumannii* in the ICU, against which colistin was the only antibiotic with activity. In 2016, with the transfer of ICU trauma patients to a new building and the implementation of preventive procedures, including the active declaration of colonized patients [22], the frequency of multi-resistant *A. baumannii* appears to have reduced.

In women with non-complicated UTI, the recommended treatment is usually a single dose of fosfomycin, which is highly active against Gram-negative microorganisms, although the MIC was below 64 mg μl^{-1} in most isolates of *A. baumannii* and *P. aeruginosa*. In general, UTIs caused by these microorganisms are often complicated because they affect hospitalized or high-risk patients; however, although fosfomycin can be useful in treating cystitis when the MIC is <32 mg l^{-1}, it does not appear to be an appropriate empirical treatment for these microorganisms in our setting,

especially for *A. baumannii*, which had an MIC for fosfomycin >64 mg l^{-1} for all isolates recorded in 2016.

The main study limitation was the lack of data on the consumption of all antibiotics, mainly for 2013, or on the functional status of the patients.

Catheter use is associated with colonization by antimicrobial-resistant organisms, but we found no differences in the distribution of catheterized individuals among the years under study. Finally, the high resistance rates in *A. baumannii* and *P. aeruginosa* observed in our study suggest the need to improve surveillance protocols to avoid the spread of these multi-resistant bacteria in our hospital.

In conclusion, in our setting, it is no longer possible to use carbapenems and piperacillin-tazobactam for empirical treatment of UTIs due to *A. baumannii* or to use piperacillin/tazobactam for empirical treatment of UTIs due to *P. aeruginosa*. Colistin was the most active antibiotic *in vitro*. There was a marked increase in the consumption of piperacillin-tazobactam.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This was a non-interventional study with no investigation additional to routine procedures. Biological material was used only for standard urinary infection diagnostics following physicians' prescriptions. No additional sampling or modification of the routine sampling protocol was performed.

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