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Trends in *Pseudomonas aeruginosa* Antibiotic Resistance: Case Study from a Portuguese Central Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Author SGP designed the study, created the database, performed part of the statistical analysis, wrote the manuscript and managed its publication. Author MM performed and author JP designed and supervised the statistical analysis. Author OC managed the data collection, collaborated in the study design and reviewed the manuscript prior to submission. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *P. aeruginosa* antibiotic resistance is increasing worldwide, greatly limiting therapeutic options, and is mainly associated to nosocomial infections, relating to high morbidity and mortality outcomes. Frequently updated regional guidelines, supported in statistically valid longitudinal information, are mandatory.
Methodology: Resistance to 11 antibiotics used to treat *P. aeruginosa* infections were surveyed in clinical isolates from a Portuguese central during 10 years (n=3778) using the MicroScan WalkAway system. Statistical analysis (Mann-Whitney tests and regression modeling) were used to determine its time evolution according to origin, specimen samples and hospital wards.

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Results: Total resistance rates fluctuated between 77.9% (ciprofloxacin), and 28.6% (amikacin and piperacillin with tazobactam). Statistical analysis revealed an increase over time for the majority of the resistance rates according to origin, specimen samples and wards of collection. Several trends were best fitted to positive power or quadratic regression models, predicting even higher overall resistance rates in the near future. Few resistance trends were best fitted to negative models, indicative of a possible decrease in the future, which is positive but reflects the discrepant success of empirical antibiotic prescription regimens in different wards from the same hospital.
Conclusion: These results, apart from indicating that the studied hospital can significantly improve its prescription policy, prove the importance of specific and local longitudinal studies of resistance trends over time to further drug prescription strategies. Similar biostatistic analysis should be performed in other hospitals and regarding other pathogens to broaden this awareness, necessary for the improvement of empirical antibiotherapy regimens.

Keywords: Resistance; antibiotics; surveillance; Pseudomonas aeruginosa; biostatistics; logistic regression.

1. INTRODUCTION

The discovery of antibiotics in the beginning of the 20th century is considered one of the most outstanding achievements in modern medicine. However, its usage was accompanied, since the beginning, by the emergence of resistant strains [1]. There are several types of antibiotics, classified according to their structure and activities but, for each one of them, bacteria may present one or more mechanisms that allow them to resist and survive their activity. Three groups of antibiotics are more currently used against *Pseudomonas aeruginosa*: beta-lactams, aminoglycosides and fluoroquinolones. This bacterium resist to several of these agents, and others, mainly due to its outer membrane low permeability, constitutive expression of efflux pumps and production of beta-lactamases [2,3,4] along with a great ability to acquire resistance genes [5].

P. aeruginosa is the fifth most relevant pathogen worldwide, the second most frequent pathogen in nosocomial pneumonia, the third in urinary infections, the fourth in post-surgical infections and the seventh in sepsis [3,6]. It is the leading cause of morbidity and mortality in patients with ventilator-acquired pneumonia and cystic fibrosis [7,8] and the most frequently found Gram-negative bacteria in pediatric patients hospitalized in intensive care units (ICU) [3]. In European ICUs, *P. aeruginosa* is considered the second most frequent pathogen, responsible for 30% of all pneumonias, 19% of all urinary infections and 10% of all sepsis [3]. Outbreaks of *P. aeruginosa* nosocomial infections are frequently reported and mainly associated to the incorrect use of medical devices [9,10] and cross-contamination. Community infections caused by *P. aeruginosa* are also frequent, mainly folliculitis and otitis, associated to water recreational usage [11], and keratitis, related to the use of contact lenses [12]. Pneumonias caused by *P. aeruginosa* have a 7% incidence in community [13] and also 90% of all endocarditis and osteomyelitis in drug-addicts [6,14].

Epidemiological surveillance of resistance is an important measure for the clinical management of antipseudomonal chemotherapy locally, as divergence in the susceptibility patterns of *P. aeruginosa* isolates over time and from different geographical locations and hospital environments are commonly known. Therefore, it is very important that physicians have updated epidemiological information for the most significant pathogens in their hospital and, if possible, considering the different wards and biological products from where the isolates are collected, in order to better adequate the empirical antibiotherapy regimens to the specific reality of their practices.

We studied the evolution of *P. aeruginosa* antibiotic susceptibility in a Portuguese central hospital during the last 10 years and predicted the trend of the resistance patterns according to their origin, specimens sample and wards of collection. With this, we intend not only to provide information about the success of antibiotic prescription policies applied in different circumstances within the same hospital over time, but also to suggest similar data analysis approaches, useful to the infection control committees of health care facilities.

2. MATERIALS AND METHODS

2.1 Study Setting and Clinical Isolates

The studied hospital is a 600 bed cluster tertiary care hospital constituted by a main central building hospital (CH), a pediatric hospital (PH), a maternity hospital (MH) and a secondary building (SH) located 30 km far. Each building is divided in several wards, to admit inpatients, and have an Emergency room (ER) and External Consult Unit (ECU), to attend outpatients.

All *P. aeruginosa* isolates collected from inpatients and outpatients of CH, PH, MH or SH between May 2003 and April 2013 were preserved for the study, in a total of 3778 isolates. 60.5% (n=2280) were from inpatients from the 4 units and 39.5% (n=1498) from outpatients of CH and PH. Isolates obtained from an inpatient after 3 days hospitalization or from outpatients attending ER or ECU with a medical history of recent hospitalization (<3 days) were classified as nosocomial. Otherwise were considered of ambulatory origin.

2.2 Antimicrobial Susceptibility Testing

Bacterial identification and antibiotic susceptibility was performed with MicroScan WalkAway (Dade Behring, Sacramento, CA), testing the susceptibility to the carbapenems imipenem (IP) and meropenem (MP), the cephalosporins ceftazidime (CAZ) and cefepime (FEP), the monobactam aztreonam (AZT), the penicillin piperacillin (PRL) and piperacillin with tazobactam (TZP), the aminoglycosides amikacin (AMK), gentamicin (GM) and tobramycin (NN) and the fluoroquinolone ciprofloxacin (CIP). Results were interpreted according to Clinical Laboratory Standards Institute (CLSI) recommendations [15], with intermediate results considered resistant.

2.3 Data Analysis

Data were analyzed using IBM SPSS® Statistics for Windows version 21.0 (IBM Corp., Armonk, NY). Specimen samples were grouped in respiratory isolates (sputum and aspirates), exudates, urine and others (blood, feces and others). CH and PH wards were grouped according to their location and overall numbers, so that each variable had at least 50 observations. CH wards were grouped into 8 categories (medicine, pneumology, neurology+neurosurgery, surgery, ICU, infectious diseases, ER+ECU, others). PH wards were grouped into 3 categories (medicine, ICU, others).

Antibiotic resistance distribution over time and according to origin (ambulatory vs nosocomial) were analyzed by non-parametric Mann-Whitney tests with a significance level of 0.05, after assessing the non-normality of the data with the Kolmogorov-Smirnov test, for each group. Similar analyzes were performed considering only the nosocomial isolates, divided according to the specimens source and wards of origin. If statistical significant results were obtained regarding a grouped category, exploratory multiple regression modeling was performed using 3 models (linear, quadratic and power – Bonferroni correction for multiple comparisons with a factor of 3 was applied), to observe the time evolution trend of antibiotic resistance in each subgroup. Whenever more than one regression model was statistically significant, the best fit model was selected according to the lower p-value and the higher R^2 value.

3. RESULTS

3.1 Study Population and Clinical Isolates

In the majority of the years, 300 to 400 isolates were collected. In 2008 and 2009, more than 500 isolates were collected (535 and 518, respectively), and in the last year only 216 isolates were obtained. Table 1 presents the distribution of all isolates according to the patient's gender and age, specimen source, hospitals and respective wards, highlighting similar information regarding the nosocomial isolates.

During the 10 years period, CIP was the antibiotic with the highest overall resistance rate (77.9%), followed by GM (59.5%) and FEP (52.8%). AMK and TZP were the antibiotics with the lowest overall resistance rate (28.6%), followed by MP (30.4%) and PIP (38.7%). All other antibiotics presented overall resistance rates between 39% and 51%. Fig. 1 presents the 10 years resistance rates discriminated between nosocomial and ambulatory isolates. Only CIP and GM presented resistance rates in the ambulatory isolates superior to 20% (30.3% and 21.7%, respectively), and all others between 13.9% (FEP) and 5.1% (MP).

3.2 Total Resistance Rates Distribution and Evolution over Time

Statistical significant differences in the resistance rates over time were observed for all antibiotics (*P*<.001) except GM (*P*=.21). A linear model with positive trend (Fig. 2a) was the best fit model for the time evolution of MP, PIP and NN resistance and a power model with positive trend (Fig. 2b) for CIP (Tables 2, 3 and 4). IP, CAZ and FEP resistance evolution were best fitted to a negative quadratic model (Fig. 2c) (Table 2). TZP, AZT and AMK resistance time evolution was not fitted to none of the three tested models (Tables 3 and 4).

When analyzing the population according to origin, nosocomial or ambulatory, similar trends of resistance evolution was observed, although some models were best fitted to different regression equations than those obtained in the overall analysis, particularly IP resistance in ambulatory isolates, whose time evolution was best fitted to a quadratic model with a positive trend (Fig. 2d). In ambulatory isolates, GM resistance rates evolution along the 10 years survey was statistically significant (*P*=.03), while remaining not statistically valid for nosocomial isolates (Table 4).

Table 1. Distribution of *P. aeruginosa* isolates collected from May 2003 to April 2013 in a Portuguese Central Hospital, according to patient gender and age, biological products, hospitals and respective wards, with discrimination of nosocomial isolates

	Total	Nosocomial
	n (%) (N=3778)	n (%) (N=2280)
Patient gender		
Male	2273 (60.2)	1458 (63.9)
Female	1505 (39.8)	822 (36.1)
Patient age		
<=18 years	665 (17.6)	230 (10.1)
18<years<65	884 (23.4)	603 (26.4)
>=65 years	2229 (59)	1447 (63.5)
Specimen samples		
Respiratory	1777 (47)	1215 (53.3)
Urine	1082 (28.7)	486 (21.3)
Exudates	609 (16.1)	406 (17.8)
Others	310 (8.2)	173 (7.6)
Hospitals		
Central	2806 (74.3)	1727 (75.7)
Pediatric	637 (16.8)	226 (9.9)
Maternity	10 (0.3)	6 (0.3)
Secondary	325 (8.6)	321 (14.1)
CH wards	*(N=2806)*	*(N=1727)*
Medicine	450 (16)	393 (22.8)
Pneumology	272 (9.7)	222 (12.8)
Neurology+Neurosurgery	372 (13.3)	357 (20.7)
Surgery	156 (5.6)	123 (7.1)
ICU	107 (3.8)	104 (6)
Infectious diseases	222 (7.9)	198 (11.5)
ER+ECU	909 (32.4)	78 (4.5)*
Others	318 (11.3)	252 (14.6)
PH wards	*(N=637)*	*(N=226)*
Medicine	130 (20.4)	98 (43.4)
ICU	69 (10.8)	61 (27)
Others	438 (68.8)	67 (29.6)
SH wards	*(N=325)*	*(N=321)*
Medicine	322 (99.1)	320 (99.7)
Others	3 (0.9)	1 (0.3)

*CH – Central hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External consult unit; PH – Pediatric hospital; SH – Secondary hospital; * Not considered in the retrospective study*

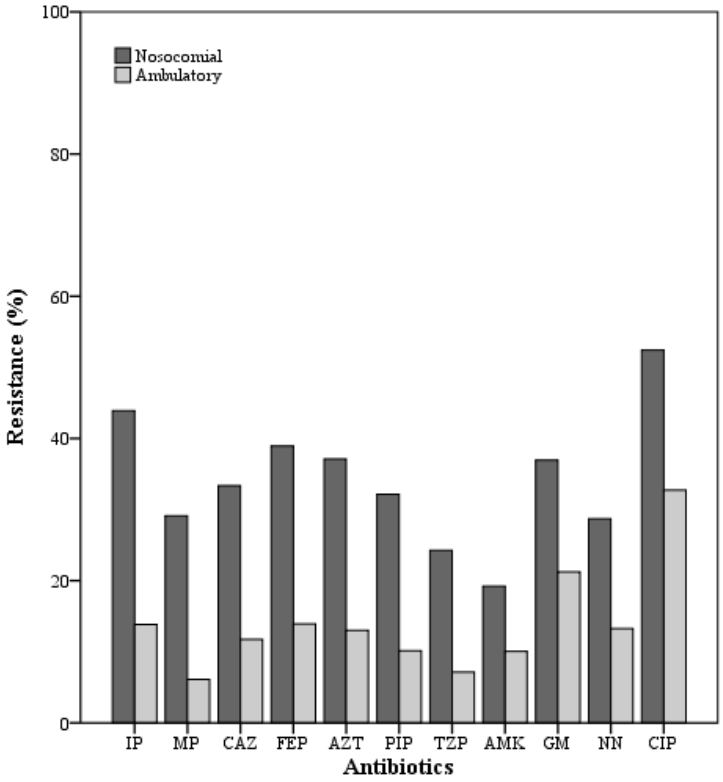

Fig. 1. Resistance rate (%) of total *P. aeruginosa* clinical isolates, discriminated according to origin (nosocomial or ambulatory)

3.3 Nosocomial Isolates Resistance Rates Distribution and Evolution over Time

Resistance rates of nosocomial isolates were studied according to the specimen source and wards of origin from the CH, PH and SH hospitals. The number of isolates collected from Maternity Hospital was not enough to allow a robust statistical analysis (Table 1). Figs. 3 and 4 present the resistance rates of each tested antibiotic according to specimen sample and wards of origin, respectively. As a reflex of its higher overall incidence, CIP was the antibiotic with higher resistance rate in all specimen samples (Fig. 3).

Similar results were observed when considering the wards of origin in CH, except in neurology+neurosurgery and ICU, where IP resistance was more prevalent (Fig. 4). Regarding PH wards, aminoglycosides resistance was more prevalent in medicine ward and IP in ICU and others wards (Fig. 4). In SH Medicine ward, CIP was the antibiotic with higher resistance rate and TZP with the lowest (Fig. 4).

Regarding specimen source, Mann-Whitney test showed statistical significant differences over time (P <0.05) on the resistance rates of all tested antibiotics in *P. aeruginosa* isolates from respiratory samples, except for GM (P=.16). Other specimen source had lower resistance rates and respective statistically significant association of its evolution over time. Tables 2 - 6 present the Mann-Whitney test p-value and the best regression fit models for each case. In the respiratory isolates, resistance to all antibiotics increased (positive trends) by power, quadratic or linear models. Similar trends were observed when considering exudates and only the urine isolates presented regression models with negative trends: IP, PIP and NN. Other samples group did not show statistical significant association with time considering the majority of the tested antibiotics. IP evolved by a power positive equation. NN, although with a statistically significant (P=.02) association, it was not explained by any of the 3 tested regression models.

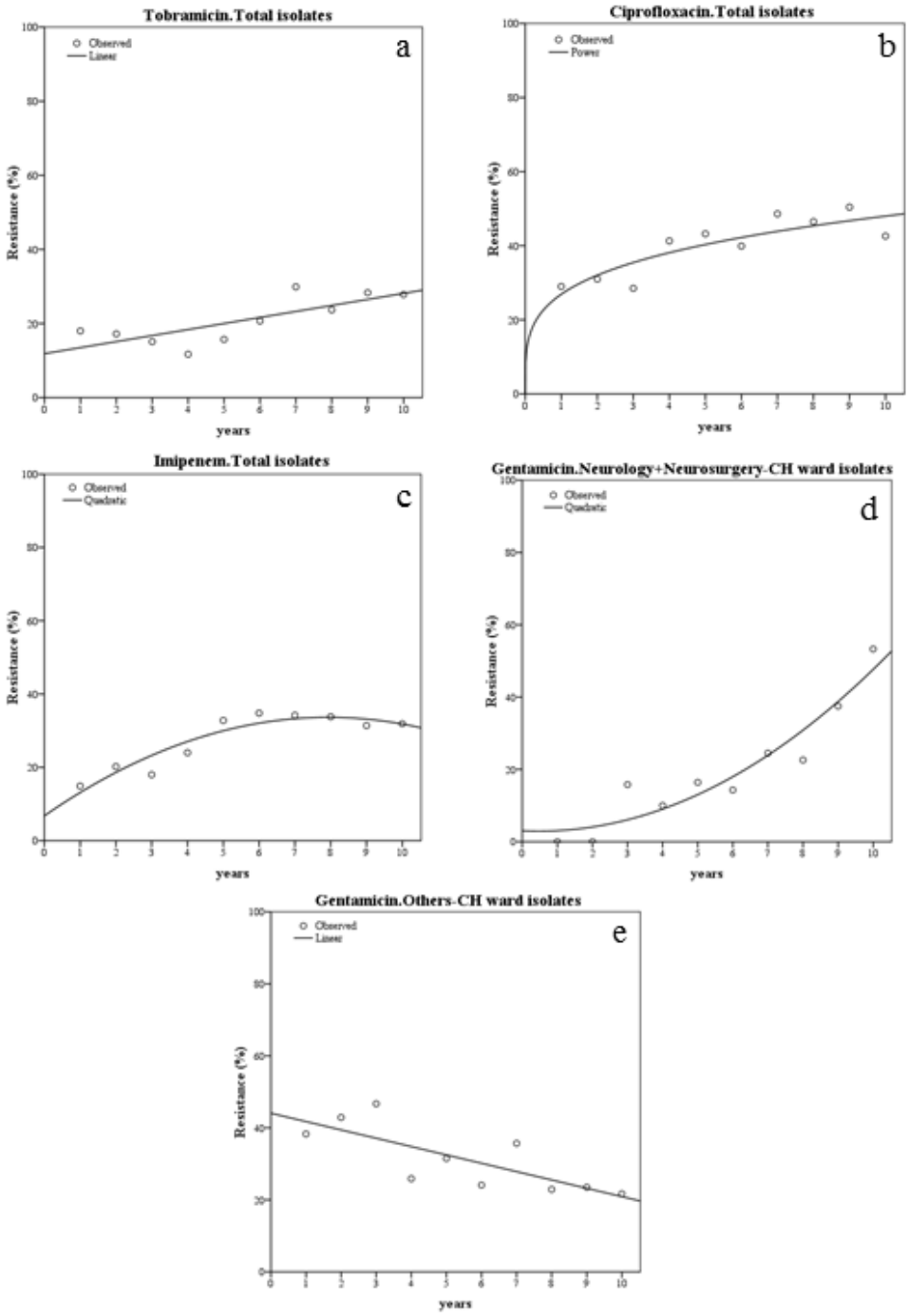

Fig. 2. Examples of the different obtained regression models in the study: a) Linear model with positive trend (evolution of tobramycin resistance in overall isolates); b) Power model with positive trend (evolution of ciprofloxacin resistance in overall isolates); c) Quadratic model with negative trend (evolution of imipenem resistance in overall isolates); d) Quadratic model with positive trend (evolution of gentamycin resistance in neurology+neurosurgery wards from Central Hospital); e) Linear model with negative trend (evolution of gentamycin resistance in others ward from Central Hospital)

Table 2. Mann-Whitney tests results (p-values) and respective best fit regression model for *P. aeruginosa* isolates carbapenem resistance time evolution. Statistical significant p-values (*P* <.05) of Mann-Whitney tests are highlighted in bold. Italicized regression models indicate those whose significance failed to survive the Bonferroni correction

	Imipenem					**Meropenem**				
	MW test	**Regression modeling**				**MW test**	**Regression modeling**			
	p-value	**Best fit**	**Trend**	**R^2**	**p-value***	**p-value**	**Best fit**	**Trend**	**R^2**	**p-value***
Total	**<.001**	Quadratic	Negative	0.879	.003	**<.001**	Linear	Positive	0.579	.03
Ambulatory	**<.001**	Quadratic	Positive	0.832	.006	**<.001**	Linear	Positive	0.668	.008
Nosocomial	**<.001**	Quadratic	Negative	0.902	<.001	**<.001**	*Power*	*Positive*	*0.572*	*.09 (.03)*
Biological products										
Respiratory	**<.001**	Power	Positive	0.891	<.001	**<.001**	*Power*	*Positive*	*0.526*	*.05 (.02)*
Exudates	.88	---	---	---	---	.82	---	---	---	---
Urine	**<.001**	Quadratic	Negative	0.855	.003	**<.001**	Power	Positive	0.616	.02
Others	**.02**	Power	Positive	0.839	<.001	.15	---	---	---	---
Wards CH										
Medicine	**<.001**	Quadratic	Negative	0.765	.02	**<.001**	n.d.	n.d.	n.d.	n.d.
Pneumology	**<.001**	Power	Positive	0.555	.04	**<.001**	Linear	Positive	0.581	.03
Neurology+neurosurgery	**.046**	Power	Positive	0.695	.009	.25	---	---	---	---
Surgery	.48	---	---	---	---	.99	---	---	---	---
ICU	.74	---	---	---	---	.28	---	---	---	---
Infectious diseases	**<.001**	Power	Positive	0.767	.003	**.002**	*Quadratic*	*Negative*	*0.617*	*.11 (.04)*
Others	.55	---	---	---	---	.17	---	---	---	---
Wards PH										
Medicine	**.012**	Power	Positive	0.571	.03	.51	---	---	---	---
ICU	.19	---	---	---	---	**.004**	*Linear*	*Positive*	*0.527*	*.051 (.02)*
Others	.64	---	---	---	---	.97	---	---	---	---
Wards SH										
Medicine	.06	---	---	---	---	**.008**	n.d.	n.d.	n.d.	n.d.

*MW – Mann-Whitney; CH – Central Hospital; PH – Pediatric Hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External Consult Unit; n.d. – n.d. – regression model not determined (none of the tested models were statistically significant, even when considering the uncorrected p-value). * - P-values are presented with Bonferroni correction when this retained significance – when significance was lost after correction, the uncorrected values are shown in parenthesis*

Table 3. Mann-Whitney tests results (p-values) and respective best fit regression model for *P. aeruginosa* isolates cephalosporines resistance time evolution. Statistical significant p-values (p<0.05) of Mann-Whitney tests are highlighted in bold. Italicized regression models indicate those whose significance failed to survive the Bonferroni correction

	Ceftazidime					Cefepime				
	MW test	Regression modeling				MW test	Regression modeling			
	p-value	Best fit	Trend	R^2	p-value*	p-value	Best fit	Trend	R^2	p-value*
Total	**<.001**	Quadratic	Negative	0.731	.03	**<.001**	Quadratic	Negative	0.891	.012
Ambulatory	**<.001**	Power	Positive	0.636	.02	**<.001**	Linear	Positive	0.668	.008
Nosocomial	**<.001**	Quadratic	Negative	0.748	.02	**<.001**	Linear	Positive	0.834	.006
Biological products										
Respiratory	**<.001**	Power	Positive	0.829	.003	**<.001**	Power	Positive	0.707	.03
Exudates	**.03**	*Linear*	*Positive*	*0.518*	*.06 (.02)*	.23	---	---	---	---
Urine	.27	---	---	---	---	**<.001**	Power	Positive	0.725	.02
Others	.32	---	---	---	---	.46	---	---	---	---
Wards CH										
Medicine	**<.001**	*Power*	*Positive*	*0.436*	*.11 (.04)*	**.02**	n.d.	n.d.	n.d.	n.d.
Pneumology	.07	---	---	---	---	**<.001**	Power	Positive	0.704	.03
Neurology+neurosurgery	.62	---	---	---	---	.26	---	---	---	---
Surgery	**.047**	n.d.	n.d.	n.d.	n.d.	**.003**	Linear	Positive	0.746	.02
ICU	.26	---	---	---	---	**.03**	Linear	Positive	0.782	.012
Infectious diseases	**<.001**	Quadratic	Negative	0.727	.03	**<.001**	Quadratic	Negative	0.935	.003
Others	.15	---	---	---	---	.28	---	---	---	---
Wards PH										
Medicine	.08	---	---	---	---	.67	---	---	---	---
ICU	.86	---	---	---	---	**.01**	n.d.	n.d.	n.d.	n.d.
Others	.96	---	---	---	---	.62	---	---	---	---
Wards SH										
Medicine	**.004**	*Linear*	*Positive*	*0.514*	*.09 (.03)*	**.02**	*Linear*	*Positive*	*0.514*	*.11 (.04)*

*MW – Mann-Whitney; CH – Central Hospital; PH – Pediatric Hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External Consult Unit; n.d. – n.d. – regression model not determined (none of the tested models were statistically significant, even when considering the uncorrected p-value). * - P-values are presented with Bonferroni correction when this retained significance – when significance was lost after correction, the uncorrected values are shown in parenthesis*

Table 4. Mann-Whitney tests results (p-values) and respective best fit regression model for *P. aeruginosa* isolates penicillin resistance time evolution. Statistical significant p-values (p<0.05) of Mann-Whitney tests are highlighted in bold. Italicized regression models indicate those whose significance failed to survive the Bonferroni correction.

	Piperacillin					Piperacillin and Tazobactam				
	MW test	Regression modeling				MW test	Regression modeling			
	p-value	Best fit	Trend	R^2	p-value*	p-value	Best fit	Trend	R^2	p-value*
Total	**<.001**	Linear	Positive	0.559	.039	**<.001**	n.d.	n.d.	n.d.	n.d.
Ambulatory	**.002**	n.d.	n.d.	n.d.	n.d.	**<.001**	*Linear*	*Positive*	*0.485*	*.075 (.025)*
Nosocomial	**<.001**	Linear	Positive	0.752	.003	**<.001**	*Linear*	*Positive*	*0.411*	*.14 (.046)*
Biological products										
Respiratory	**<.001**	Linear	Positive	0.595	.03	**<.001**	n.d.	n.d.	n.d.	n.d.
Exudates	**<.001**	Power	Positive	0.693	.009	**.011**	Linear	Positive	0.696	.009
Urine	**.002**	*Quadratic*	*Negative*	*0.656*	*.07 (.02)*	.15	---	---	---	---
Others	.12	---	---	---	---	.87	---	---	---	---
Wards CH										
Medicine	**<.001**	Quadratic	Negative	0.761	.02	**.012**	Power	Positive	0.580	.03
Pneumology	**<.001**	Linear	Positive	0.576	.03	**.001**	n.d.	n.d.	n.d.	n.d.
Neurology+neurosurgery	**.02**	n.d.	n.d.	n.d.	n.d.	.06	---	---	---	---
Surgery	**<.001**	Quadratic	Positive	0.806	.009	**.012**	n.d.	n.d.	n.d.	n.d.
ICU	**.04**	*Linear*	*Positive*	*0.514*	*.09 (.03)*	**.03**	Linear	Positive	0.633	.03
Infectious diseases	**<.001**	*Quadratic*	*Negative*	*0.648*	*.078 (.026)*	**<.001**	n.d.	n.d.	n.d.	n.d.
Others	.22	---	---	---	---	.52	---	---	---	---
Wards PH										
Medicine	.50	---	---	---	---	**.048**	n.d.	n.d.	n.d.	n.d.
ICU	.15	---	---	---	---	.36	---	---	---	---
Others	.07	---	---	---	---	.39	---	---	---	---
Wards PH										
Medicine	.99	---	---	---	---	.45	---	---	---	---

*MW – Mann-Whitney; CH – Central Hospital; PH – Pediatric Hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External Consult Unit; n.d. – n.d. – regression model not determined (none of the tested models were statistically significant, even when considering the uncorrected p-value). * - P-values are presented with Bonferroni correction when this retained significance – when significance was lost after correction, the uncorrected values are shown in parenthesis*

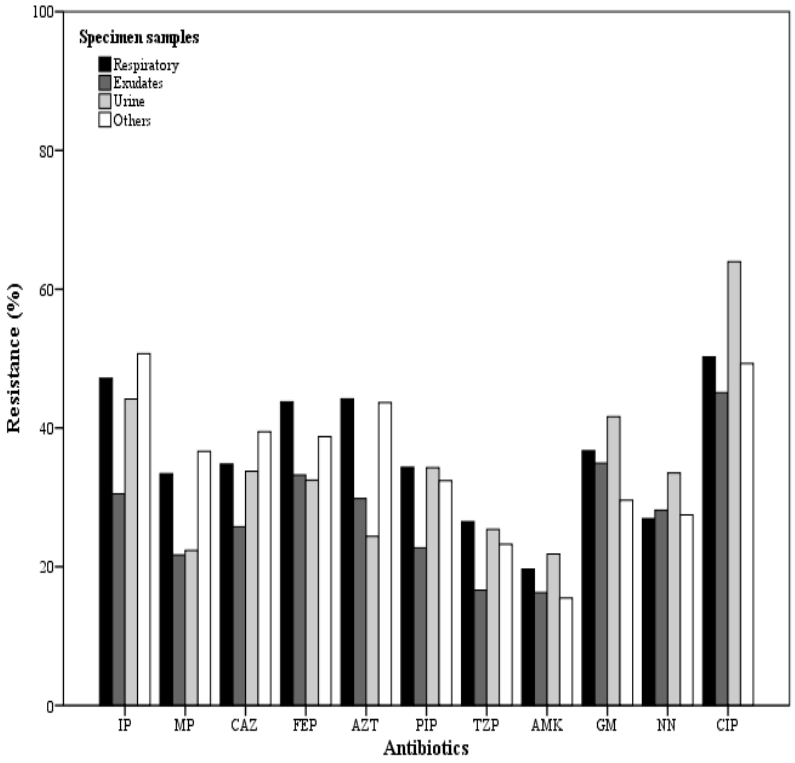

Fig. 3. Resistance rate (%) of total *P. aeruginosa* clinical isolates, discriminated according to specimen samples

When analyzing the time evolution of resistance according to CH wards, all the 11 tested antibiotics resistance rates were statistically associated to time in the isolates collected from the infectious diseases ward ($P<0.05$) and in medicine and pneumology wards only one was not: GM in medicine ward, CAZ in pneumology ward. ICU was the ward with fewer statistical significant associations of antibiotic resistance and time. It was observed a total of 46 statistically significant associations, with 22 best fitted to regression models with positive trends, 8 with negative trends and 16 not described by any of the 3 used models. PIP and CIP were the antibiotics with more associations to CH wards and MP, CAZ, GM and NN the least (Tables 2 - 6). In PH wards, medicine and ICU wards presented 4 statistically significant associations to time for IP, TZP, AMK and GM, and MP, FEP, AZT and GM, respectively, with positive trends, best fitted to power or linear models, except GM in ICU, which best fitted to a negative power model (Fig. 2e). Four statistically significant associations were not described by any of the 3 used models (Tables 2 - 6). Finally, in SH medicine ward, it was observed linear positive trends of CAZ and FEP resistance time and MP, CIP and NN resistance evolution were not described by any of the three tested regression models. All other resistance rates did not presented statistical significant association with time (Tables 2 - 6).

4. DISCUSSION

Antibiotic resistance in *P. aeruginosa* is increasing all over the world. Infections caused by resistant *P. aeruginosa* are related to an increase in mortality and morbidity rates, along with an increase in hospital-stay and chronic care [16]. In the studied hospital high rates of antibiotic resistance and different trends of evolution over time according to different wards and specimen samples were observed. The majority presented positive trends of evolution, reflecting the rapid increase of resistance rates, which is concerning. However, IP and CAZ resistance rates in overall and nosocomial isolates showed negative trends, as well as FEP in overall isolates, possibly indicating a higher effort in controlling the use of these antibiotics in the hospital in study.

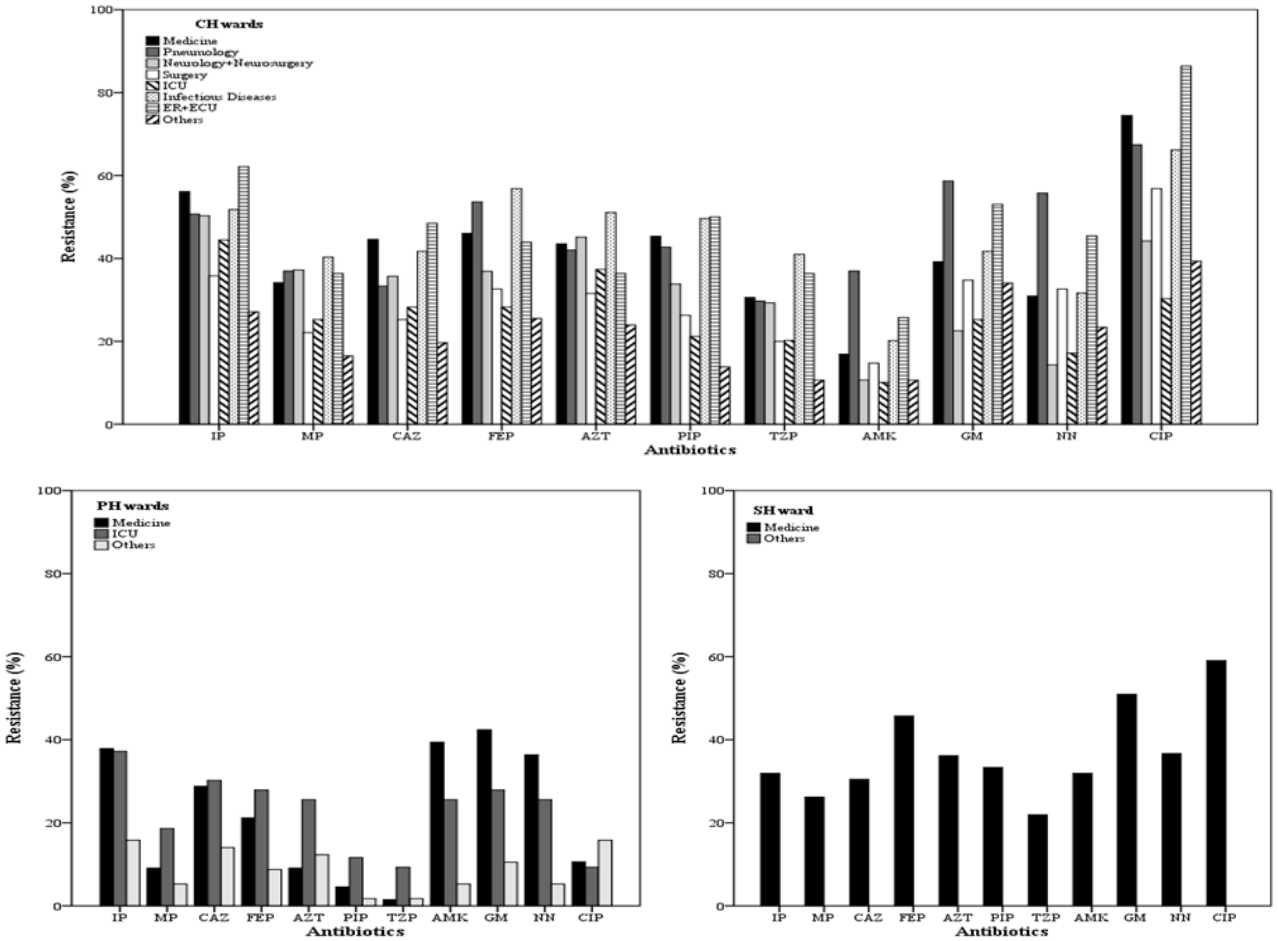

Fig. 4. Resistance rate (%) of nosocomial *P. aeruginosa* clinical isolates, discriminated according to wards of origin: a) Central Hospital (CH); b) Pediatric Hospital (PH); c) Secondary Hospital (SH)

Table 5. Mann-Whitney tests results (p-values) and respective best fit regression model for *P. aeruginosa* isolates aminoglycoside resistance time evolution. Statistical significant p-values (p<0.05) of Mann-Whitney tests are highlighted in bold. Italicized regression models indicate those whose significance failed to survive the Bonferroni correction

	Amikacin					**Gentamycin**					**Tobramycin**				
	MW	**Regression modeling**				**MW**	**Regression modeling**				**MW**	**Regression modeling**			
	p-value	**Best fit**	**Trend**	**R^2**	**p-value***	**p-value**	**Best fit**	**Trend**	**R^2**	**p-value***	**p-value**	**Best fit**	**Trend**	**R^2**	**p-value***
Total	**<.001**	n.d.	n.d.	n.d.	n.d.	.21	---	---	---	---	**<.001**	Linear	Positive	0.609	.02
Ambulatory	**.001**	n.d.	n.d.	n.d.	n.d.	**.03**	n.d.	n.d.	n.d.	n.d.	**<.001**	Linear	Positive	0.583	.03
Nosocomial	**.001**	*Quadratic*	*Negative*	*0.583*	*.14 (.047)*	.21	*Power*	*Positive*	*0.572*	*.09 (.03)*	**<.001**	Quadratic	Positive	0.728	.03
Biol. products															
Respiratory	**.004**	Quadratic	Positive	0.769	.02	.16	---	---	---	---	**.001**	Quadratic	Positive	0.723	.03
Exudates	.17	---	---	---	---	.51	---	---	---	---	.07	---	---	---	---
Urine	.54	---	---	---	---	.88	---	---	---	---	**<.001**	Quadratic	Negative	0.794	.012
Others	.11	---	---	---	---	.14	---	---	---	---	**.02**	n.d.	n.d.	n.d.	n.d.
Wards CH															
Medicine	**.02**	n.d.	n.d.	n.d.	n.d.	.15	---	---	---	---	**<.001**	Linear	Positive	0.613	.02
Pneumology	**.014**	*Quadratic*	*Positive*	*0.586*	*.14 (.046)*	**.003**	n.d.	n.d.	n.d.	n.d.	**<.001**	n.d.	n.d.	n.d.	n.d.
Neuros(l+s)	**.004**	*Linear*	*Positive*	*0.440*	*.11 (0.04)*	**<.001**	Quadratic	Positive	0.897	<.001	**<.001**	Quadratic	Positive	0.800	.012
Surgery	.12	---	---	---	---	.45	---	---	---	---	.29	---	---	---	---
ICU	.97	---	---	---	---	.46	---	---	---	---	.08	---	---	---	---
Inf. diseases	**.04**	n.d.	n.d.	n.d.	n.d.	.20	---	---	---	---	**.003**	n.d.	n.d.	n.d.	n.d.
Others	.65	---	---	---	---	**.02**	Linear	Negative	0.594	.03	.36	---	---	---	---
Wards PH															
Medicine	**.005**	Power	Positive	0.597	.03	**.006**	*Power*	*Positive*	*0.526*	*.054 (.02)*	.054	---	---	---	---
ICU	.47	---	---	---	---	**.016**	*Linear*	*Negative*	*0.449*	*.10 (.03)*	.60	---	---	---	---
Others	.09	---	---	---	---	.97	---	---	---	---	.51	---	---	---	---
Wards SH															
Medicine	.20	---	---	---	---	.36	---	---	---	---	**.001**	n.d.	n.d.	n.d.	n.d.

*MW – Mann-Whitney; CH – Central Hospital; PH – Pediatric Hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External Consult Unit; n.d. – n.d. – regression model not determined (none of the tested models were statistically significant, even when considering the uncorrected p-value). * - P-values are presented with Bonferroni correction when this retained significance – when significance was lost after correction, the uncorrected values are shown in parenthesis*

Table 6. Mann-Whitney tests results (p-values) and respective best fit regression model for *P. aeruginosa* isolates monobactam (aztreonam) and fluoroquinolone (ciprofloxacin) resistance time evolution. Statistical significant p-values (p<0.05) of Mann-Whitney tests are highlighted in bold. Italicized regression models indicate those whose significance failed to survive the Bonferroni correction

	Aztreonam					Ciprofloxacin				
	MW test	Regression modeling				MW test	Regression modeling			
	p-value	Best fit	Trend	R^2	p-value*	p-value	Best fit	Trend	R^2	p-value*
Total	**<.001**	n.d.	n.d.	n.d.	n.d.	**<.001**	Power	Positive	0.754	.003
Ambulatory	**<.001**	Power	Positive	0.738	.003	**<.001**	*Linear*	*Positive*	*0.518*	*.06 (.02)*
Nosocomial	**<.001**	*Quadratic*	*Negative*	*0.598*	*.12 (.04)*	**<.001**	Quadratic	Negative	0.807	.003
Biological products										
Respiratory	**<.001**	Linear	Positive	0.769	.003	**<.001**	Quadratic	Negative	0.800	.012
Exudates	.27	---	---	---	---	.09	---	---	---	---
Urine	**.003**	*Power*	*Positive*	*0.490*	*.07 (.024)*	.07	---	---	---	---
Others	.34	---	---	---	---	.10	---	---	---	---
Wards CH										
Medicine	**.002**	n.d.	n.d.	n.d.	n.d.	**<.001**	n.d.	n.d.	n.d.	n.d.
Pneumology	**<.001**	Linear	Positive	0.732	.006	**<.001**	Power	Positive	0.758	.003
Neurology+neurosurgery	**.006**	Quadratic	Negative	0.765	.03	**<.001**	Linear	Positive	0.724	.006
Surgery	.24	---	---	---	---	.89	---	---	---	---
ICU	.42	---	---	---	---	**.003**	n.d.	n.d.	n.d.	n.d.
Infectious diseases	**.001**	n.d.	n.d.	n.d.	n.d.	**.03**	n.d.	n.d.	n.d.	n.d.
Others	.52	---	---	---	---	**.02**	Power	Positive	0.829	<.001
Wards PH										
Medicine	.35	---	---	---	---	.22	---	---	---	---
ICU	**.02**	n.d.	n.d.	n.d.	n.d.	.88	---	---	---	---
Others	.12	---	---	---	---	**.002**	n.d.	n.d.	n.d.	n.d.
Wards SH										
Medicine	**.02**					.20	---	---	---	---

*MW – Mann-Whitney; CH – Central Hospital; PH – Pediatric Hospital; ICU – Intensive care unit; ER – Emergency room; ECU – External Consult Unit; n.d. – n.d. – regression model not determined (none of the tested models were statistically significant, even when considering the uncorrected p-value). * - P-values are presented with Bonferroni correction when this retained significance – when significance was lost after correction, the uncorrected values are shown in parenthesis*

There are several surveillance programs regarding the major pathogens all over the world. The European Antimicrobial Resistance Surveillance Network (EARS-Net), promoted by the European Centre for Disease Control and Prevention (ECDC), is the most important surveillance program in Europe. Recent data from 2012 reports resistance rates in *P. aeruginosa* clinical invasive isolates (collected from blood or cerebrospinal fluid) from several Portuguese hospitals of 20.4% for carbapenems (n=116 out of 568), 15.3% for CAZ (n=90 out of 587), 19.8% for PIP, associated or not with tazobactam (n=116 out of 586), 14.7% for aminoglycosides (n=86 out of 586) and 25.6% for fluoroquinolones (n=150 out of 587) [17]. Trends of antibiotic resistance between 2006 and 2012 show carbapenem resistance rates decreased between 2006 and 2010 and are increasing since 2011, with the same happening with CAZ and fluoroquinolone resistance rates. PIP, with or without tazobactam, resistance rates decreased until 2008 but are increasing since 2010, and aminoglycosides resistance rates increased from 2008 to 2011, remaining stable in 2012 [17,18]. Although this data only considers invasive isolates, it is similar to the observed in this study regarding the majority of other specimen samples, proving the overall effectiveness of EARS-Net strategy for the general resistance evolution characterization using only invasive isolates. However, particular deviations from that general trend are important to study within each hospital and its subdivisions, to allow the most efficient prescription policies possible, that should be as tightly tuned as possible to the reality of each ward. Outbreaks of resistance pathogens are frequently described across the world, with evidences of having its origin in specific wards and spreading to other specific wards within the same hospital [1]. This evidences the totally different reality in terms of drug resistance and vulnerability to outbreaks within the same healthcare unit. Thus, it is extremely important to promote a specific knowledge of resistance trends within each hospital, to allow the best possible adjustment of its prescription policies to its specific reality, which we believe will be more effective in the long term control of drug resistance than what we are currently observing.

To test this hypothesis, we studied the resistance to several antibiotics in *P. aeruginosa* isolates over time according to origin and specimen sample and also according to the wards from where they were collected. It was possible to observe that for half of the tested antibiotics, at least one negative trend of resistance evolution was observed in different wards, proving the discrepancies in the hospital's infection control and drug prescription policies efficiency between wards, regarding specific antibiotics. A review of these policies, supported in the obtained data, could be done by the respective committee to extinguish or diminish these discrepancies and promote the rapid shift of resistance trends in those hospital wards. Acquisition of resistance during treatment is a highly concerning problem that duplicates hospital-stay and increases therapy costs [19]. Stringent control of its use must be implemented and optimized regularly, according to the data obtained from each hospital, to better direct therapy.

The used data aggregation, regression method and respective interpretation have limitations, inherent to all statistical analysis, but we believe it gave relevant information on the trends of antibiotic resistance rates in the studied hospital that may be helpful to optimize its antibiotherapy policy. From the 100 statistically significant association of antibiotic resistance to time in the Mann-Withney tests determined, more than half [55] were described by statistically valid regression models, 20 were valid without Bonferroni correction, and only 25 were not statistically significantly explained by any of the 3 used models (table 2 to 6). If a greater number of models were used, we would have lost statistical significance [20], and would still not be able to model all statistical significant associations observed in Mann-Whitney tests. Acknowledging this, we think that similar retrospective analysis could be performed in other Portuguese and European hospitals, to allow a better understanding of the resistance distribution and evolution in Europe. ECDC, and other disease control centers worldwide, could also promote the increase in the number of antibiotics, specimen samples and select specific wards to be reported amongst the participating hospitals. This would allow the profiling and respective analysis of a broader *P. aeruginosa* resistance trend in other relevant samples, as they represent an increasing concern, and information about their prevalence in Europe and over the world is limited.

To our knowledge, it is not available in the literature a study as detailed and extended to many samples and antibiotics as the one here presented. Castanheira et al. [21] presented epidemiological data only on *P. aeruginosa*

carbapenem resistance of 14 European and Mediterranean hospitals, indicating an overall resistance rate of 25.6% (n=2066). Hocquet et al. [22] reported 6.0% CAZ resistance in 2326 *P. aeruginosa* clinical isolates in France. Fonseca et al. [23] revealed resistance rates of IP, AZT, PIP, TZP, CIP and AMK in only 88 *P. aeruginosa* isolates from a central hospital in North of Portugal, ranging between 15.0% (AMK) and 59.0% (CAZ). Cavallo et al. [24] reported high resistance rates for IP, CAZ, FEP, AZT, PIP, TZP, AMK, NN and CIP, ranging from 50.0% (AZT) to 86.0% (AMK) in 15 French hospitals in 2004 (n=450). Strateva et al. [25] studied several antibiotic resistance from different specimen samples and wards of 5 hospitals in Sofia, Bulgaria, during 5 years, but in a very low sample of 203 *P. aeruginosa* isolates, observing resistance rates ranging from 42.3% (IP) to 89.6% (NN). But none of the referenced works studied the resistance rate evolution over time, which we consider highly important and relevant to better interpret drug resistance. Finally, literature reports that ICU deserve special attention regarding resistance in *P. aeruginosa*, since it is detected higher resistance rates in patients from these units than in patients hospitalized in other units [3]. Due to their general low health condition and higher use of invasive medical devices in these units, ICU patients have increased risk of acquiring *P. aeruginosa* nosocomial infections [11], and thus, more care should be take in these units regarding its epidemiology, to better help physicians in the empirical antibiotherapy for these patients. In current study, the incidence of *P. aeruginosa* in ICU from CH or PH was 3.8% and 10.8%, respectively (Table 1) and resistance rates ranged from 11.5% (AMK) to 43.3% (IP) in ICU from CH and from 6.6% (TZP) and 39.3% (IP) in ICU PH. In CH, ICU resistance rates were not the highest observed for any of the tested antibiotics, contradicting the literature, but in PH, ICU presented the higher resistance rate for MP, CAZ, FEP, AZT, PIP and TZP (Fig. 4), which is more coincidental to current literature.

5. CONCLUSION

In conclusion, current work presents an exhaustive analysis of the resistance rates distribution and trends of 11 antibiotics frequently used to treat *P. aeruginosa* infections in a Portuguese central hospital, revealing a consistent and broadly distributed increase in the resistance rates of all antibiotics, which is of great concern. However, some antibiotics resistance rates presented a negative trend of evolution, which indicates that it is possible to diminish resistance in hospitals. Efforts to achieve this in a higher rate should be undertaken, and we propose that similar analysis are performed in other hospitals and countries, to better address the concerning problem of antibiotic resistance and thus, help improve the empirical prescription regimens that are of major importance for the good practice in each specific medical center.

COMPLIANCE WITH ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees and with the Helsinki Declaration of 1975, as revised in 2008. According to the ethical committee of the institution that provided the bacterial isolates and data for this study, formal consent from the patients is not required, as their identity is preserved and research does not involve any tissue, cell or genetic material from the patients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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