RESEARCH ARTICLE

TWENTY-YEAR TRENDS IN ANTIMICROBIAL RESISTANCES AMONG *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

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ABSTRACT: We retrospectively analyzed the antimicrobial data of *P. aeruginosa* strains isolated from hospitalized subjects and outpatients over a 20-year period (2000-2019). A total of 2,588 unique *P. aeruginosa* strains, 588 from outpatients (23 %) and 2,000 from hospitalized subjects (77 %), were retrieved. Except gentamicin and ciprofloxacin, which showed significant antibiotic decreasing trends, all the antimicrobial agents tested did not show significantly changes in both groups ($p < 0.01$). There were significant increasing resistance trends for all antibiotics, except gentamicin and ciprofloxacin in *P. aeruginosa* strains isolated from respiratory tract samples ($p < 0.05$), and for meropenem and piperacillin-tazobactam in urine samples from subjects with and without urinary catheter ($p < 0.05$). Moreover, there was a significant increase in multidrug resistant isolates ($p < 0.05$). Monitoring antibiotic resistances at local and regional levels are required in order to reduce inappropriate antimicrobial consumption, to increase the focus on antimicrobial stewardship.

KEYWORD: Antibiotic resistance, *Pseudomonas aeruginosa*, Epidemiology, Carbapenem resistance, Surveillance

INTRODUCTION:

The inappropriate and excessive use of antimicrobial agents and the limited availability of new antibiotics has increased the emergence of resistances, thus representing the biggest public health problem in the world. [1-3] *Pseudomonas aeruginosa* is a Gram-negative rod-shaped opportunistic microorganism that causes severe nosocomial infections mainly in immunocompromised subjects, critically ill patients affected by cystic fibrosis, and patients on mechanical ventilation in the intensive care unit (ICU). [4-6] *P. aeruginosa* is resistant to several antibiotics due to its intrinsic and acquired antimicrobial resistance. *P. aeruginosa* is one of the "ESKAPE" microorganisms, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* species, which are known to escape the

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activity of antibiotics.[4] Therefore, *P. aeruginosa* is inserted by World Health Organization (WHO) in the priority list of microorganisms for which it is mandatory to research and develop new antibiotics.[4,7] The pathogenicity of *P. aeruginosa* comprises different features, such as the production of several virulence factors, metabolic versatility, and the formation of biofilms, which are all controlled by the transcriptional, post-transcriptional, and post-translational regulation of numerous systems.[4] The increasing trends of antibiotic resistance of *P. aeruginosa* strains have contributed to a higher mortality rate of infected subjects, a longer hospitalization, and higher costs of treatment.[4,6] A few works on antibiotic resistances of *P. aeruginosa* have been published, particularly over a long-time period.[4-6] In this work, we aimed to retrospectively investigate the antimicrobial data of *P. aeruginosa* strains isolated from the Italian Hospital of Desio over a 20-year period, 2000-2019. The antimicrobial resistance trends were assessed to provide useful information to clinicians to prescribe a more appropriate therapy.

MATERIALS AND METHODS:

Study design and setting

In this retrospective observational study, antibiotic resistance patterns of *P. aeruginosa* strains were analyzed. Data were retrieved from the database of the Laboratory of Microbiology of Desio Hospital, Italy, over a 20-year period (from January 1, 2000 to December 31, 2019). In the case of multiple *P. aeruginosa* isolates in one subject, showing the same antibiotic resistance pattern, only the first one was used for the analysis. Specimens presenting multiple isolates other than *P. aeruginosa* were excluded.

Bacterial isolates and antimicrobial susceptibility testing

The antimicrobial susceptibility of *P. aeruginosa* isolates was determined by the VITEK® 1 and 2 systems (bioMérieux, Marcy l'Étoile, France) using Antimicrobial Susceptibility Testing (AST) cards.

For this retrospective study, resistances to the following 13 antibiotics were analyzed: piperacillin/tazobactam, amikacin, ciprofloxacin, cefepime, ceftazidime, Fosfomycin, gentamicin, imipenem, and meropenem. From 2000 to 2010, the results were interpreted using the criteria recommended by the Clinical & Laboratory Standards Institute (CLSI).[8] From June 2011 to December 2019, results were interpreted using the criteria recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[9] The identification of bacteria was performed by VITEK® 1 and 2 systems, and from 2014 by Vitek® matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Escherichia coli* ATCC® 8739 was used as a control.

Definition

We defined a *P. aeruginosa* isolate as Multi-Drug Resistant (MDR) if it exhibited a non-susceptibility to at least one agent in three or more antimicrobial categories. Resistant and intermediate resistant *P. aeruginosa* isolates were combined, as previously reported. [10]

Statistics

All statistical analyses were performed using Stata (Stata Statistical Software: Release 16).[11] A chi-square test was applied to compare the antimicrobial susceptibilities among inpatient and outpatient results over 20 years, and to determine whether there were statistically significant trends over the study period, which was divided into four intervals of time, 2000–2004, 2005–2009, 2010–2014 and 2015-2019. A p-value < 0.05 was considered statistically significant.

RESULTS:

We identified a total of 2,588 unique *P. aeruginosa* strains from positive samples, 588 from outpatients (23 %) and 2,000 from hospitalized subjects (77 %). The median age of patients was 64 years (interquartile range (IQR): 55-78 years). The majority of isolates were from males (64.4 % compared to 35.6 % females). The most common specimen type from which *P. aeruginosa* strains were isolated was bronchoalveolar lavage (BAL) (24 %, $n = 614$), followed by urine samples from subjects with catheter (18 %, $n = 459$), sputum (15 %, $n = 401$), midstream urines (13 %, $n = 337$), skin swabs (11 %, $n = 288$), and ear swabs (8 %, $n = 200$) (**Figure 1**).
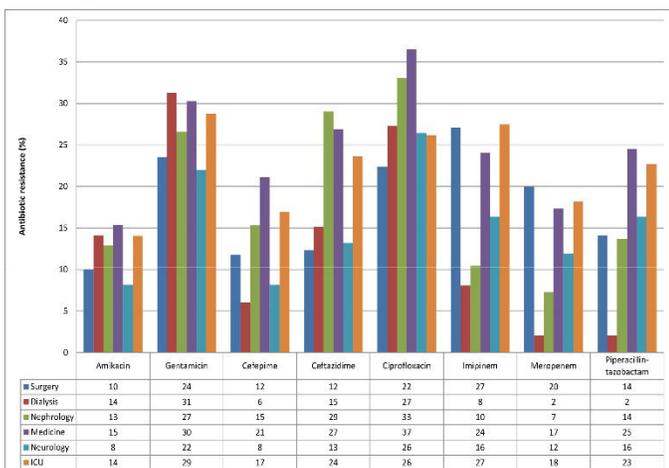

**Figure 1: Distribution of *Pseudomonas aeruginosa* isolates by infection type.**
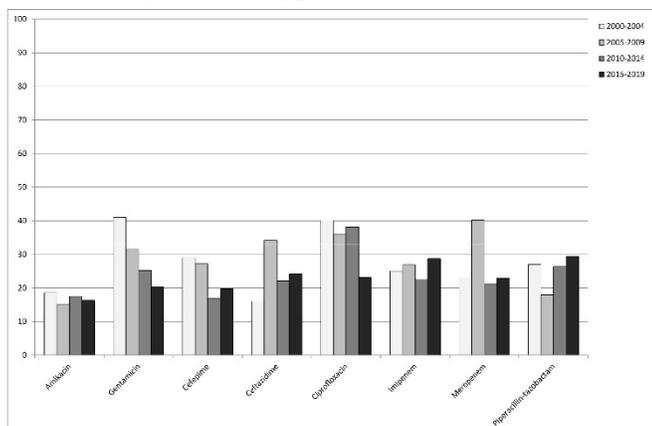
Figure 2: Resistant percentages of *Pseudomonas aeruginosa* isolates by 5-year period over 20 years of

the study for selected antimicrobials: amikacin, gentamicin, cefepime, ceftazidime, ciprofloxacin, imipenem, meropenem, and piperacillin/tazobactam.

Figure 2 shows the *P. aeruginosa* susceptibilities to the 8 antimicrobial agents tested among all subjects enrolled in the study. From 2000-2004 to 2015-2019, except gentamicin (from 41 % to 29 %) and ciprofloxacin (from 40 % to 23 %) which showed significant decreasing resistance rates (p trend < 0.01), all the other antibiotics (mean values: amikacin 17 %, cefepime 23 %, ceftazidime 24 %, imipenem 26 %, meropenem 27 %, and piperacillin-tazobactam 25 %) did not show significant changes over the time (p trend > 0.05). We noted significant decreased resistance rate values for third- and fourth-generation cephalosporins, carbapenems, particularly meropenem, from 2005-2009 to the last period 2015-2019, while significant increased levels for piperacillin-tazobactam (p trend < 0.01). Considering all antibiotics, mean resistance rate increased from 27 % in 2000-2004 to 29 % in 2005-2009, and decreased to 23 % in the last period 2015-2019, but the differences were not statistically significant (p trend > 0.05).

Concerning these results, we analyzed the relationship between the data of the first, 2000-2004, and the last period, 2015-2019, with the aim to assess the antibiotic resistance trends in the most common specimens positive to *P. aeruginosa* infection isolated in hospitalized and non-hospitalized subjects. **Tables 1 and 2** show the antibiotic resistance rates of *P. aeruginosa* strains isolated from respiratory tract samples, particularly bronchoalveolar lavage (BAL) and sputum, among hospitalized subjects and outpatients. Our data reported statistically significant increasing trends in resistance rates for all antibiotics both for hospitalized and community-related subjects (p < 0.05), except for gentamicin and ciprofloxacin which presented decreasing trends in hospitalized subjects (p < 0.05).

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Table 1: Antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from respiratory tract specimens* among hospitalized patients in 2000-2004 and 2015-2019.

Antibiotic	% in 2000-2004 (n = 214)	% in 2015-2019 (n = 93)
Amikacin	9	17**
Gentamicin	41	14**
Cefepime	3	14**
Ceftazidime	20	22**
Ciprofloxacin	43	19**
Imipinem	21	29**
Meropenem	2	32**
Piperacillin-tazobactam	1	39**

*Bronchoalveolar lavage (BAL) and sputum specimens.
**p < 0.05, see *Materials and methods* for details.

Table 2: Antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from respiratory tract specimens* among outpatients in 2000-2004 and 2015-2019.

Antibiotic	% in 2000-2004 (n = 13)	% in 2015-2019 (n = 16)
Amikacin	0	56**
Gentamicin	23	44
Cefepime	0	38**
Ceftazidime	0	25**
Ciprofloxacin	0	13**
Imipinem	0	31**
Meropenem	0	13**
Piperacillin-tazobactam	0	19**

*Bronchoalveolar lavage (BAL) and sputum specimens.
**p < 0.05, see *Materials and methods* for details.

Tables 3 and 4 show the antibiotic resistance rates of *P. aeruginosa* strains isolated from urine specimens, except those from subjects with urinary catheter, among hospitalized subjects and outpatients. Our data reported statistically significant decreasing trends in resistance rates for all antibiotics in hospitalized subjects (p < 0.05), except for meropenem and piperacillin-tazobactam which presented increasing trends (p < 0.05). In *P. aeruginosa* strains isolated from community-related subjects we observed the same results obtained in hospitalized patients.

Table 3: Antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from positive urine samples (except subjects with urinary catheter) among hospitalized patients in 2000-2004 and 2015-2019.

Antibiotic	% in 2000-2004 (n = 8)	% in 2015-2019 (n = 17)
Amikacin	25	12*
Gentamicin	50	13*
Cefepime	13	6*
Ceftazidime	25	6*
Ciprofloxacin	63	29*
Imipinem	25	6*
Meropenem	0	18*
Piperacillin-tazobactam	0	6

*p < 0.05, see *Materials and methods* for details.

Table 5: Antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from positive urine samples (except subjects with urinary catheter) among outpatients in 2000-2004 and 2015-2019.

Antibiotic	% in 2000-2004 (n = 35)	% in 2015-2019 (n = 27)
Amikacin	14	0*
Gentamicin	3	7
Cefepime	3	0
Ceftazidime	20	11
Ciprofloxacin	37	26*
Imipinem	0	1
Meropenem	0	7*
Piperacillin-tazobactam	0	7*

*p < 0.05, see *Materials and methods* for details.

Table 5 shows the antibiotic resistance rates of *P. aeruginosa* strains isolated from urine specimens of hospitalized subjects with urinary catheter. Our data reported statistically significant decreasing trends in resistance rates for gentamicin, ceftazidime, and ciprofloxacin (p < 0.05), while increasing trends for cefepime, meropenem and piperacillin-tazobactam (p < 0.05).

Table 4: Antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from positive urine samples among hospitalized patients with urinary catheter in 2000-2004 and 2015-2019.

Antibiotic	% in 2000-2004 (n = 73)	% in 2015-2019 (n = 116)
Amikacin	14	16
Gentamicin	40	24*
Cefepime	1	20*
Ceftazidime	29	20
Ciprofloxacin	48	25*
Imipinem	23	21
Meropenem	0	20*
Piperacillin-tazobactam	0	36*

*p < 0.05, see *Materials and methods* for details.
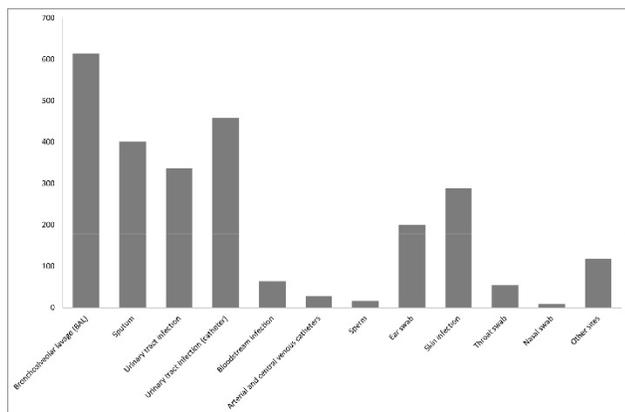
Figure 3: Distribution of mean antibiotic resistance percentages of *Pseudomonas aeruginosa* isolates by unit ward over the study period for selected antimicrobials: amikacin, gentamicin, cefepime, ceftazidime, ciprofloxacin, imipenem, meropenem, and piperacillin/tazobactam.

Figure 3 presents the proportion of resistant *P. aeruginosa* isolates according to the different hospital wards, such as Surgery, Dialysis, Nephrology, Medicine, Neurology and ICU, where most of *P. aeruginosa* strains were isolated during the study period. The analysis reported that the higher percentages of antibiotic resistance rates were detected in Medicine and ICU wards, 25 and 22 %, respectively; whereas, the lower values were detected in Dialysis and Neurology wards, with 15 and 13 %, respectively (p < 0.05). The highest mean values of antibiotic resistance were for ciprofloxacin and gentamicin, 29 and 27 %, respectively, followed by ceftazidime (20 %), imipenem (19 %), and piperacillin-tazobactam (16 %).

Of all 2,588 *P. aeruginosa* strains, 172 (7 %) were from Medicine, of which 59 (34 %) resulted MDR; whereas of 158 (6 %) isolated from ICU, 32 (20 %) were MDR. Generally, there was a significant increase in MDR-positive isolates over the study period (p < 0.05).

DISCUSSION:

The inappropriate prescription and use of antibiotics have promoted the spread of antimicrobial resistance in most bacteria, causing nearly 700,000 people death every year worldwide. [4] Surveillance studies have key importance in the identification of bacterial changes in susceptibility patterns, to critically review the empiric treatment protocols. The present study is one of the largest database of susceptibility data of *P. aeruginosa* clinical isolates over a long period time, thus allowing for reliable assessments of the resistance trends. Switching from CLSI to EUCAST criteria, most antimicrobial susceptibility percentages did not change, although a few works reported a decrease in aminoglycoside susceptibility of *P. aeruginosa* in the application of the EUCAST guidelines.[12] *P. aeruginosa* is intrinsically resistant to several antimicrobials, mainly thanks to a combination of intrinsic, acquired and adaptive systems, such as low outer membrane permeability, expression of efflux pumps, AmpC overexpression, and biofilm formation.[4] Eight classes of antibiotics are most frequently administered to treat *P. aeruginosa* infections: penicillins with β-lactamase inhibitors (ticarcillin-clavulanic acid, piperacillin-tazobactam), cephalosporins (ceftazidime, cefepime), carbapenems (imipenem, meropenem, doripenem), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (gentamicin, amikacin, netilmicin, tobramycin), monobactams

(aztreonam), phosphonic acids (fosfomycin) and polymyxins (colistin and polymyxin B).[4] Over the whole study period, we did not observe significant increasing trends of antibiotic resistance rates of *P. aeruginosa* clinical isolates. However, considering the comparison between 2000-2004 and 2015-2019 periods, resistance rates of *P. aeruginosa* strains isolated from the respiratory tract and urine specimens increased for β-lactams, third-generation cephalosporins, and carbapenems, particularly meropenem, both in community and hospital-related infections, as previously observed.[4] Conversely, it was important to observe that there were small but significant decreasing trends of resistance rates in the hospitalized population for fluoroquinolones, and aminoglycosides, while in outpatients the trends for most of antimicrobial agents markedly increased, more likely as a consequence of the different therapies administered. Our data agree with the Italian surveillance report 2015-2019 which described that the resistance trends decreased for all the antibiotics used in *P. aeruginosa* infections, and that the greater values of non-susceptibility were observed for penicillins with β-lactamase inhibitors, followed by fluoroquinolones, cephalosporins, carbapenems, and aminoglycosides.[13] Moreover, the European surveillance report of antimicrobial resistance in the same period described that the highest EU/EEA resistance percentages were also observed for fluoroquinolones, followed by penicillins with β-lactamase inhibitors, carbapenems, and cephalosporins.[14]

In Italy, fluoroquinolones were the most common antibiotics prescribed in 2019, preceded only by β-lactams and macrolides.[15] In 2018, following the Pharmacovigilance Risk Assessment Committee (PRAC) recommendations, the European Medicines Agency (EMA) suspended the marketing authorization of quinoline-containing medicines, such as cinoxacin, flumequine, nalidixic acid and pipemidic acid, and restricted the fluoroquinolone-containing antibiotics usage, such as ciprofloxacin, due to serious, disabling and potentially permanent

side effects.[16] In 2019, Italy implemented these recommendations and our data confirmed the decreasing resistance trends for fluoroquinolones due to a diminished clinical usage in the last period, 2015-2019. On the other hand, as a consequence, the greater administration of β-lactams and cephalosporins increased resistance rates to these drugs, particularly ceftazidime, a fourth-generation cephalosporin, and piperacillin-tazobactam. These antibiotic resistances are interrelated, since the inducible over-expression of AmpC and efflux pumps, due to the adaptive ability of *P. aeruginosa*, is responsible not only for the resistance to penicillins and cephalosporins, but also to carbapenems, mainly imipenem. Moreover, further specific mutations which induce over-expression of efflux pumps reduce susceptibility to another carbapenem, meropenem.[4] Carbapenems are very important in human health and are considered the last choice for the treatment of multi-drug resistant Gram-negative bacteria, particularly in ICU.[4] Carbapenemases are not intrinsically produced by *P. aeruginosa*, but rather expressed by genes acquired by horizontal gene transfer.[4] Therefore, the presence of carbapenem-resistant *P. aeruginosa* strains represents a serious health problem. Our data did not show any significant change over the study period, with mean resistance percentages in accordance with the data of Italian and European surveillance reports.[13,14] However, the increasing carbapenem resistance trends observed in *P. aeruginosa* strains isolated in non-hospitalized subjects highlight the importance to follow national and international guidelines for the prudent use of antimicrobials in human health. [17]

The present study analyzed the MDR *P. aeruginosa* strains isolated over 20 years. A significant increasing trend was observed, as previously reported in other countries.[18-20] A recent European survey, including Italy, provided targets for the reduction of unnecessary and inappropriate antibiotic use in human healthcare, to reduce the development and spread of multi-resistant strains.[21] It is noteworthy to report that, based on ESAC-Net 2018,

Italy presented a statistically significant decreasing trend on antimicrobial consumption during the period 2009–2018.[22]

As far as the association between antibiotic resistance rates and hospital wards is concerned, most of *P. aeruginosa* strains was isolated in Medicine and ICU, where serious ill patients are subjected to a long length of stay and often to invasive medical procedures, such as mechanical ventilation, central venous and arterial catheter, urinary catheterization, which are known to be a source of several infections.[23,24] A strong modulation and adequacy of antimicrobial therapy based on host characteristics, and more attention to the different routes of transmission that include (I) from environment to patient, (II) from colonized patients to the environment and (III) between patients, are needed.

Our study presents a few limitations that should be considered: (A) the work is retrospective and was performed in a single hospital; (B) the lack of clinical data cannot provide a more comprehensive representation of resistance trends; (C) the lack of a comparative analysis with the antibiotic consumption.

Collectively, the major strength of our work is the large sample size and long study period with which we performed our analyses. We demonstrated that *P. aeruginosa* resistance rates did not significantly change during the 20 years considered, except for decreased values for fluoroquinolones and aminoglycosides, and increased values for carbapenems in strains isolated from outpatients. Therefore, it is important to continuously study and monitor antibiotic non-susceptibilities at local and regional levels, being essential in order to reduce antibiotic consumption, to detect alarming resistance mechanisms, and to contribute to new antimicrobial stewardships.

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