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Evolution of the antimicrobial resistance of *Pseudomonas aeruginosa* in Spain: Second National Study (2003)

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SUMMARY

The second national prevalence study of Pseudomonas aeruginosa *has been carried out in Spain. A total of 1250 clinical isolates of* P. aeruginosa *were collected from 127 hospitals in 1 week in 2003 and the resistance data gathered from the isolates was compared with those of the first study in 1998 (1014 isolates from 136 hospitals). Antimicrobial susceptibility testing was performed in both studies in the same laboratory. The most active antimicrobials were piperacillin, piperacillin-tazobactam, and amikacin (≤10% resistant) and resistance to these antimicrobials did not change over the time. The least active were ofloxacin and gentamicin (≥30% resistant). From 1998 to 2003, resistance increased significantly to ciprofloxacin (23% vs. 28%, respectively, p = 0.015); ofloxacin (30%* vs. *37%, p = 0.002); imipenem (14%* vs. *18%, p = 0.017) and meropenem (8%* vs. *13%, p <0.001). Resistance to aztreonam (23%), ceftazidime (16%), cefepime (20%), ticarcillin (13%) and tobramycin (11%) remained stable. Isolates from inpatients were significantly more resistant than those from outpatients to all antimicrobials, with the exception of fluoroquinolones and aminoglycosides (p <0.01). Isolates from outpatients were significantly more resistant to these two groups (p <0.05) than to other antimicrobials. In Spain, from 1998 to 2003, the susceptibility pattern of* P. aeruginosa *to antimicrobial agents has changed. Isolates have become significantly more resistant to fluoroquinolones and carbapenems. However, resistance to beta-lactams and aminoglycosides remains stable.*

Key words: *Pseudomonas aeruginosa* - National prevalence study - Antimicrobial resistance

Evolución de la resistencia a los antimicrobianos de Pseudomonas aeruginosa *en España: Segundo estudio nacional (2003)*

RESUMEN

Se ha realizado el segundo estudio nacional de prevalencia de Pseudomonas aeruginosa en España. Durante una semana en el año 2003 se recuperaron 1250 aislamientos clínicos de esta bacteria en 127 hospitales españoles y los datos de resistencia se compararon con los obtenidos en el primer estudio realizado en 1998 (1014 aislamientos de 136 hospitales). Las pruebas de sensibilidad se realizaron en el mismo laboratorio en ambos estudios. Los antimicrobianos que se mostraron más activos fueron piperacilina, piperacilina-tazobactam y amikacina

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(<10% resistencia), y sus porcentajes de resistencia no cambiaron a lo largo de los dos estudios. Por el contrario, los antimicrobianos menos activos fueron ofloxacino y gentamicina (>30% resistencia). Desde el año 1998 al 2003 se observó un aumento de resistencias significativo en ciprofloxacino (23% vs. 28%, respectivamente, p = 0.015), ofloxacino (30% vs. 37%, p = 0.002), imipenem (14% vs. 18%, p = 0.017) y meropenem (8% vs. 13%, p <0.001). Los porcentajes de resistencia a aztreonam (23%), ceftazidima (16%), cefepima (20%), ticarcillina (13%) y tobramicina (11%) permanecieron estables. Los aislamientos de pacientes ingresados fueron significativamente más resistentes que los de los pacientes ambulatorios para todos los antimicrobianos ensayados excepto para las fluoroquinolonas y los aminoglucosidos (p <0.01). Los aislamientos procedentes de la comunidad fueron más resistentes significativamente en esos dos grupos (p <0.05) que en el resto de los antimicrobianos. En España, los patrones de sensibilidad de P. aeruginosa *a los antimicrobianos ha cambiado desde 1998 a 2003. Los aislamientos son más resistentes a las fluorquinolonas y los carbapenemes, y se mantienen estables los porcentajes de resistencia para betalactámicos y aminoglucósidos.*

Palabras clave: *Pseudomonas aeruginosa* - Estudio de prevalencia nacional - Resistencia antimicrobiana

INTRODUCTION

In 1998, the first national prevalence study of *Pseudomonas aeruginosa* in Spain was carried out; 136 hospitals participated and 1014 isolates were studied (1). The treatment of infections caused by *P. aeruginosa* gives cause for concern, because of its intrinsic resistance to many antimicrobial agents. In addition, *P. aeruginosa* has a high level of ability to acquire resistance via mutations to all relevant antimicrobials and it is frequently seen in severe infections (2-6). The high and increasing rates of local resistance require surveillance studies so that its antimicrobial resistance is known at a given point. However, most studies of the resistance of *P. aeruginosa* to antimicrobial agents are based on the situation in particular institutions or on particular types of patients and clinical syndromes, and thus provide information which is biased. Data about antimicrobial susceptibility without a previous selection are scarce. The results of the second national study, performed in 2003, are reported here and show the evolution of the resistance to antimicrobial agents of *P. aeruginosa* in Spain.

MATERIAL AND METHODS

Participating hospitals

A total of 136 randomly selected Spanish hospitals which represented all types and sizes of public hospitals in Spain and which participated in the first study (1) were invited to take part in the present study, by sending all clinical isolates of *P. aeruginosa* identified during a particular week, without duplication of strains from the same patient and sample, to a reference laboratory. Of the 136 hospitals, 127 participated in this second study. The protocol followed, the reference laboratory, and the identification and susceptibility testing procedures were the same as those used in the first study (1). All isolates were accompanied by a uniform protocol which included the characteristics of the hospital of origin (the number of beds, ward), site of isolation, and acquisition from outpatients (<48 h hospital admission) or inpatients. Information was requested about the population treated by the hospital; the number of admissions per year; the number of samples received by the local microbiology laboratory during the week of the study; and the local susceptibility of *P. aeruginosa* to selected antimicrobial agents. Hospital size was classified into three categories: less than 500 beds; 500-1000 beds; and more than 1000 beds. The ward of isolation was grouped into four categories: high risk of infection (intensive care, units for newborns and immunocompromised patients); surgery; medical units (including pediatrics); and low risk (psychiatry and obstetrics units). The origin of the isolates were classified as: blood; urine; lower respiratory tract; wounds and abscesses; sterile fluids (bile, peritoneal, pleural and cerebrospinal fluid [CSF]); mucosa (conjunctiva, pharyngeal, nasal and urethral); and others.

Identification of the isolates and susceptibility testing

All isolates were identified again at the reference laboratory. Identities and minimum inhibitory concentrations (MICs; mg/l) were determined using an automated microdilution system using MicroScan Neg Combo 1S panels (MicroScan, Baxter Diagnostics, Inc., CA, USA) and following the manufacturer's guidelines. Isolates whose identification was inconclusive were identified again using standard procedures (7) and by the use of the API 20NE system (Biomerieux, Marcy l'Etoile, France). The antimicrobials and concentrations tested were: ticarcillin and piperacillin 16 and 64 mg/l; piperacillin-tazobactam 16/4 mg/l and 64/4 mg/l; ceftazidime, cefepime and aztreonam 1-2 mg/l and 8-16 mg/l; imipenem 1-8 mg/l; meropenem 4-8 mg/l; ciprofloxacin 0.12 and 1-2 mg/l; ofloxacin 0.5 and 2-4 mg/l; gen-

tamicin and tobramycin 4-8 mg/l; and amikacin 8-16 mg/l. Each panel was inoculated with an appropriate dilution of an exponential phase culture of a microorganism. Readings were performed after overnight incubation at 35 °C. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used daily as control strains. Breakpoints were applied following Clinical and Laboratory Standards Institute (CLSI) recommendations (8). MICs (mg/l) in both the intermediate and resistant ranges (CLSI criteria) were considered as nonsusceptible in this study.

Statistical analysis

Susceptibility data were compared by using a Chi-square test (qualitative variables). Statistical analysis was performed with SPSS software version 11.5. In all cases, $p \leq 0.05$ was considered statistically significant. All p-values were calculated as two-sided.

RESULTS

Demographic and identification data

A total of 1270 clinical isolates were received. Of these, 1250 were identified as *P. aeruginosa*. According to the data provided by the participating hospitals, it was estimated that Spanish microbiology laboratories identified 207 *P. aeruginosa* isolates per 100,000 inhabitants of population per year (33 isolates per 1000 hospital admissions per year). *P. aeruginosa* was recovered in 8% of all the samples received in the microbiology laboratory with bacterial isolates.

The majority of the isolates (43%) were recovered from hospitals with 500-1000 beds. *P. aeruginosa* was recovered in all wards, although the medical wards provided the greatest number of isolates (40%). Nevertheless, considering the smaller number of beds belonging to high-risk areas, the proportion of isolates in these areas was greater. The lower respiratory tract represented the most frequent origin of the isolates (32%), followed by wounds and abscesses (31%) and urine (21%). Thirty per cent of the isolates were recovered from outpatients.

Evolution of resistance of *P. aeruginosa* to antimicrobials

Comparative data regarding antimicrobial resistance in both studies (1998 and 2003) are summarized in Table 1. No one antimicrobial was uniformly active against all *P. aeruginosa* isolates. The most active antimicrobial agents were piperacillin, piperacillin-tazobactam and amikacin (≤10% resistant), and the percentages of resistance to these antimicrobials did not changed over time. In contrast, the least active were ofloxacin and gentamicin (≥30% resistant). Resistance rates increased significantly between 1998 and 2003 to ciprofloxacin (23% vs. 28%, respectively, p=0.015); ofloxacin (30% vs. 37%, p=0.002); imipenem (14% vs. 18%, p=0.017) and meropenem (8% vs. 13%, p<0.001). Re-

Table 1. *In vitro* activities of antimicrobial agents against *P. aeruginosa*.

	Year of study						
	1998 (n=1014 isolates)			2003 (n=1250 isolates)			
Antimicrobial agent	Range (mg/l)	MIC_{90} (mg/l)	%R*	Range (mg/l)	MIC_{90} (mg/l)	%R*	p-value
Amikacin	≤8->16	16	9	≤8->16	16	8	NS
Aztreonam	≤1->16	16	23	≤1->16	16	23	NS
Cefepime	≤1->16	16	17	≤1->16	16	20	NS
Ceftazidime	≤1->16	16	15	≤1->16	16	16	NS
Ciprofloxacin	≤0.12->2	>2	23	≤0.12->2	>2	28	0.015
Gentamicin	≤4->8	>8	31	≤4->8	>8	30	NS
Imipenem	≤1->8	8	14	≤1->8	>8	18	0.017
Meropenem	≤4->8	4	8	≤4->8	8	13	<0.001
Ofloxacin	≤0.5->4	>4	30	≤0.5->4	>4	37	0.002
Piperacillin	≤16->64	64	10	≤16->64	64	10	NS
Piperacillin-tazobactam	≤16->64	64	7	≤16->64	64	7	NS
Ticarcillin	≤16->64	>64	13	≤16->64	>64	13	NS
Tobramycin	≤4->8	4	10	≤4->8	>8	11	NS

MIC: minimum inhibitory concentration.
*Percentage of resistance, includes all nonsusceptible (intermediate and resistant) isolates. NS: statistically nonsignificant.

Table 2. Resistance* of *P. aeruginosa* isolates from inpatients and outpatients.

Antimicrobial agent	Inpatients (n=875)	Outpatients (n=375)	p-value
Amikacin	8	7	NS
Aztreonam	26	17	0.002
Cefepime	23	15	0.004
Ceftazidime	20	9	<0.001
Ciprofloxacin	29	26	NS
Gentamicin	32	28	NS
Imipenem	24	9	<0.001
Meropenem	18	5	<0.001
Ofloxacin	39	34	NS
Piperacillin	13	6	<0.001
Piperacillin-tazobactam	10	2	<0.001
Ticarcillin	16	8	<0.001
Tobramycin	13	9	NS

*Percentage of resistance, includes all nonsusceptible (intermediate and resistant) isolates. NS: statistically nonsignificant.

sistance to aztreonam (23%), ceftazidime (16%), cefepime (20%), ticarcillin (13%), and tobramycin (11%) remained stable. Isolates from inpatients were significantly more resistant than those from outpatients to all antimicrobials, with the exception of fluoroquinolones and aminoglycosides (Table 2). Isolates from outpatients were significantly more resistant to these two groups of antimicrobials than to others ($p<0.05$). Resistance to aminoglycosides, with the exception of amikacin, and to carbapenems were significantly higher in hospitals with more than 1,000 beds ($p<0.05$). Isolates from high-risk areas were more resistant to carbapenems and beta-lactams than those from other wards ($p<0.01$). Isolates from catheter tips were significantly more resistant to all antimicrobials tested ($p<0.01$) than those from other samples; only 17 isolates came from this origin. In contrast, isolates from urine were the most susceptible to all antimicrobials with the exception of fluoroquinolones: a significant increase in the resistance to ciprofloxacin of urine isolates was observed in comparison with the results obtained in the first study (28% in 1998 vs. 38% in 2003, $p=0.020$). For outpatient isolates, urine was the most common site of isolation (31%); this was significantly more common than lower respiratory tract ($p<0.05$). In contrast, in strains from inpatients lower respiratory tract was the most common site of isolation and it was significantly more common than urine ($p<0.05$). The cross-resistance of *P. aeruginosa* isolates to antimicrobial agents is summarized in Table 3. The majority (93%) of meropenem-resistant isolates were also resistant to imipenem; however, 33% of imipenem-resistant isolates were susceptible to meropenem. About half of the imipenem-resistant isolates were susceptible to ceftazidime, cefepime, aztreonam and ciprofloxacin, and 75% were sus-

Table 3. Crossresistance of *P. aeruginosa* isolates.

Drug(s) to which isolates were resistant	% Resistance* to												
	AK	AZT	CPE	CAZ	CIP	GN	IMP	MER	OFL	PI	P/T	TI	TO
AK		45	59	47	66	100	45	37	78	36	25	37	58
AZT	15		60	51	53	50	41	38	72	37	27	51	24
CPE	36	92		92	56	77	66	62	71	74	68	83	34
CAZ	28	94	95		56	61	58	53	67	81	61	78	32
CIP	28	43	43	33		62	34	27	99	21	15	29	35
GN	25	27	46	32	57		36	29	67	22	16	27	36
IMP	19	52	53	47	54	61		67	67	30	25	34	35
MER	22	67	68	61	60	68	93		76	39	34	46	43
OFL	16	45	40	29	76	56	33	27		18	13	28	28
PI	28	83	92	89	60	67	52	50	67		64	78	42
P/T	29	90	98	93	61	73	65	64	70	96		92	39
TI	23	91	83	69	64	64	48	47	81	62	48		36
TO	40	48	67	59	87	97	55	49	91	38	23	40	

*Includes all nonsusceptible (intermediate and resistant) isolates.
AK: amikacin; AZT: aztreonam; CPE: cefepime; CAZ: ceftazidime; CIP: ciprofloxacin; GN: gentamicin; IMP: imipenem; MER: meropenem; OFL: ofloxacin; P/T: piperacillin-tazobactam; PI: piperacillin; TI: ticarcillin; TO: tobramycin.

ceptible to piperacillin-tazobactam. About 40% of the ceftazidime-resistant isolates were susceptible to carbapenems, piperacillin-tazobactam and ciprofloxacin. All amikacin nonsusceptible isolates were resistant to gentamicin, but 42% were susceptible to tobramycin (MIC ≤4 mg/l). Around 75% of the gentamicin-resistant isolates were susceptible to amikacin and tobramycin. Approximately two-thirds of the ciprofloxacin-resistant isolates were susceptible to ceftazidime, piperacillin-tazobactam, carbapenems, tobramycin and amikacin.

DISCUSSION

This study demonstrates that important changes have occurred in the susceptibility pattern of *P. aeruginosa* to antimicrobial agents in a short period of time in Spain. Isolates have become significantly more resistant to fluoroquinolones and carbapenems, however, resistance to beta-lactams and aminoglycosides has remained stable. Infections due to *P. aeruginosa* are often difficult to treat due to its virulence and to the relatively limited choice of effective antimicrobial agents (9, 10). In recent years, several studies have reported an increasing resistance of *P. aeruginosa* to different antimicrobial agents (11-15). Most studies, however, report the resistance of *P. aeruginosa* to a particular class of antimicrobials (16-18), in special units or diseases (19-21), or in special types of patients (22, 23). Our study is not limited to specific aspects and includes all isolates without *a priori* selection. In a multicenter study performed in Belgium and Luxembourg in 1999 and in which 40 hospitals participated, the percentages of resistance of *P. aeruginosa* to meropenem (9.5%) and to ciprofloxacin (24%) were similar to those of the present study (24). Another study in Italy showed similar percentages of resistance to ciprofloxacin (29% in 1998 and 28% in 2002) (25). In a multicenter study in the United States, resistance to fluoroquinolones was 29% in 1999 and 36% in 2001 (26). In our study, a high percentage of *P. aeruginosa* isolates were obtained from outpatients, of which 26% were resistant to ciprofloxacin and 34% to ofloxacin. The low percentages of susceptibility to fluroquinolones may reflect the high use of these antimicrobials in the community. The present study demonstrates that beta-lactams, despite having been in use for a longer time, have higher *in vitro* activity than the fluoroquinolones against *P. aeruginosa;* resistance to beta-lactams has not increased in comparison with the first study performed in 1998. It is noteworthy that the percentages of resistance to ceftazidime (16%) and piperacillin-tazobactam (7%) have remained stable. Resistance to cefepime showed a tendency to increase (16% in 1998 vs. 20% in 2003), but this did not reach statistical significance. The SENTRY Antimicrobial Surveillance Program (1998-2003) performed in U.S. medical centers reported 11.6% of ceftazidime-resistant *P. aeruginosa* (27), while another SENTRY study (1998-2004) reported 16.9% of isolates with resistance to ceftazidime (28). Other studies have demonstrated similar values of resistance to carbapenems, ciprofloxacin and aminoglycosides to those found in the present study (29-31). Isolates from inpatients were significantly more resistant to all antimicrobials with the exception of fluoroquinolones and aminoglycosides, but isolates from outpatients presented high levels of resistance to these antimicrobials as well. We observed significantly higher percentages of resistance to carbapenems and aminoglycosides in hospitals with more than 1000 beds, but found no relevant data in the literature. Significantly higher percentages of resistance to carbapenems and beta-lactams were also observed in isolates from high-risk areas and that may reflect the higher use of these antimicrobial agents in the intensive care units (ICUs). The high rate of resistance to all antimicrobials in isolates from catheter tips may be due to the scarce number of isolates with this origin in the present study. On the other hand, isolates from urine were the most susceptible to all antimicrobials tested, with the exception of fluoroquinolones. These data are in accordance with previous publications (32). Cross-resistance data indicate that a high number of isolates probably have resistance due to either a combination of multiple unrelated mechanisms of resistance or the involvement of efflux pump overexpression in association with fluoroquinolone resistance.

CONCLUSION

The intrinsic resistance of *P. aeruginosa* to many antimicrobial agents and the high ability of this microorganism to acquire new mechanisms of resistance make its management problematic in the hospital setting. However, in our study, there are still several antimicrobials (carbapenems, cefepime, ceftazidime, tobramycin and amikacin) that have good activity against *P. aeruginosa,* with piperacillin-tazobactam being the most active agent. Care must be taken in the overuse of carbapenems as this may lead to increasing resistance to these drugs, as observed in the present study.

In summary, in a very short period of time significant changes have been observed in the resistance of *P. aeruginosa* to antimicrobial agents in Spain that are not attributable to a selected institution or to a selected type of patient. Periodical surveillance studies are essential to determine the current susceptibility of *P. aeruginosa* to different antimicrobial agents and to provide an overview of the situation in a given country.

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