Bacterial Resistance to Ciprofloxacin in Greece: Results from the National Electronic Surveillance System

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According to 1997 susceptibility data from the National Electronic System for the Surveillance of Antimicrobial Resistance, Greece has high rates of ciprofloxacin resistance. For most species, the frequency of ciprofloxacin-resistant isolates (from highest to lowest, by patient setting) was as follows: intensive care unit > surgical > medical > outpatient. Most ciprofloxacin-resistant strains were multidrug resistant.

Soon after the broad-spectrum, highly effective antibiotics fluoroquinolones were introduced, their extensive use and misuse in hospitals and communities, as well as in veterinary medicine, have led to the emergence and spread of resistant strains (1,2). Highly divergent rates of fluoroquinolone resistance in both community-acquired and nosocomial pathogens have been reported worldwide (2). Many factors, including patient characteristics, local epidemiologic factors, antibiotic policies, over-the-counter use (which often leads to inadequate use), lower standard of living in developing countries, lack of information on the prudent use of antibiotics, and use of antibiotics in animal husbandry may contribute to the emergence of quinolone-resistant organisms.

Surveillance is an integral part of controlling resistance, and local and national surveys to identify, monitor, and study the epidemiology of the emergence and spread of resistant isolates are needed (3). To identify national trends and local differences in the epidemiology of quinolone resistance in Greece, we report 1997 ciprofloxacin susceptibility data from the National Electronic System for the Surveillance of Antimicrobial Resistance.

The National Electronic System for the Surveillance of Antimicrobial Resistance was introduced in Greece 3 years ago. Involving 17 hospitals throughout Greece, the system analyzes the routine results of the antibiotic sensitivity tests performed in hospital microbiology laboratories by using WHONET software (4).

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In our analysis we included 11,097 isolates (4,204 from medical wards, 2,897 from surgical wards, 1,724 from intensive care units [ICU], and 2,272 from outpatient departments) (Table 1). We focused on the bacteria most frequently encountered in Greek hospitals (National Electronic System for the Surveillance of Antimicrobial Resistance [www.mednet.gr/whonet]; N.J. Legakis, Enare Sentry, unpub. data): *Escherichia coli, Klebsiella pneumoniae, Enterobacter* species, *Pseudomonas aeruginosa,*

Table 1. Isolates included in the analysis[a]

	Type of ward				
Species	Medical	Surgical	ICU[b]	Outpatients	All
Escherichia coli	2,100	1,114	94	1,571	4,879
Pseudomonas aeruginosa	672	527	570	195	1,964
Staphylococcus aureus	452	467	318	248	1,485
Enterobacter spp.	396	332	198	142	1,068
Klebsiella pneumoniae	419	224	177	96	916
Acinetobacter spp.	165	233	367	20	785
All	4,204	2,897	1,724	2,272	11,097

[a]One isolate per species per patient (the first isolated) is shown. [b]ICU, intensive care unit.

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Acinetobacter baumanii, and *Staphylococcus aureus*. These species are also the most important nosocomial pathogens in most parts of the world in terms of rate of isolation, pathogenicity, and virulence (5,6).

Isolation and identification were performed by standard methods at the microbiology laboratories of each hospital participating in the network. The susceptibility testing methods were Kirby-Bauer disk diffusion (7 hospitals); Sensititre (Sensititre, Salem, NH) (1); Pasco (Difco, Detroit, MI) (8); and VITEK (Bieux-Merieux Marcy l'Etoile, France) (1). The actual zone diameters or MICs (not the interpretations of the tests) were entered into WHONET. The chi-square test was used to evaluate differences in resistance rates between types of wards, as well as between clinical specimens. Pearson's correlation coefficients were calculated for possible associations between resistance rates and hospital size.

The resistance rate to ciprofloxacin by type of ward, clinical specimen, and bacterial species is shown in Table 2. There is a stepwise decrease in the frequency of isolation of ciprofloxacin-resistant isolates (ciprofloxacin resistance in

Table 2. Ciprofloxacin resistance by specimen and type of ward[a]

	Outpatients		Medical		Surgical		ICU	
	No.	%R[b]	No.	%R	No.	%R	No.	%R
Escherichia coli								
Urine	1,191	5.0	1,572	5.5	597	8.5	39	10.2
Blood	-		195	6.9	14	18.1	5	0.0
Respiratory	-		56	2.1	-		23	9.0
Pus	-		33	12.1	203	8.4	11	27.8
Other	380	4.5	244	7.5	300	6.5	16	20.0
All	1,571	3.7	2,100	5.6	1,114	8.2	94	13.3
Salmonella spp.								
Stool	195	0.7						
Klebsiella pneumoniae								
Urine	62	6.6	254	15.5	85	19.8	28	64.0
Blood	-		45	11.3	10	9.8	18	72.3
Respiratory	-		62	9.8	12	50.0	90	69.8
Pus	-		14	50.0	42	19.0	0	0.0
Other	34	3.1	44	18.5	79	28.3	41	65.4
All	96	5.4	419	15.8	226	23.9	177	67.7
Serratia marcences								
All			76[c]	7.7[c]			20	45.2
Enterobacter spp.								
Urine	76	12.0	190	29.7	85	32.0	24	75.4
Blood	-		37	21.8	13	54.2	24	66.6
Respiratory	-		76	6.3	10	40.2	58	48.6
Pus	-		22	36.8	138	18.5	27	67.6
Other	66	10.8	71	16.9	86	23.3	65	69.0
All	142	11.6	396	22.2	332	24.8	198	62.2
Pseudomonas aeruginosa								
Urine	51	31.0	270	44.0	171	40.7	70	79.3
Blood	0	0.0	24	20.6	13	46.5	29	75.6
Respiratory	11	18.2	258	34.4	29	44.6	379	62.9
Pus	18	11.3	35	31.6	147	22.6	16	69.5
Ear	72	1.7	7	47.3	30	3.7	0	0.0
Other	43	18.8	78	26.9	137	25.9	76	66.9
All	195	16.7	672	37.5	527	28.2	570	66.4
Acinetobacter spp.								
Urine	-		72	62.6	32	65.9	34	94.4
Blood	-		18	38.7	16	69.0	40	92.3
Respiratory	-		38	49.7	11	100.0	190	91.0
Pus	-		13	61.8	87	60.1	19	94.8
Other	-		24	62.5	87	69.1	84	78.9
All	20	45.1	165	56.8	233	66.6	367	88.4
Staphylococcus aureus								
Urine	-		37	32.9	16	31.0	-	
Blood	-		101	51.0	15	67.0	40	62.7
Respiratory	-		123	45.3	28	57.1	221	65.8
Pus	104	18.2	88	21.6	272	30.8	14	71.4
Ear	52	3.8	-		-		-	
Other	92	10.3	103	25.6	136	31.4	43	67.4
All	248	12.8	452	30.5	467	33.0	318	63.6
MRSA[d]	40	56.7	140	69.1	176	75.3	375	94.3
MSSA[e]	184	1.7	256	12.4	219	6.5	92	4.6

[a]One isolate per patient (the first isolated) is shown. [b]R, resistant. [c]Medical and surgical wards combined. [d]MRSA, methicillin-resistant *S. aureus*. [e]MSSA, methicillin-sensitive *S. aureus*.

isolates from ICU patients > isolates from surgical patients > isolates from medical patients > isolates from outpatients). These differences were significant ($p < 0.01$), with the exception of decreases in resistance rates for *E. coli* between surgical wards and ICUs; for *Enterobacter* spp. between medical and surgical wards; for *Acinetobacter* spp. between outpatients, medical, and surgical wards; and for *S. aureus* between medical and surgical wards. Moreover, for *P. aeruginosa,* the resistance rates were significantly higher in medical than in surgical wards ($p = 0.00097$).

As for clinical specimens, each bacterial species followed a different pattern (Table 2). In medical wards, enterobacterial strains isolated from purulent infections were more often resistant to ciprofloxacin, but this difference was statistically significant only for *K. pneumoniae* ($p = 0.012$). In surgical wards, blood and respiratory isolates were more often resistant, but this difference was significant only for *Enterobacter* spp. ($p = 0.02$). On the other hand, ciprofloxacin-resistant *P. aeruginosa* strains were more frequently isolated ($p = 0.0021$) in medical wards from urine and in surgical wards from urine and blood as opposed to all other specimens ($p = 0.0005$). No significant differences were observed in the rate of isolation of ciprofloxacin-resistant *A. baumanii* strains among the various clinical specimens. *S. aureus* strains resistant to ciprofloxacin were mostly methicillin-resistant (MRSA) (Table 2). Very low resistance rates were observed in *P. aeruginosa* isolated from ear infections, especially from outpatients.

Approximately 75% of *K. pneumoniae*, 87% of *Enterobacter* spp., 55% of *P. aeruginosa*, 76% of *A. baumanii*, and 75% of MRSA strains were drug resistant to at least three different classes (Table 3). However, 15% of the ciprofloxacin-resistant *E. coli* were resistant only to this antibiotic, and 25% had additional resistance only to cotrimoxazole. Moreover, 48% of ciprofloxacin-resistant but methicillin-sensitive *S. aureus* were resistant only to chloramphenicol.

When we plotted resistance rates to ciprofloxacin against the number of beds in each hospital, we found no correlation (Figure). The rate of isolation of ciprofloxacin-resistant isolates varied greatly by hospital for all species examined: from 1% to 15% for *E. coli*, 1% to 23% for *K. pneumoniae*, 1% to 33% for *Enterobacter* spp., 11% to 33% for *P. aeruginosa*, 29% to 73% for

Table 3. Resistant phenotypes of ciprofloxacin-resistant isolates to other classes of antibiotics[a]

Klebsiella pneumoniae			*Enterobacter* spp			*Escherichia coli*		
Phenotype[b]	No.	%	Phenotype	No.	%	Phenotype	No.	%
F	4	3.7	F	0	0	F	25	15.1
DBXF	9	8.4	IF	4	2.5	IDBXF	16	9.6
IDB F	16	15.0	IDB F	7	4.4	IXF	29	17.5
IDBXF	64	59.8	IDBXF	131	82.9	XF	42	25.3
all other	14	13.1	all other	16	10.1	all other	54	32.5
All	107	100.0	All	158	100.0	All	166	100.0

Pseudomonas aeruginosa			*Acinetobacter baumanii*		
Phenotype	No.	%	Phenotype	No.	%
F	10	7.3	F	0	0.0
1DM F	14	10.2	SMD XF	5	10.0
1DMNF	23	16.8	D XF	15	30.0
1 M F	40	29.2	MD XF	23	46.0
all other	50	36.5	all other	7	14.0
All	137	100.0	All	50	100.0

Staphylococcus aureus					
MRSA			MSSA		
Phenotype	No.	%	Phenotype	No.	%
F	0	0.0	F	7	10.3
OG E F	23	11.3	E F	9	13.2
OG ECF	44	21.7	CF	33	48.5
OGXECF	84	41.4			
all other	52	25.6	all other	19	27.9
All	203	100.0	All	68	100.0

[a]All wards, intensive care units isolates are not included. [b]1, piperacillin; B, tobramycin; C, chloramphenicol; D, ceftazidime; E, erythromycin; F, ciprofloxacin; G, gentamicin; I, cefoxitin; M, amikacin; N, imipenem; O, oxacillin; S, amoxicillin/sulbactam; X, cotrimoxazole; MRSA, methicillin-resistant *S. aureus;* MSSA, methicillin-sensitive *S. aureus*.

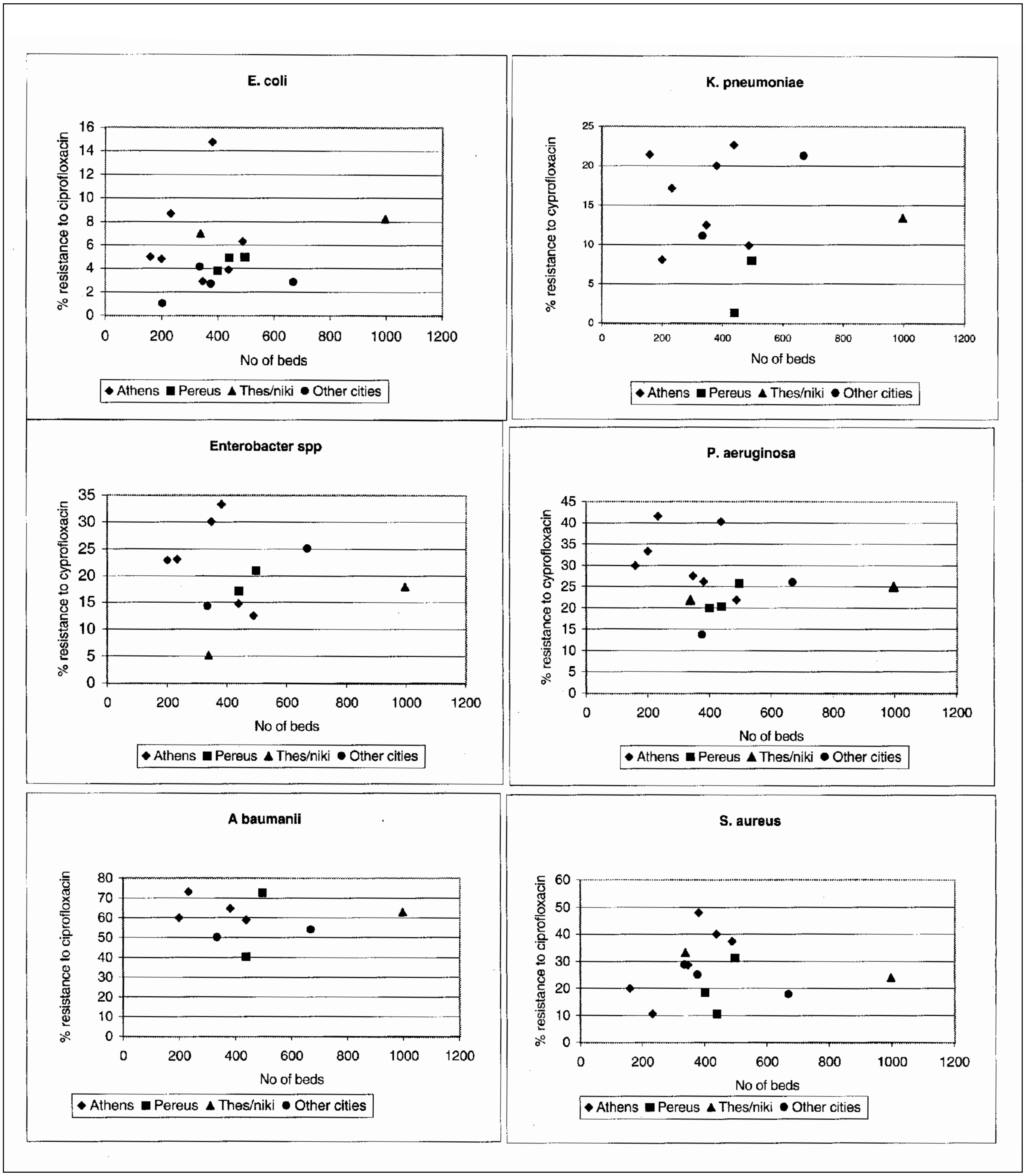

Figure. Resistance rates to ciprofloxavin in each hospital by number of beds and geographic area of the hospital. Only hospitals with more than 20 isolates are included. (Isolates from all wards but not intensive care units.)

A. baumanii, and 11% to 48% for *S. aureus*. Ciprofloxacin resistance was observed in hospitals throughout Greece.

In Europe and North America, a striking difference in the incidence of bacterial resistance to quinolones has been observed between nosocomial and community-acquired infections; resistance is only rarely encountered among the latter (2,7). The incidence of resistance to fluoroquinolones in bacteria isolated from hospital-acquired infections varies among bacterial species, clinical settings, and countries and may be related to local epidemic spread of a few clones (2). The highest incidence of resistance is among *P. aeruginosa, Acinetobacter* spp., *Serratia marcescens,* and particularly MRSA strains (8). Our results place Greece among the countries with high resistance levels to quinolones. Although quinolones are among the antibiotics restricted by the Greek Ministry of Health and Welfare, the mean national level of quinolone resistance has increased in most bacterial species during the last 5 years (9).

The 3.7% quinolone resistance rate among *E. coli* isolated from outpatients is almost double that in other industrialized countries (2). This high rate may be due to the use of quinolones, and especially norfloxacin, as a first-line antibiotic in Greece to treat uncomplicated urinary tract infections in the outpatient setting. Free access to fluoroquinolones has also been incriminated in increased quinolone resistance in industrialized and developing countries (10,11). The low rate of quinolone resistance in salmonellas, compared with other countries (12,13), may be due to infrequent use of quinolones in farm animals in Greece. Among *Enterobacteriaceae,* quinolone resistance seems to be higher in *K. pneumoniae* and *Enterobacter* spp. than in *S. marcescens*.

The high level of resistance in ICUs was expected since ICUs are well-known focuses of antimicrobial resistance (14). Hospitalization in ICUs was an independent risk factor for acquiring infection by multidrug-resistant strains in Greece (15). Moreover, ICU patients are often colonized with endemic, multidrug-resistant strains, which often spread to other wards (16).

We found higher rates of isolation of quinolone-resistant strains of some species in the surgical wards than in medical wards. Patients at high risk for a resistant nosocomial infection (e.g., cancer patients, immunosupressed patients) are usually in medical wards. High resistance in the surgical wards could be the result of nursing practices or unnecessary prophylactic administration of antibiotics, both of which should be further evaluated.

Most quinolone-resistant strains in Greece are also resistant to other clinically relevant antibiotics. The possible clinical and epidemiologic importance of the newly described multidrug efflux pumps in multidrug resistance, mainly in *P. aeruginosa,* is under investigation worldwide (17). Moreover, the marginal susceptibility of *S. aureus* to quinolones and the ease with which mutations affecting susceptibility can occur in this species contribute to the observed high rates of quinolone resistance. MRSA strains are no more likely to develop resistance to quinolones than other staphylococci (8). In any case, the favorable accumulation of different traits in quinolone-resistant strains or, alternatively, the favorable potential for mutation to quinolone resistance in multidrug-resistant strains has not been proved. Epidemiologic parameters, and more specifically the sequential introduction of various antibiotic classes in most of the world and in Greek hospitals, could explain multidrug resistance. The extensive aminoglycoside and beta-lactamase use in the 1980s is responsible for the high prevalence of multidrug-resistant plasmids and transposons found in the nosocomial strains of various bacterial genera in Greek hospitals (18-20). The strains harboring these plasmids can survive in the hospital environment and become the best candidates for selection of resistant mutants under the pressure of quinolones.

That quinolone-resistant strains are found in hospitals in all parts of Greece and resistance is not associated with the size of the hospital or its geographic area are consistent with the high prescription rate for quinolones. However, the isolation rate of resistant strains varied considerably by hospital, perhaps because of local epidemiologic factors (e.g., prescribing or nursing habits) or possible (epidemic) spread of strains among patients.

This study has limitations. First, it is based on routine data generated in the microbiology laboratories of participating hospitals. Sometimes different antibiotics are tested in each hospital, which limits the possibility for interhospital comparisons. Moreover, different methods for susceptibility testing are used in each hospital. Data such as antibiotic consumption or days of hospitalization are not available since they are not included as information in the WHONET software and they are difficult and time-consuming to collect routinely.

Quinolone use is a well-proven independent risk factor for resistance (21,22). Nevertheless, local differences indicate that other epidemiologic parameters should be further evaluated.

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The following hospitals participate in the system: Polycliniki General Hospital, Agia Olga General Hospital, Elpis General Hospital, First IKA Hospital of Athens, Agios Savas Cancer Hospital, Sismanoglion General Hospital, Hippocration General Hospital, Areteion University Hospital, Venizelio General Hospital, University Hospital of Alexandroupolis, University Hospital of Ioannina, General Hospital of Xanthi, Threassio General Hospital, Tzannio General Hospital, Asclepeion Voulas General Hospital, Theagenio Cancer Hospital, and Hippocration Hospital Thessaloniki.

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