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Resistance Status and Evolution Trends of *Klebsiella pneumoniae* Isolates in a University Hospital in Greece: Ineffectiveness of Carbapenems and Increasing Resistance to Colistin

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Key Words
Resistance patterns · *Klebsiella pneumoniae* · Colistin · Carbapenems

Abstract
Background: Due to its increased non-susceptibility rates, *Klebsiella pneumoniae* has emerged as one of the most problematic pathogens. ***Methods:*** The level of resistance to 25 antimicrobials of *K. pneumoniae* isolates from a teaching hospital in Greece and the evolution trends during 2 decades were examined. ***Results:*** A statistically significant increase in non-susceptibility rates was found for almost all antimicrobials examined. During 2008, the isolates presented non-susceptibility rates to aminoglycosides >50% and to quinolones >60%. Nowadays, 1 out of 10 isolates is non-susceptible to colistin. Moreover, the isolates non-susceptible to imipenem were almost doubled between 2007 (29%) and 2008 (50%). Among the imipenem-resistant isolates, 1 out of 4 was also resistant to colistin. ***Conclusion:*** The effectiveness of carbapenems has been compromised and the increase in resistance to colistin is rapid and steep.

Introduction

Over the last decade, *Klebsiella pneumoniae* has emerged as one of the most problematic pathogens, as treatment has largely been limited to only a few antimicrobials. During the 1990s, the production of extended-spectrum β-lactamases by *K. pneumoniae* was not uncommon. Extended-spectrum β-lactamases conferred resistance to all β-lactams, except carbapenems, leading to extensive use of these molecules. The increased selection pressure asserted to *K. pneumoniae* strains by carbapenems resulted in the acquisition of an additional resistance mechanism, namely that of carbapenemase production.

The majority of carbapenemases are metallo-β-lactamases of the VIM and IMP type, whereas lately, carbapenemases of the KPC or OXA type have been reported [1–3]. Usually, metallo-β-lactamase genes are carried on plasmids as gene cassettes inserted into class 1 integrons [1, 4]. The production of a carbapenamase by Enterobacteriaceae is usually presented as intermediate or full resistance to carbapenems, whereas often, the minimal in-

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hibitory concentrations of carbapenemase-producing Enterobacteriaceae are below the resistance breakpoints in routine susceptibility testing [4, 5].

Greece has been shown to be a 'hot spot' of antimicrobial resistance [5–7]. In order to determine the status and the trends of resistance in our region, we conducted the present study by analyzing the in vitro susceptibilities of all *K. pneumoniae* isolates consecutively collected from clinical specimens over 2 time periods: a 4-year period of the present decade (2005–2008) designated as 'period A', and a 3-year period of the previous decade (1996–1998) designated as 'period B'.

Materials and Methods

The University Hospital of Heraklion is the referral 750-bed, tertiary hospital of the Mediterranean island of Crete. The admissions recorded during the study years were: 42,711 during 1996, 48,559 during 1997, 51,733 during 1998, 67,525 during 2005, 67,663 during 2006, 67,031 during 2007 and 65,952 during 2008. All *K. pneumoniae* strains isolated in the microbiology laboratory of the hospital over the study periods were included. To avoid any bias due to duplication, only the first case among identical resistance phenotypes per patient was examined. A total of 959 and 345 clinical isolates were examined for period A and B, respectively. *K. pneumoniae* strains were isolated from urine (41.1%), blood (18.6%), pus (11.5%), respiratory secretions (11.4%) and catheters (5.5%).

Bacteria were identified by conventional methods and the API 20E system (Biomérieux, Marcy-l'Etoile, France) or the Vitek II system (Biomérieux). Susceptibilities were determined by either the disk diffusion method following the recommendations of the Clinical and Laboratory Standards Institute [8], or by the Vitek II system. Vitek II was introduced in 2006 and used according to the manufacturer's instructions. Since then, the API 20E and the disk diffusion method are used only when there are no available positions in Vitek II. Quality controls rule out the possibility of discrepancies in the results of the 2 methods. *Escherichia coli* 25922 and *E. coli* 35218 were used as control strains. Bacteria were classified as susceptible, intermediately resistant and resistant according to the recommendations of the Clinical and Laboratory Standards Institute [9]. When necessary, editing of the results was performed, according to standard guidelines [9, 10]. Isolates with reduced susceptibility to antimicrobials (full or intermediate resistance) were grouped together and were designated as non-susceptible isolates.

The antimicrobials tested were: amikacin, amoxicillin-clavulanic acid, aztreonam, cefalotin, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, colistin (CS), gentamicin, imipenem (IPM), meropenem, nitrofurantoin, norfloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, tetracycline, ticarcillin, tobramycin and trimethoprim-sulfamethoxazol.

The statistical significance of the differences in non-susceptibility observed during 2005–2008 for each antimicrobial was determined by the distribution-free non-parametric Kruskal-Wallis H test. Probability was considered significant at the level of 0.05. Statistical analysis was performed using the SPSS 11.5 software (SPSS, Chicago, Ill., USA).

Results

The resistance rates of *K. pneumoniae* isolates to 25 selected antimicrobials over periods A (2005–2008) and B (1996–1998) are shown in table 1, as well as the statistical analysis of non-susceptibility rates over period A, the mean percentages of non-susceptibility over periods A and B, and the mean percentages of non-susceptibility in the intensive care unit (ICU) alone over period A. With the exception of chloramphenicol, a statistically significant increase in non-susceptibility proportions over period A was found for all other antimicrobials examined (table 1). *K. pneumoniae* isolates had already shown a very high level of reduced susceptibility (>97%) to piperacillin and ticarcillin over period A (2005–2008). Therefore, no statistically significant increase was found. The increase in non-susceptibility rates to many antibiotics was more steep and dramatic in the last 2 years of period A (2007–2008). Especially, the rate of non-susceptibility to IPM isolates was almost doubled between 2007 and 2008 (from 29 to 50%). As shown in table 1, with the exception of piperacillin, the mean rates found in the ICU were much higher than the mean rates found in the hospital during period A. It should be noted that the rates of the hospital were calculated with the inclusion of the ICU cases. The mean percentages of non-susceptibility to each antibiotic of period A were much higher than the corresponding percentages of period B, with the exception of tetracycline, for which a decrease was noticed from 51 to 44%. No strains with reduced susceptibility to either CS or IPM were observed over the last decade, whereas nowadays, 1 out of 10 and 1 out of 3 isolates have reduced susceptibility to CS and IPM, respectively.

The total number of *K. pneumoniae* isolates resistant to IPM over period A (2005–2008) was 181. Susceptibilities of these IPM-resistant isolates to other antimicrobials over period A are shown in table 2. Among these isolates, 1 out of 4 (24%) was also resistant to CS, an agent representing our last therapeutic option in cases of IPM resistance.

Trends of the index of *K. pneumoniae* isolates per 1,000 admissions (index A) and of the index of *K. pneumoniae* isolates non-susceptible to IPM per 1,000 admissions (index B) for the study periods are shown in figure 1.

Table 1. Non-susceptibility rates (%) of *K. pneumoniae* to 25 selective antimicrobials over periods A (2005–2008) and B (1996–1998), as well as statistical analysis of non-susceptibility rates over period A, mean percentages of non-susceptibility over periods A and B, and mean percentages of non-susceptibility in the ICU alone over period A

Antimicrobial	1996 (n = 114) I+R	1997 (n = 105) I+R	1998 (n = 126) I+R	2005 (n = 143) I+R	2006 (n = 194) I+R	2007 (n =293) I+R	2008 (n = 329) I+R	Period A p value[1]	Period A mean (n = 959)	Period B mean (n = 345)	Period A ICU mean (n = 158)
Amox/Cla	34	24	34	41	45	53	64	<0.0001	54	31	81
Aztreonam	24	10	16	39	43	50	63	<0.0001	51	17	80
Cefalotin	32	15	26	48	50	55	66	<0.0001	57	24	82
Cefepime		9	16	36	43	50	63	<0.0001	51	13	79
Cefotaxime	24	10	15	39	43	49	63	<0.0001	51	16	80
Cefoxitin	29	22	24	43	45	52	64	<0.0001	54	25	82
Ceftazidime	25	10	18	40	43	50	63	<0.0001	51	17	80
Ceftriaxone			15	39	43	49	63	<0.0001	51	15	80
Cefuroxime	31	31	30	45	46	52	65	<0.0001	54	30	81
Piperacillin	40	27	32	98	100	99	100	NS	99	33	99
Pip/Taz		16	21	40	45	53	64	<0.0001	53	19	81
Ticarcillin	99	99	99	100	100	100	100	NS	100	99	100
IPM	0	0	0	14	26	29	50	<0.0001	33	0	65
Meropenem				9	27	33	52	<0.0001	37		68
Amikacin	17	9	14	24	39	45	51	<0.0001	43	13	68
Gentamicin	16	9	14	18	38	46	51	<0.0001	42	13	64
Tobramycin	18	8	16	28	41	46	53	<0.0001	45	14	71
Ciprofloxacin	5	10	2	38	43	50	64	<0.0001	52	6	82
Norfloxacin	13	15	5	40	43	50	64	<0.0001	52	11	82
Ofloxacin	13	20	6	39	43	50	64	<0.0001	52	13	82
Chloramph	16	20	14	39	29	48	58	NS	44	17	62
CS	0	0	0	1	4	10	19	<0.0001	10	0	17
Nitrofurantoin	47	54	40	81	78	83	88	0.016	83	47	92
Tetracycline	48	47	56	30	42	37	57	<0.0001	44	51	59
Trim/Sulf	35	15	33	41	45	49	64	<0.0001	52	28	80

I = Intermediate; R = resistant; Amox = amoxicillin; Cla = clavulanic acid; Pip = piperacillin; Taz = tazobactam; Chloramph = chloramphenicol; Trim = trimethoprim; Sulf = sulfamethoxazole; NS = not significant.

[1] The statistical significance of the increase in the percentage of non-susceptible cases during the years 2005–2008 was evaluated for each antimicrobial using the Kruskal-Wallis H test (d.f. = 2). The Mann-Whitney U test was also performed for evaluating the statistical significance of the increase in resistance rates observed per year pair-wise with similar results (data not shown).

Discussion

The present study examined the antimicrobial susceptibility patterns of a large number of *K. pneumoniae* isolates from the University Hospital of Heraklion, the only tertiary general hospital on the island of Crete. The island has an indigenous population of almost 650,000 and attracts millions of tourists from all over the world on a year-round basis.

One of the most striking findings of the present study is that during 2008, 1 out of 2 (50%) *K. pneumoniae* isolates was non-susceptible to IPM, a finding consistent with another report from Greece [6]. These findings reveal a tremendous difference between Greece and other European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) for 2007 [6, 11]. As presented by EARSS, the rates of *K. pneumoniae* isolates non-susceptible to IPM remained <2% in almost all countries including Germany, the United Kingdom, France, Italy, Austria, Spain, Portugal, the Czech Republic, Switzerland, Croatia, Slovenia and the Scandinavian countries [6]. The rate reported from Turkey was 2.2%, and the only country in the vicinity of Greece with a high rate of non-susceptibility was Israel (21.9%) [6]. The high non-susceptibility rates found in Greece can probably be explained by the dissemination of VIM- or KPC-producing strains of *K. pneumoniae* and the ineffectiveness of the infection control policies implemented [5].

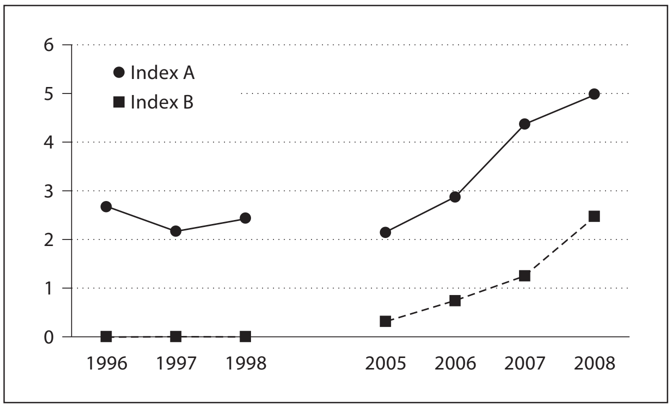

Fig. 1. Trends of index A (number of *K. pneumoniae* isolates per 1,000 admissions) and index B (number of *K. pneumoniae* isolates non-susceptible to IPM per 1,000 admissions) over the years of the study.

Table 2. Non-susceptibilities (%) to other antimicrobials in the cases of *K. pneumoniae* strains with resistance to IPM over period A (2005–2008)

Antimicrobial	2005 (n = 14) I+R	2006 (n = 43) I+R	2007 (n = 36) I+R	2008 (n = 88) I+R	Total (n =181) I+R
Aztreonam	100	100	97	100	100
Cefepime	93	100	97	100	99
Cefotaxime	100	100	97	100	99
Ceftazidime	100	100	100	100	100
Piperacillin/Taz	100	100	100	100	100
Amikacin	86	100	97	90	93
Gentamicin	64	88	97	85	87
Ciprofloxacin	100	100	97	100	100
Chloramphenicol	83	31	87	100	76
CS	14	0	31	34	24
Nitrofurantoin	100	100	100	100	100
Tetracycline	21	88	72	82	77
Trimethoprim/Sulf	100	100	94	98	98

I = Intermediate; R = resistant; Taz = tazobactam; Sulf = sulfamethoxazole.

As shown in the present study, the increase in non-susceptibility rates is rapid and steep and reveals the ability of these strains to prevail in a hospital setting within only a few years. The prevalence of these multi-resistant strains and their ability to overcome the usually administered empirical chemotherapies most probably explains the increase in the total number of *K. pneumoniae* isolates encountered over the last years (fig. 1).

The compromised effectiveness of carbapenems has a dramatic impact, since the armamentarium against isolates such as those described in the present study is almost exhausted. Practically, 2 therapeutic options are left: CS, and lately, tigecycline. CS was introduced in the 1950s, and recently, has been 'rediscovered' [12, 13]. However, there are reports describing increasing resistance of Gram-negative organisms to this agent [14]. Tigecycline is a new semisynthetic glycylcycline introduced in 2005 with promising results [12, 15, 16]. In the present study, we assessed the number of isolates exhibiting resistance to CS among the isolates that were also resistant to IPM over the last 4 years. The percentage rose from 14% in 2006 to 34% in 2008. For the last 2 years of the study (2007 and 2008), almost 1 out of 3 IPM-resistant isolates had additional resistance to CS. Despite the fact that no *K. pneumoniae* isolate has been found to be resistant to tigecycline so far [17], the presence of *K. pneumoniae* isolates with parallel resistance to both IPM and CS is extremely worrisome, leaving us literally with only 1 last weapon: tigecycline.

Moreover, it should be emphasized that the overall resistance to CS among all *K. pneumoniae* isolates rose from 1% in 2005 to 19% in 2008. This statistically significant increase indicates that unless effective policies will be designed and implemented, CS will no more be a therapeutic option against *K. pneumoniae* isolates within the next few years.

The mean percentages of *K. pneumoniae* isolates non-susceptible to aminoglycosides ranged from 42% (gentamicin) to 45% (tobramycin) over period A. These percentages were much lower than others reported from Greece (59.8%) [5]. However, it should be noted that during the last year of the study (2008), all 3 aminoglycosides tested presented non-susceptibility rates >50%. The mean rates found over period A were similar to those found in Israel (46.4%), the Czech Republic (43.5%) and Croatia (39.8%) and much higher than those found in the rest of Europe (Germany 8.7%, United Kingdom 8.8%, France 11.6%, Sweden 1.1%) [6].

Regarding quinolones and third-generation cephalosporins, the rates found in 2008 were similar to those reported previously from Greece (ciprofloxacin 64 vs. 58%, ceftazidime 63 vs. 63.2%) [6]. These levels of non-susceptibility are the highest found in Europe and differ substantially from those of all other countries [6]. An exception was the Czech Republic, which was reported to have a percentage of non-susceptibility to quinolones of 48.5% and to third-generation cephalosporins of 45.7%, along with Israel, with a reported percentage of non-susceptibility to quinolones of 42.6% and to third-generation cephalosporins of 43.7% [6].

One drawback of the present study is the lack of molecular analysis, which could specify the underlying resistance genes and investigate the possibility of clonal expansion of the resistant isolates. A second drawback is the lack of data concerning the antibiotic prescription policy in our hospital during the 2 study periods. It is due to the fact that all these data (especially those of period B) are not in a form that could allow us to handle them.

In conclusion, the present study has shown high levels of resistance of *K. pneumoniae* isolates to all classes of antimicrobials. A worrisome increase in non-susceptibility rates has been observed between the 2 time periods heralding that within the next few years, the usefulness of both carbapenems and CS will be compromised. Effective policies should be designed and implemented with no delay by the responsible authorities.

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