Containment of carbapenem resistance rates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in a Greek hospital with a concomitant increase in colistin, gentamicin and tigecycline resistance

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---- **SUMMARY** ----

In 2010 the Hellenic center for disease control and prevention launched the "Prokroustes" nationwide action plan to tackle the increasing rates of carbapenem resistance among gram-negative nosocomial pathogens. In the present report, data from a Greek tertiary-care hospital are presented three years after the adoption of the infection control measures. Carbapenem resistance rates have been contained for *Klebsiella pneumoniae* and *Acinetobacter baumannii* but not for *Pseudomonas aeruginosa*. More worryingly, in accordance with their overuse against carbapenem-resistant bacteria, resistance rates to colistin and tigecycline have risen significantly.

KEY WORDS: Infection control, Carbapenems, Colistin, Tigecycline.

Health care-associated infections due to multidrug-resistant and especially carbapenem-resistant Gram-negative microorganisms are recognized as a public health issue of major importance, accounting for a significant rise in morbidity and mortality, increased periods of hospitalization and consequent economic impact (Schwaber *et al.*, 2011). Greece, among the European countries, presents the highest rates of carbapenem resistance, is considered endemic for KPC and VIM carbapenemases (Grundmann *et al.*, 2010) and is a hot spot for the dissemination of resistant bacteria due to patient transfer to the rest of Europe (Meletis *et al.*, 2014). Especially during the 2010s a worrying increase in carbapenem resistance rates among *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* occurred in Greek hospitals (Miyakis *et al.*,

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2011). Moreover, data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed an almost steady increase in carbapenem resistance rates of *K. pneumoniae* from 2005 to 2010 (Magiorakos *et al.*, 2013). Therefore, the "Prokroustes" nationwide action plan involving infection control measures (http://www.keelpno.gr) was launched by the Hellenic center for disease control and prevention (HCDCP) in October 2010 aiming at two major objectives:

1) the surveillance of infections attributed to multidrug-resistant strains of the three aforementioned species via compulsory notification to the HCDCP, for estimation and follow-up of the incidence of these infections in hospitalized patients;

2) the implementation of infection control measures, emphasizing the isolation or cohorting of patients with infection or colonization and compliance with hand hygiene and contact precautions. Our hospital, a 250-bed tertiary-care institution, was one of the first to adopt this plan.

The present report reviewed the microbiology laboratory records three years before (1 November 2007 - 31 October 2010) and three years after (1 November 2010 - 31 October 2013) the implementation of the infection control measures. More precisely, non-duplicate isolations of *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* were calculated for intensive care unit (ICU) and non-ICU patients. Carbapenem-resistant (CR) isolates were defined as resistant to both imipenem and meropenem. Furthermore, colistin, gentamicin and tigecycline resistance rates for CR *K. pneumoniae* as well as colistin and gentamicin resistance rates for CR *A. baumannii* and CR *P. aeruginosa* in the two study periods were determined.

Results were associated with the consumption of carbapenems (imipenem, meropenem and ertapenem), colistin, gentamicin and tigecycline in the hospital expressed as defined daily doses (DDDs) per 1000 patient-days (www.whocc.no/atc_ddd_index), for each year starting from 2007 until 2013.

Species identification and susceptibility testing were performed by the VITEK2 automated system (bioMérieux, Marcy l'Étoile, France) until September 2012. After that, due to reduced funding because of the Greek economic crisis, organisms were identified using conventional phenotypic assays and susceptibility testing was performed by the disc diffusion method for gentamicin, E-test for colistin and tigecycline and both methods for carbapenems.

Results were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (EUCAST, 2013). Tigecycline was introduced in the susceptibility testing panel only after July 2008 and this was taken into consideration for the calculation of its resistance rates.

The combination meropenem disc test was performed for the phenotypic detection of *K. pneumoniae* carbapenemase (KPC) and/or metallo-beta-lactamase (MBL) production in all carbapenem-resistant *K. pneumoniae* (Tsakris *et al.*, 2010) and *P. aeruginosa* (Pasteran *et al.*, 2011).

Statistical comparisons between groups of the two study periods were made with the χ^2 or Fisher's-Exact test where appropriate, using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Overall, 110 *K. pneumoniae*, 166 *A. baumannii* and 171 *P. aeruginosa* isolations were made three years before and 346, 270, 305 three years after the Prokroustes plan commenced, respectively. Carbapenem resistance was contained for *K. pneumoniae* as well as for *A. baumannii* however, the opposite was observed for *P. aeruginosa* (Table 1 and Figure 1). More worryingly, CR *K. pneumoniae* resistance to colistin, tigecycline and gentamicin as well as CR *A. baumannii* resistance to gentamicin rose significantly in the last three study years despite the fact that infection control measures were applied. Of interest is also that resistance rates of *P. aeruginosa* to colistin and gentamicin did not increase and that *A. baumannii* remained 100% susceptible to colistin during the last six years.

The DDDs of carbapenems, colistin, gentamicin and tigecycline per year are shown in Figure 2. Colistin was increasingly prescribed whereas the use of tigecycline, in accordance with the rapid emergence of resistance, was notably reduced in 2013.

Three years after the introduction of the infection control measures in our hospital, it seems that the increasing rates of carbapenem resistance, even though still high, have been contained for *K. pneumoniae* and *A. baumannii* but not for *P. aeruginosa*. This observation could partially be attributed to the different genetic armamentarium and specific carbapenem resistance mechanisms of *P. aeruginosa* (Meletis *et al.*, 2012), many more factors however could have played an important role such as the introduction of patients previously hospitalized elsewhere or possible specific clinical approaches to *P. aeruginosa* infections.

It is reasonable that in an area in which carbapenemase-encoding genes are endemic (our phenotypic tests revealed 122/197 (61.92%) KPC-, 40/197 (20.30%) MBL- and 6/197 (3.04%) KPC+MBL-producers among CR *K. pneumoniae* and 94/164 (57.31%) MBL-producers among CR *P. aeruginosa*) colistin, as well as combinations of carbapenems with colistin, gentamicin or tigecycline are considered last resort treatments. Consequently, the rise of colistin, gentamicin and tigecycline resistance rates among CR isolates could be considered somehow predictable. Under these circumstances, CR *K. pneumoniae* showed the highest ability to de-

TABLE 1 - *Resistance rates of clinical isolates to carbapenems, colistin, tigecycline and gentamicin for the two study periods.*

Species	Resistance rates to	Isolated from	Period 2007-2010	Period 2010-2013	p
K. pneumoniae	Carbapenems	ICU patients	21/35 (60%)	92/157 (58.59%)	0.879
		Non-ICU patients	24/75 (32%)	60/189 (31.70%)	0.968
	Colistin (among CR isolates)	ICU patients	0/21 (0%)	20/92 (21.73%)	0.022
		Non-ICU patients	0/24 (0%)	5/60 (8.33%)	0.315
	Tigecycline (among CR isolates)	ICU patients	0/15 (0%)	30/92 (32.60%)	0.010
		Non-ICU patients	0/16 (0%)	18/60 (30%)	0.009
	Gentamicin (among CR isolates)	ICU patients	4/21 (19.04%)	29/92 (31.52%)	0.257
		Non-ICU patients	2/24 (8.33%)	10/60 (16.66%)	0.495
A. baumannii	Carbapenems	ICU patients	90/100 (90%)	132/156 (84.61%)	0.216
		Non-ICU patients	50/66 (75.75%)	75/114 (65.78%)	0.162
	Colistin (among CR isolates)	ICU patients	0/90 (0%)	0/132 (0%)	NA
		Non-ICU patients	0/50 (0%)	0/75 (0%)	NA
	Gentamicin (among CR isolates)	ICU patients	56/90 (62.22%)	117/132 (88.63%)	<0.001
		Non-ICU patients	34/50 (68%)	66/75 (88%)	0.006
P. aeruginosa	Carbapenems	ICU patients	18/47 (37.50%)	88/156 (56.41%)	0.022
		Non-ICU patients	22/123 (17.88%)	36/149 (24.16%)	0.209
	Colistin (among CR isolates)	ICU patients	1/18 (5.55%)	2/88 (2.27%)	0.444
		Non-ICU patients	1/22 (4.54%)	0/36 (0%)	0.197
	Gentamicin (among CR isolates)	ICU patients	11/18 (61.11%)	50/88 (56.81%)	0.737
		Non-ICU patients	16/22 (72.72%)	25/36 (69.44%)	0.790

CR: carbapenem-resistant; ICU: intensive care unit; NA: not applicable.
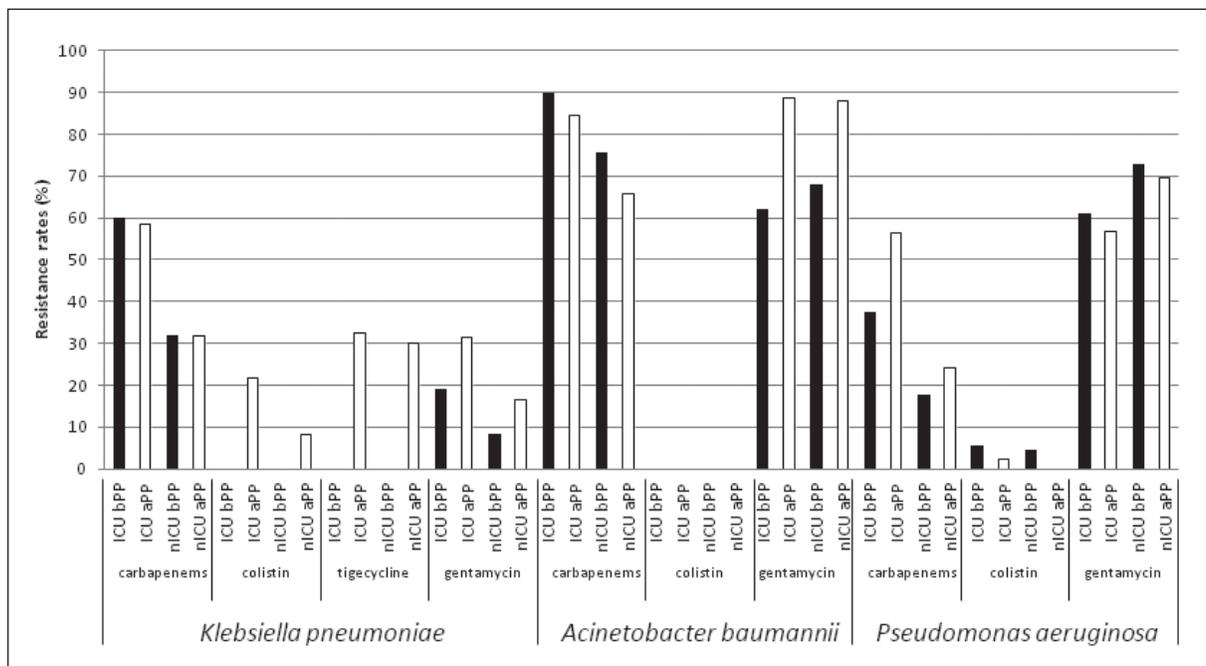

FIGURE 1 - *Total carbapenem resistance rates and colistin, gentamicin and tigecycline resistance rates among carbapenem-resistant bacteria for ICU and non-ICU isolations before and after the Prokroustes action plan. ICU: intensive care unit, bPP: before the adoption of the Prokrustes plan, aPP: after the adoption of the Prokroustes plan.*
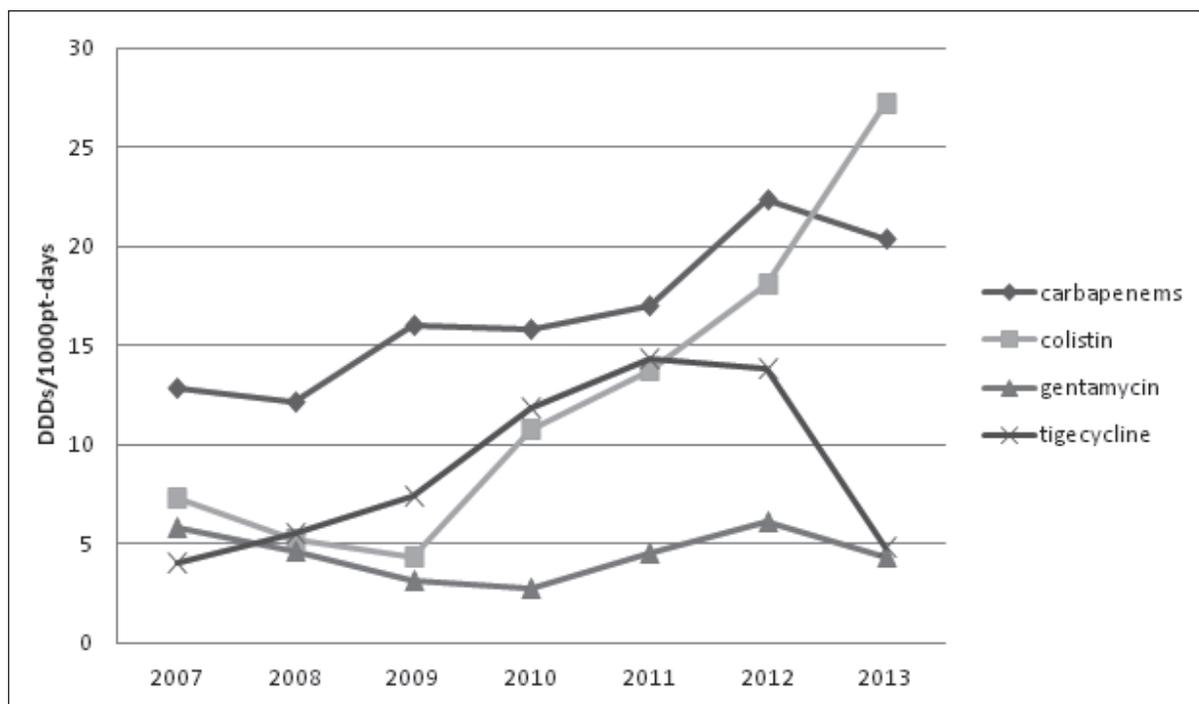
FIGURE 2 - *Trends of carbapenems, colistin, gentamicin and tigecycline defined daily doses (DDD) from 2007 to 2013.*

velop resistance to the non-beta lactam study agents whereas *A. baumannii* did not develop resistance to colistin despite the fact that CR *A. baumannii* infections are commonly treated with this agent.

According to the Greek System for the Surveillance of Antimicrobial Resistance (http://www.mednet.gr/whonet/), non susceptibility rates of *K. pneumoniae* and *A. baumannii* to imipenem during 2007 to 2013 varied among different hospitals and presented a noteworthy overall decrease only in the second semester of 2013. Imipenem non susceptibility rates of *P. aeruginosa* were similar (51.3% and 51.1%) between the second semester of 2010 and the same semester of 2013 for ICU isolates but increased (27.8% to 33.3%) for medical wards. Unfortunately, the national network for continuous monitoring of bacterial antibiotic resistance in Greek hospitals has not collected data on colistin, gentamicin and tigecycline resistance.

A successful control of the incidence of hospital-acquired carbapenemase-producing *K. pneumoniae* despite the fact that the number of imported cases remained stable was reported in 2012 from Serres General Hospital (Poulou *et al.*, 2012). In that hospital the infection control measures where intensified by the active surveillance of their implementation.

Our report aims to communicate the difficulties that are faced in addressing the problem of multidrug-resistance once carbapenem resistance is well established in a specific geographic area. It also presents useful data on the response of *K. pneumoniae* and *A. baumannii* in our hospital to the almost compulsory overuse of colistin and tigecycline. In the light of these observations as well as those of the overall nationwide action plan, the intensification and close monitoring of the infection control measures emphasizing active surveillance of the health care personnel compliance with contact precautions and hand hygiene has been decided and implemented in our hospital.

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