TITLE:

Empiric monotherapy with meropenem or combinated therapy: microbiologic point of view

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ABSTRACT

The increase in clinical isolates of broad-spectrum betalactamases or carbapenemases Enterobacteriaceae producers and carbapenems resistant *Pseudomonas aeruginosa* rises problems when it comes to putting empirical treatments in severe gram-negative bacilli associated infections. Our work aims to meet the resistance of meropenem in our field and the situation of the combination of this compound and two antibiotics of different family: amikacin and ciprofloxacin.

Between 2009 and 2013, a total of 81.310 clinical isolates belonging to the main species of Enterobacteriaceae and 39.191 of *P. aeruginosa* were analyzed through data provided

by RedMiva, isolated in 28 hospitals from the Valencian Community, in the South Eastern Mediterranean Coast of Spain ~~between 2009 and 2013~~.

Resistance to meropenem in Enterobacteriaceae has increased from 0.16% in 2009 to 1.25% in 2013. Very few Enterobacteriaceae strains resistant to meropenem were sensitive to ciprofloxacin; in contrast, the combination of meropenem and amikacin decrease strongly the risk that the microorganisms were resistant to both compounds (RR=34 in 2013)

Concerning to *P. aeruginosa*, resistance to meropenem has also increased (from 14.32% in 2009 to 24.52% in 2013). Most meropenem resistant *P. aeruginosa* isolates were also resistant to fluoroquinolones. However, addition of amikacin decreased more than three times resistance risk.

In our sector, empiric treatment with meropenem is suitable against enterobacterial infections but poses difficulties in the event of suspected infections originated by *P.aeruginosa*, being the addition of amikacin the combination with higher success rate.

INTRODUCTION

Unsuitable empirical antimicrobial treatment of serious infections has been directly linked to mortality, primarily by bacteremia where its incidence reaches 30-35% in different series of literature (1). Although the concept of unsuitability includes different factors as the dose used, route of drug administration and duration, the most important factor is the lack of sensitivity of the organism to the antibiotic; in the design of empirical treatment protocols is therefore essential to know the local epidemiology and evolution of antibiotic resistance for different microorganisms (2)

The increase in clinical isolates of Enterobacteriaceae carriers of broad-spectrum beta-lactamases and carbapenemases as well as *P.aeruginosa* resistant to carbapenems are one of the main causes of inadequate of empirical antibiotic treatment both in bacteremia as in other serious infectious processes, and therefore, our study seeks to analyze the situation of the resistance in Enterobacteriaceae and *P.aeruginosa* in our environment to meropenem; also studies the co-resistance to this compound and ciprofloxacin, or amikacin.

MATERIAL AND METHODS

Retrospective study (2009-2013) of antibiotic resistance to the most prevalent Enterobacteriaceae (*Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp and *Proteus* sp.) and *P.aeruginosa*. (Single isolate per patient)

Data from the antibiotic resistance on the Valencian Community (VC) was collected through the RedMiva (network of microbiological monitoring of the Valencian Community); this network collects data automatically and daily (all studies of antibiotic sensitivity of each hospital); analyzes information of 28 microbiology laboratories covering more than 90 per cent of the population of the region. The quality control of the same is ensured through a system of alerts, supervised by a Commission of microbiologists (3). The Valencian Community, located in the southeast of Spain has a population of approximately 5 million people. The antibiotic resistance data at national and European level was obtained from the EARSS reports of the years 2009-2013 (4). We define that a clinical isolated to each combination co-resistance presents if is intermediate or resistant to both compounds studied.

For the analysis of the data for each group of microorganism (Enterobacteriaceae and *P.aeruginosa*), percentages of resistances to meropenen (risk of resistance) were estimated for each year, according to type of antimicrobial resistance: single meropenem resistance versus combined resistance (meropenem plus ciprofloxacin or meropenem plus amikacin). As impact measure, Risk Differences (RD) with their 95% confidence intervals (95%CI) was estimated. As association measures, risk ratios (RR) with their 95%CI were estimated. The combined resistance was treated as reference category. The level of statistical significance was set at 0.05 and all tests were two-tailed. All analyzes were performed with SPSS v.21.0 and Epidat 3.1.

RESULTS

81310 Enterobacteriaceae clinical isolates as well as 39191 *P.aeruginosa* clinical isolates were studied.

Resistance to third-generation cephalosporins for Enterobacteriaceae has increased throughout the period studied from 9,39% to 14,43% (See table 1a). For the most prevalent species, *Escherichia coli*, the average resistance in the VC, Spain and Europe was quite similar (12,11%; 12,14% and 10,28% respectively) but for the second most prevalent species, Klebsiella sp, it was lower than the Spanish average and much lower than the European (9,08%; 14,24% and 24,8%) (Tables 1b and 1c).

Resistance to meropenem in Enterobacteriaceae was less frequent, although a progressive increase of resistance was observed during the study period (from 0.16% in 2009 to 1.25% in 2013). The risks of resistance for each year according to type of meropenem resistance (single versus combined resistance) are presented in table 2.

Very few Enterobacteriaceae resistant to meropenem strains were sensitive to ciprofloxacin, so RR was not of clinical relevance (RR≤1.8 for all studied years). In contrast, the combination of meropenem and amikacin decreased strongly the risk that the microorganism was resistant to both compounds (RR=34 in 2013) although clinically the difference of risk only denotes a RD of 1.3% (95% CI 1.03-1.55).

Figure 1a, shows the evolution of resistance along the studied years. Resistance increases strongly for meropenem and for co-resistance to meropenem-ciprofloxacin, while the co-resistance to the combination of amikacin-meropenem hasmaintained or has even declined slightly in recent years.

As for *P.aeruginosa*, resistance to meropenem in the VC has progressively increased from 14,32% in 2009 to 24,52% in 2013. Comparing these data with those published in the EARSS study for the same period, we observed that from a lower level than the average Spanish and European resistance, in the last year, the resistance has increased at an alarming rate exceeding the national rate and European (table 1d).

Table 3, shows the risk of resistance for each year according to type of meropenem resistance for *P.aeruginosa*.

As in the case of Enterobacteriaceae, most of the meropenem resistant *P.aeruginosa* strains were also resistant to fluoroquinolones, so RR was neither of clinical relevance (RR<1.5 also for all studied years) with a RD of 3,7% for the year 2013 (95%CI 2,6-4,8). However, the addition of amikacin diminished more than three times the risk of resistance for any of studied years, with RD of 19,2% for the year 2013 (95%CI 18,0-20,3)

Figure 1b shows the *P.aeruginosa* evolution of resistance along the studied period. The increase of resistance to meropenem and the co-resistance with ciprofloxacin runs in

parallel, while the co-resistance with amikacin and meropenem presents a smaller increase throughout the studied period.

DISCUSSION

The suitability of the empirical treatment is one of the most important parameters to evaluate at the time of healing of patients with severe infectious processes associated with gram negative bacilli, and is directly associated with their survival, not only in relation to the selection of the antibiotic therapy but also in the properness of initiation, the dosing, the duration, the route of administration, etc. Concerning the antibiotics choice, the local rates of resistance must be taken into account by the existence of geographical variability of the those resistances (5, 6, 7)

Our study shows that, from the microbiological point of view, the Protocol of empirical treatment for severe Enterobacteriaceae infection should take into account the high proportion of strains with resistance to third generation cephalosporins present in our sector so, patients with clinical factors associated with this resistance (8, 9), should undergo meropenem monotherapy. In contrast, in our environment the presence of meropenem resistant strains is very uncommon so, excluding special circumstances, it would not be required to resort to the use of combined therapy with other compounds, opposing to what has been described in other geographical areas of Europe, with serious problems of multidrug resistance in *Klebsiella pneumoniae* (10, 11, 12). Only in unusual circumstances the addition of a second drug should be considered, being amikacin the most advised option.

If the involvement of *P.aeruginosa* is suspected, the progressive increase of strains resistant to carbapenems, which in 2013 arose to 24,5%, implies that the monotherapy

with these compounds can be microbiologically inadequate in nearly a quarter of the patients, as has been described in other geographical areas (13). Therefore, the combination with others antimicrobials, is required for proper empirical handling in severe infection. When choosing the most suitable treatment combination, account must be taken of the little usefulness of combination therapy with ciprofloxacin by the increase of resistance detected, both in our environment such as in other geographical areas (14). In contrast, our data shows that the use of a combined therapy of meropenem with amikacin is the safest, as it is unlikely to find organisms that are resistant to both antibiotics.

On the other hand, our study shows an important increase of antibiotic resistance in the past few years in our environment, as it has happened in other regions of Spain (15) which suggests the need to implement measures that help control this phenomenon (16). These measures should include both programs of stewardship to ensure the correct use of antibiotics such as rapid microbiological systems, to decrease the time in which the patient takes wrong empirical treatments through a quick adjustment incorrect on the basis of the microbiological data in order to control the use of broad-spectrum antibiotics, shrinkage toxicities and control the rise in antibiotic resistance (17, 18).

Our data confirms that in our location, the available therapeutic options for the management of severe gram-negative bacilli infections by are rather limited, especially if the involvement of *P.aeruginosa* is suspected, where the use of combined therapies may be needed for most of the severe cases (19, 20). However, this strategy is not free of complications, so well-designed randomized trials should be implemented to elucidate the effectiveness of the multiple combination routines, (21, 22, 23) as well as

161 the development of standardized and validated systems to assess the activity of several
162 compounds in combination with the aim of characterizing major the phenomena of
163 antagonism and synergy (24).

164 REFERENCES

165 1.- Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein
166 D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M. Duration of hypotension before
167 initiation of effective antimicrobial therapy is the critical determinant of survival in
168 human septic shock. Crit Care Med. 2006;34:1589–1596.

169 2.- Dantas RC, Ferreira ML, Gontijo-Filho PP, Ribas RM. Pseudomonas aeruginosa
170 bacteraemia: independent risk factors for mortality and impact of resistance on outcome.
171 J Med Microbiol. 2014;63:1679-87.

172 3.- Muñoz, I., H. Vanaclocha, and F. González. The importance of microbiological
173 surveillance networks in monitoring resistant bacteria. RedMIVA. Rev. Esp. Quimioter.
174 2007;20:193-202.

175 4.- *European Centre for Disease Prevention and Control. EARSS* Annual Report of
176 antimicrobial resistance surveillance in Europe. Available at
177 http://ecdc.europa.eu/en/publications/surveillance_reports/arhai/Pages/arhai.aspx (15
178 January 2016, date last accessed).

179 5.-Retamar P, Luisa Martín M, Molina J, Del Arco A. Evaluating the quality of
180 antimicrobial prescribing: Is standardisation possible?. Enferm Infecc Microbiol Clin.
181 2013;31 Suppl 4:25-30.

182 6.- Calbo E, Alvarez-Rocha L, Gudiol F, Pasquau J. A review of the factors influencing
183 antimicrobial prescribing. Enferm Infecc Microbiol Clin. 2013;31 Suppl 4:12-5.

7.- Yokota PK, Marra AR, Martino MD, Victor ES, Durão MS, Edmond MB, dos Santos OF. Impact of appropriate antimicrobial therapy for patients with severe sepsis and septic shock--a quality improvement study. PLoS One. 2014;9:e104475.

8.- Faine BA, Harland KK, Porter B, Liang SY, Mohr N. A clinical decision rule identifies risk factors associated with antimicrobial-resistant urinary pathogens in the emergen cy department: a retrospective validation study. Ann Pharmacother. 2015;49:649-55.

9.- Bielicki JA, Lundin R, Sharland M; ARPEC Project. Antibiotic Resistance Prevalence in Routine Bloodstream Isolates from Children's Hospitals Varies Substantially from Adult Surveillance Data in Europe. Pediatr Infect Dis J. 2015;34:734-41.

10.- Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. J Intern Med. 2015;277:501-12.

11.- Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A. 2015;112:E3574-81.

12.- Tumbarello M, Trecarichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, Losito AR, Bartoletti M, Del Bono V, Corcione S, Maiuro G, Tedeschi S, Celani L, Cardellino CS, Spanu T, Marchese A, Ambretti S, Cauda R, Viscoli C, Viale P; ISGRI-

208 SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia
209 Antinfettiva). Infections caused by KPC-producing Klebsiella pneumoniae: differences
210 in therapy and mortality in a multicentre study. J Antimicrob Chemother. 2015;70:2133-
211 43.

212 13.- Bassetti M, De Waele JJ, Eggimann P, Garnacho-Montero J, Kahlmeter G,
213 Menichetti F, Nicolau DP, Paiva JA, Tumbarello M, Welte T, Wilcox M, Zahar JR,
214 Poulakou G. Preventive and therapeutic strategies in critically ill patients with highly
215 resistant bacteria. Intensive Care Med. 2015;41:776-95.

216 14.- Brigmon MM, Bookstaver PB, Kohn J, Albrecht H, Al-Hasan MN. Impact of
217 fluoroquinolone resistance in Gram-negative bloodstream infections on healthcare
218 utilization. Clin Microbiol Infect. Rapid nucleic acid diagnostics for the detection of
219 antimicrobial resistance in Gram-negative bacteria: is it time for a paradigm shift?
220 2015;21:843-9.

221 15.- Oteo J, Saez D, Bautista V, Fernández-Romero S, Hernández-Molina JM, Pérez-
222 Vázquez M, Aracil B, Campos J; Spanish Collaborating Group for the Antibiotic
223 Resistance Surveillance Program. Carbapenemase-producing enterobacteriaceae in
224 Spain in 2012. Antimicrob Agents Chemother. 2013;57:6344-7.

225 16.- Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of
226 antibiotics. Clin Infect Dis. 2013;56:1445-50.

227 17.- Tuite N, Reddington K, Barry T, Zumla A, Enne V. Rapid nucleic acid diagnostics
228 for the detection of antimicrobial resistance in Gram-negative bacteria: is it time for a
229 paradigm shift?J Antimicrob Chemother. 2014;69:1729-33.

230 18.- Kuehn BM. IDSA: Better, faster diagnostics for infectious diseases needed to curb
231 overtreatment, antibiotic resistance. JAMA. 2013;310:2385-6.

19.- Maruyama T, Fujisawa T, Okuno M, Toyoshima H, Tsutsui K, Maeda H, Yuda H, Yoshida M, Kobayashi H, Taguchi O, Gabazza EC, Takei Y, Miyashita N, Ihara T, Brito V, Niederman MS. A new strategy for healthcare-associated pneumonia: a 2-year prospective multicenter cohort study using risk factors for multidrug-resistant pathogens to select initial empiric therapy. Clin Infect Dis. 2013;57:1373-83.

20.- Bass SN, Bauer SR, Neuner EA, Lam SW. Impact of combination antimicrobial therapy on mortality risk for critically ill patients with carbapenem-resistant bacteremia.Antimicrob Agents Chemother. 2015;59:3748-53.

21.- Kmeid JG, Youssef MM, Kanafani ZA, Kanj SS. Combination therapy for Gram-negative bacteria: what is the evidence? Expert Rev Anti Infect Ther. 2013;11:1355-62.

22.- Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence. Antimicrob Agents Chemother. 2014;58:654-63.

23.- Viale P, Giannella M, Tedeschi S, Lewis R. Treatment of MDR-Gram negative infections in the 21st century: a never ending threat for clinicians. Curr Opin Pharmacol. 2015;24:30-37.

24.- Hsu AJ, Carroll KC, Milstone AM, Avdic E, Cosgrove SE, Vilasoa M, Tamma PD. The Use of a Combination Antibiogram to Assist with the Selection of Appropriate Antimicrobial Therapy for Carbapenemase-Producing Enterobacteriaceae Infections. Infect Control Hosp Epidemiol. 2015;36:1458-60.

Table 1. a) Resistance (%) to third generation cefalosporine for enterobacteria b) Resistance (%) to third generation cefalosporine for *E. coli*. c) Resistance (%) to third generation cefalosporine for Klebsiella pneumoniae.d) Resistance (%) to meropenem for *P. aeruginosa*.

a)

Year	VC	Spain	Europe
2009	9.39	NA	NA
2010	11.42	NA	NA
2011	12.61	NA	NA
2012	13.91	NA	NA
2013	14.43	NA	NA

b)

Year	VC	Spain	Europe
2009	9.41	11.3	7.9
2010	11.26	12.1	9.5
2011	12.49	12.0	9.6
2012	13.75	13.5	11.8
2013	13.65	13.3	12.6

c)

Year	VC	Spain	Europe
2009	6.8	11.1	21.4
2010	8.15	10.2	22.8
2011	8.69	13.4	24.2
2012	10.1	16.7	25.6
2013	11.7	19.8	30.0

d)

Year	VC	Spain	Europe
2009	14.32	16.1	17.2
2010	13.22	17.8	17.0
2011	12.96	16.3	16.9
2012	14.95	16.4	17.1
2013	24.52	17.6	17.6

VC: Valencian Community; NA: Not available

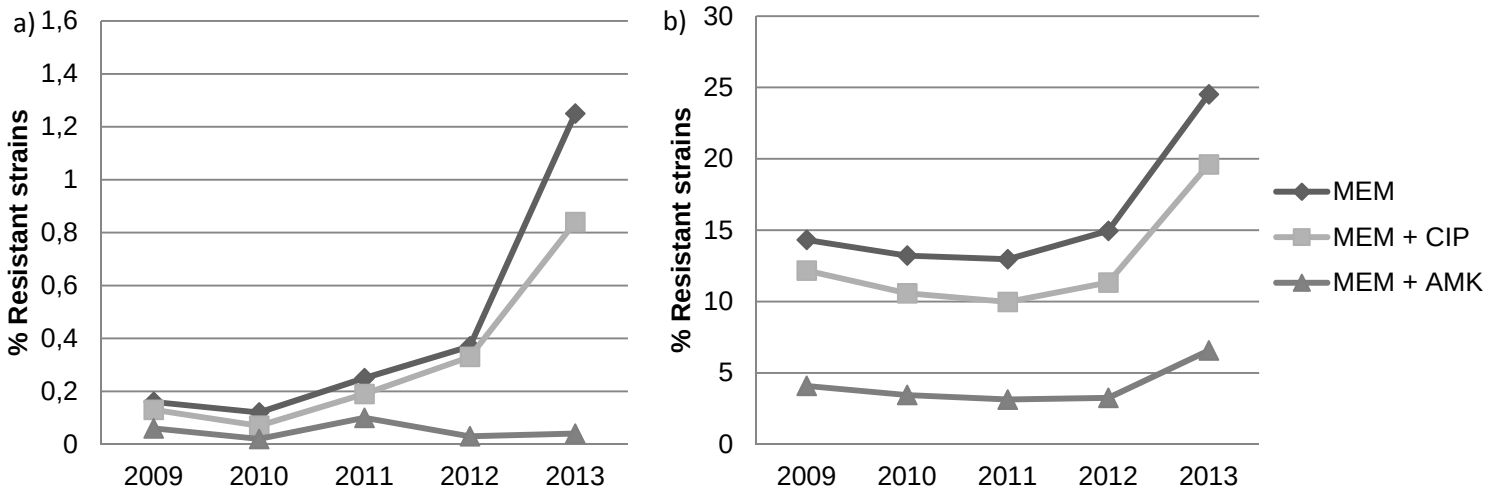

Figure 1. Evolution of resistances according to type of Meropenem use (single versus combined therapy plus Ciprofloxacin or Amikacin) . Valencian Community (years 2009-2013). a) *Enterobacteriaceae* b) *P.aeruginosa*.

Table 2. Risks of resistance according to type of meropenem use (single versus combined therapy plus ciprofloxacin or Amikacin) for *Enterobacteriaceae*. Valencian Community (years 2009-2013).

Year	Type of Meropenem use	Resistant N	Non Resistant N	Risk (%)	RD	95%	CI	RR	95%	CI	p
2009	CIP + MEM	32	25143	0.13	0	--	--	1	--	--	
	only MEM	42	25133	0.17	0.04%	-0.03	1.1	1.31	0.83	2.08	0.245
2010	CIP + MEM	13	19108	0.07	0	--	--	1	--	--	
	only MEM	23	19098	0.12	0.05%	-0.01	0.11	1.8	0.90	3.49	0.095
2011	CIP + MEM	31	16708	0.19	0	--	--	1	--	--	
	only MEM	42	16697	0.25	0.07%	-0.03	0.16	1.35	0.85	2.15	0.197
2012	CIP + MEM	37	11037	0.33	0	--	--	1	--	--	
	only MEM	42	11032	0.34	0.05%	-0.11	0.20	1.14	0.73	1.76	0.573
2013	CIP + MEM	77	9124	0.84	0	--	--	1	--	--	
	only MEM	107	9094	1.16	0.33%	0.04	0.61	1.39	1.04	1.86	0.026
2009	AMK + MEM	14	23067	0.06	0	--	--	1	--	--	
	only MEM	36	23045	0.16	0.10%	0.03	0.15	2.57	1.39	4.77	0.002
2010	AMK + MEM	3	17578	0.02	0	--	--	1	--	--	
	only MEM	20	17561	0.11	0.10%	0.04	0.15	6.7	1.98	22.43	p<0.001
2011	AMK + MEM	15	15358	0.10	0	--	--	1	--	--	
	only MEM	37	15336	0.24	0.14%	0.05	0.23	2.47	1.35	4.49	0.002
2012	AMK + MEM	3	9868	0.03	0	--	--	1	--	--	
	only MEM	35	9836	0.35	0.3%	0.20	0.45	11.67	3.59	37.92	p<0.001
2013	AMK + MEM	3	7675	0.04	0	--	--	1	--	--	
	only MEM	102	7576	1.33	1.3%	1.03	1.55	34	10.79	107.13	p<0.001

Risk: Risk of resistance; RD: Risk difference; RR: Relative Risk; 95%CI: 95% Confidence interval; p: p value of statistical significance; MEM: Meropenem; CIP: Ciprofloxacin; AMK: Amikacin

Table 3. Risks of resistance according to type of Meropenem use (single versus combined therapy plus Ciprofloxacin or Amikacin) for *P. aeruginosa*. Valencian Community (years 2009-2013).

Year	Type of Meropenem use	Resistant N	Non Resistant N	Risk (%)	RD	95%	CI	RR	95%	CI	p
2009	CIP + MEM	831	6000	12.1	0	--	--	1	--	--	
	only MEM	1034	5797	15.1	2.9%	1.8	4.1	1.24	1.14	1.35	p<0.001
2010	CIP + MEM	659	5571	10.6	0	--	--	1	--	--	
	only MEM	845	5385	13.6	3.0%	1.8	4.1	1.28	1.16	1.41	p<0.001
2011	CIP + MEM	740	6681	10.0	0	--	--	1	--	--	
	only MEM	970	6451	13.1	3.1%	2.1	4.1	1.31	1.20	1.43	p<0.001
2012	CIP + MEM	969	7581	11.3	0	--	--	1	--	--	
	only MEM	1271	7279	14.9	3.5%	2.5	4.5	1.31	1.21	1.41	p<0.001
2013	CIP + MEM	1992	8167	19.6	0	--	--	1	--	--	
	only MEM	2369	7790	23.3	3.7%	2.6	4.8	1.19	1.13	1.25	p<0.001
2009	AMK + MEM	277	6493	4.1	0	--	--	1	--	--	
	only MEM	914	5856	13.5	9.4%	8.5	10.3	3.30	2.90	3.76	p<0.001
2010	AMK + MEM	219	6125	3.4	0	--	--	1	--	--	
	only MEM	817	5527	12.9	9.4%	8.5	10.4	3.73	3.23	4.13	p<0.001
2011	AMK + MEM	226	6993	3.1	0	--	--	1	--	--	
	only MEM	928	6291	12.9	9.7%	8.9	10.6	4.11	3.56	4.73	p<0.001
2012	AMK + MEM	238	7090	3.2	0	--	--	1	--	--	
	only MEM	1101	6227	15	11.8%	10.9	12.7	4.63	4.04	5.30	p<0.001
2013	AMK + MEM	511	7262	6.6	0	--	--	1	--	--	
	only MEM	2000	5773	25.7	19.2%	18	20.3	3.91	3.57	4.29	p<0.001

Risk: Risk of resistance; RD: Risk difference; RR: Relative Risk; 95%CI: 95% Confidence interval; p: p value of statistical significance MEM: Meropenem; CIP: Ciprofloxacin; AMK: Amikacin