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Editorial

Carbapenemases: The never-ending story

Carbapenemasas: la historia interminable

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Carbapenems have traditionally been used to treat infections caused by Gram-negative bacteria resistant to many other agents, as they usually escaped the hydrolytic activity of most clinically relevant β-lactamases and are little affected (in terms of absolute MIC changes) by permeability and active efflux mechanisms. For years, resistance to these agents was mainly confined to non-fermentative Gram-negative bacteria resistant to ertapenem, and a few species (most notably *Stenotrophomonas maltophilia*) with intrinsic resistance to all carbapenems, because of a chromosomal encoded carbapenemase.

In 1991, a transferable carbapenemase was reported in *Pseudomonas aeruginosa* (strain GN17203).[1] Shortly after, OXA-23 (ARI-1)[2] in *Acinetobacter baumannii* and KPC-1 in *Klebsiella pneumoniae*[3] were also described. In the following years, organisms producing plasmid-mediated carbapenemases have rapidly spread worldwide. A pooled analysis of nine studies comparing mortality in infections caused by *Enterobacteriaceae* (including bacteraemia) found that mortality was significantly higher in patients with carbapenem-resistant infections and that a considerable number of deaths was linked to carbapenem resistance, probably because of a greater likelihood that initial empirical therapy was inadequate.[4] This situation has led the WHO to include carbapenem-resistant *A. baumannii*, carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *Enterobacteriaceae* as critical problems in its priority pathogens list for research and development of new antibiotics (accessible in: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf; last accessed Dec 9, 2018). Carbapenemases have caused not only clinical problems but even socio-political issues, as when considering the implication of naming the metallo-β-lactamase (MBL) "from New-Delhi" (NDM) in medical tourism in India,[5] or the pioneering plan by the Israeli Ministry of Health to control at a national level the spread of carbapenem-resistant enterobacteria.[6]

Transferable carbapenemases are included in all four molecular classes of β-lactamases. Their main representatives correspond to variants of KPC (class A, more frequently identified in enterobacteria, but also in *P. aeruginosa*, from USA and some European countries such as Greece and Italy), NDM and VIM (class B or metallo-β-lactamases, MBL; also in enterobacteria and *P. aeruginosa*, with NDM present worldwide and endemic in the Indian subcontinent, and VIM most often identified in Italy, Greece and Russia), oxacillinases (class D, with OXA-48 and related enzymes, particularly frequent in Mediterranean countries, and several other enzymes most common in *A. baumannii*), and CMY-10 (class C, a rare enzyme hydrolyzing imipenem).[7]

In Spain, VIM-1 producing enterobacteria were reported in 2003. All major enzymes have also subsequently been identified. In a multicenter study on enterobacteria in Spain in 2009,[8] only 0.04% of the evaluated organisms (including 0.2% *K. pneumoniae* and 0.001%, *E. coli*) produced a carbapenemase (most frequently VIM-1 and IMP-22), but just four years later[9] these figures increased to 1.7% in *K. pneumoniae* and 0.03% in *E. coli*, with a predominance of OXA-48 observed in the second study, but also VIM enzymes broadly distributed in the country, and KPC variants causing important outbreaks in some centers.[10] The importance of *K. pneumoniae* producing OXA-48 is well documented in the multicenter study in Catalonia by Argente et al. in this issue of EIMC.[11] In two multicenter studies in Spain,[12,13] resistance to carbapenems in *A. baumannii* increased from 48% (2001) to 82% (2010). In 2007, carbapenemase-producing *P. aeruginosa* only represented 0.4%[14] but in a multicenter study in 2015, evaluating (only) extremely drug-resistant isolates, 21% of them produced a carbapenemase (mostly VIM).[15]

Microbiological, epidemiological and clinical factors have contributed to the carbapenemase perfect storm.[16] Genes coding for carbapenemases are commonly located into very efficient mobile elements in conjugative plasmids: Tn4401 (with the bla_{KPC} gene) presents high frequency of transposition. *blaOXA-48* is located in *Tn*1999, which disrupt a gene (*tir*) responsible for coding a cell-to-cell transfer inhibitor protein of broad–host-range conjugative IncL/M plasmids, and increases plasmid transference between microorganisms.[16] These efficient vehicles will ensure distribution of the carbapenemase genes among multiples

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lineages of *K. pneumoniae* and other species, including the so-called high-risk bacterial clones.[17] Argente et al.[11] have documented in their report the importance of the *K. pneumoniae* ST-405 producing OXA-48 in Catalonia, and Cuaical-Ramos et al.,[18] also in this issue of EIMC, document a broad geographical spread of KPC-producing *K. pneumoniae* clones (as defined by pulsed-field gel electrophoresis) and their temporal persistance in different centers in Venezuela. Internationally, there has been a considerable spread of *K. pneumoniae* isolates of the clonal complex 258 with KPC carbapenemases.

In spite of multiple available phenotypic and genotypic methods for carbapenemase detection, CPO might not be easily recognized. This is because of multiple reasons: carbapenem resistance can be unrelated to carbapenemase production, MICs of carbapenems against CPO can be lower than the breakpoint for the susceptible category, and commercial panels of semi-automatic susceptibility testing devices used in many clinical laboratories frequently lack carbapenem concentrations reaching the EUCAST screening breakpoint for CPO (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf). This is clearly presented in the study by Argente et al.,[11] where 50.3% of *K. pneumoniae* with a positive screening test for carbapenemase production actually produced these types of enzymes, and only 23.5% of isolates producing OXA-48 were resistant to imipenem. Failure to detect CPO (in clinical or in surveillance samples) allows these organisms to spread in the hospital, reaching a difficult to solve situation when it is finally recognized. This can even represent serious legal issues.[19] With independence of the level of the hospital hosting a microbiology laboratory, it is necessary that clinical microbiologists can recognize CPO, applying interpretative reading of the antibiogram and having available the needed resources to ensure that efficient detection tools are actually applied, due to the high importance of early intervention with effective control measures. Then, once an isolate is identified as a CPO, characterization of the involved enzyme and definition of the possible clonal relationship of additional isolates can be performed in the same or in a reference (regional, national) laboratory.

CPO frequently produce other β-lactamases and contains additional mechanisms of resistance to multiple antimicrobial families. The association of OXA-48 and CTX-M-15 has been well documented in multiple studies, as in the report by Argente et al.[10] Similarly, Cuaical-Ramos et al.[18] have documented that 27% of KPC-producing *K. pneumoniae* also produce a CTX-M enzyme. This has the major negative impact of reducing available therapeutic options for CPO infections. From another perspective, observation of an ESBL phenotype or piperacillin/tazobactam resistance may alert for the simultaneous production of a poorly hydrolytic carbapenemase (i.e., OXA-48 and other enzymes).

No doubts that using non β-lactam agents to treat infections by CPO will translate, presumably in the short term, into increasing resistance rates to those agents, as already noted for colistin and *K. pneumoniae*.[20] New compounds are expected to contribute to a better therapy of patients with CPO infections.[21] Ceftazidime-avibactam can represent a convenient option against KPC- and OXA-48-producing organisms, imipenem-relebactam and meropenem-vaborbactam are also active against class A (but not OXA-48) enzymes, and aztreonam-avibactam is expected to be clinically useful against MBL-producing CPO. Cefiderocol, plazomycin, eravacycline and other compounds under clinical development will also represent clinically relevant options against CPO infections. Unfortunately, the problem is far to be solved. *K. pneumoniae* resistant to ceftazidime-avibactam have been identified shortly after the introduction of this combination into clinical use, and this has been due to the selection of organisms with a mutated KPC-3.[22] This new mutated enzyme determines an ESBL-like phenotype and the organism appears as carbapenem "susceptible"; however, new studies have shown that carbapenems can select for additional mutants with resistance to both carbapenems and ceftazidime-avibactam.[23] Even worse, in a patient never treated with ceftazidime-avibactam resistance to this compound has emerged because of porin mutations.[24]

New problems can be anticipated as the previously indicated agents are introduced into clinical practice. On the other hand, the universe of new carbapenemases (FRI enzymes, PAD-1, ...) and new alleles of already known enzymes (NDM-21, GES-16, OXA-427, ...) is also still expanding, and the recognition of uncommon enzymes (i.e. GES-, IMP-, ... types) is improving in clinical laboratories. In these circumstances, it would be difficult to anticipate an end to the growing spiral of carbapenemase-mediated resistance.

The increasingly worrying situation of ESBL-producing enterobacteria (one of many negative examples of multiresistance) during the last decades has not translated into efficient plans to control bacteria resistant to antimicrobial agents. It is not much surprising that a new wave of β-lactamases, now capable of inactivating carbapenems (commonly used to treat infections by ESBL-producing organisms), has spread worldwide. Should the rates of carbapenemase-production in *Escherichia coli* reach the level now observed in *K. pneumoniae*, it is anticipated that this problem will be into a more problematic dimension. It is imperative that sufficient resources for recognition, surveillance, control, treatment and research of CPO are immediately available to avoid a disastrous situation with predictable clinical consequences.

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Conflicts of interest

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