

# Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control

Y. Carmeli<sup>1</sup>, M. Akova<sup>2</sup>, G. Cornaglia<sup>3</sup>, G. L. Daikos<sup>4</sup>, J. Garau<sup>5</sup>, S. Harbarth<sup>6</sup>, G. M. Rossolini<sup>7,8</sup>, M. Souli<sup>9</sup> and H. Giamarellou<sup>9</sup>

1) Division of Epidemiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, 2) Hacettepe Universitesi Tıp Fakültesi, İç Hastalıklar Anabilim Dalı, Enfeksiyon Hastalıkları Ünitesi, Ankara, Turkey, 3) Dipartimento di Patologia, Università di Verona, Verona, Italy, 4) First Department of Propaedeutic Medicine, University of Athens, Athens, Greece, 5) Hospital Universitari Mutua de Terrassa, University of Barcelona, Barcelona, Spain, 6) Infection Control Programme, Geneva University Hospitals and Medical School, Geneva, Switzerland, 7) Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, 8) Dipartimento dei Servizi, Unita' Operativa di Microbiologia e Virologia, Azienda Ospedaliera-Universitaria Senese, Siena, Italy and 9) Fourth Department of Internal Medicine, Athens University Medical School, Athens, Greece

## Abstract

Although the rapid spread of carbapenemase-producing Gram-negatives (CPGNs) is providing the scientific community with a great deal of information about the molecular epidemiology of these enzymes and their genetic background, data on how to treat multidrug-resistant or extended drug-resistant carbapenemase-producing *Enterobacteriaceae* and how to contain their spread are still surprisingly limited, in spite of the rapidly increasing prevalence of these organisms and of their isolation from patients suffering from life-threatening infections. Limited clinical experience and several *in vitro* synergy studies seem to support the view that antibiotic combinations should be preferred to monotherapies. But, in light of the data available to date, it is currently impossible to quantify the real advantage of drug combinations in the treatment of these infections. Comprehensive clinical studies of the main therapeutic options, broken down by pathogen, enzyme and clinical syndrome, are definitely lacking and, as carbapenemases keep spreading, are urgently needed. This spread is unveiling the substantial unpreparedness of European public health structures to face this worrisome emergency, although experiences from different countries—chiefly Greece and Israel—have shown that CPGN transmission and cross-infection can cause a substantial threat to the healthcare system. This unpreparedness also affects the treatment of individual patients and infection control policies, with dramatic scarcities of both therapeutic options and infection control measures. Although correct implementation of such measures is presumably cumbersome and expensive, the huge clinical and public health problems related to CPGN transmission, alongside the current scarcity of therapeutic options, seem to fully justify this choice.

**Keywords:** Antimicrobial resistance, carbapenemases, Gram-negatives, infection control, metallo- $\beta$ -lactamases, review, therapy

*Clin Microbiol Infect* 2010; **16**: 102–111

**Corresponding author and reprint requests:** G. Cornaglia, Dipartimento di Patologia, Università di Verona, Strada Le Grazie, 8, 37124 Verona, Italy  
**E-mail:** giuseppe.cornaglia@univr.it

## Introduction

The rapid spread of carbapenemase-producing Gram-negatives (CPGNs) is providing the scientific community with a great deal of information about the molecular epidemiology of carbapenemases and their genetic background. At the same time, this spread is unveiling the substantial unpreparedness of European public health structures to face this worrisome emergency. This unpreparedness equally affects

the treatment of individual patients and infection control policies, with dramatic scarcities in both therapeutic options and infection control measures.

## Clinical and Therapeutic Issues

Carbapenemase production is normally associated with generalized resistance to carbapenems, penicillins and cephalosporins, and these strains usually also harbour mechanisms

of resistance to aminoglycosides and quinolones. This multi-drug resistance often leaves tigecycline (apart from the treatment of *Pseudomonas* spp.) and colistin as the only agents available.

#### Preclinical data

The few compounds to which carbapenemase-producing non-fermenters most often prove susceptible in *in vitro* tests have been used in time-kill studies and animal models of infections with these pathogens, but, overall, the resulting data are limited and rather patchy.

Aztreonam administered in high doses was found to significantly decrease the viable counts of VIM-2-producing *Pseudomonas aeruginosa* in lung tissue of treated rats [1], but the accuracy of the model is seriously put into question by the fact that all of the other  $\beta$ -lactams tested produced a similar effect, despite their high MICs. In fact, time-kill studies on 12 metallo- $\beta$ -lactamase (MBL)-producing *P. aeruginosa* isolates performed with aztreonam, either alone or in combination with ceftazidime and amikacin, revealed bactericidal activity against one and eight isolates, respectively, whereas colistin proved to be bactericidal against all 12 isolates in the same study [2]. The combination of aztreonam and various other  $\beta$ -lactams was synergistic against four of nine MBL-producing *P. aeruginosa* isolates and against four of five MBL-producing *Acinetobacter baumannii* isolates, whereas antagonism was noted in two isolates and one isolate, respectively [3]. In neutropenic mice infected with IMP-type-producing *P. aeruginosa*, high-dose polymyxin B, but not imipenem or aztreonam, significantly improved survival as compared with saline-treated controls [4].

Colistin was bactericidal against nine OXA-58-producing *A. baumannii* isolates, and synergism was observed in some of these isolates with combinations of rifampin and imipenem, rifampin and ampicillin-sulbactam, or colistin and rifampin [5].

In a mouse pneumonia model, imipenem was still the best option for infections caused by *A. baumannii* with moderate levels of imipenem resistance, preferably combined with aminoglycosides. For isolates that were highly resistant to imipenem, a combination of rifampin and imipenem, tobramycin or colistin proved useful [6].

As the current breakpoints do not classify many carbapenemase-producing *Enterobacteriaceae* as 'resistant' to carbapenems but only as being endowed with reduced susceptibility to these compounds (MIC  $\leq 4$  mg/L), the additional problem arises of whether *in vivo* non-susceptibility actually predicts *in vivo* resistance to treatment. Thus, for these pathogens, carbapenem compounds have also been

considered as potential therapeutic agents in time-kill studies and animal models of infection.

The activity of imipenem against VIM-1-producing *Klebsiella pneumoniae* was evaluated in a thigh infection model in neutropenic mice. The efficacy of imipenem was significantly higher against the control (i.e. non-VIM-1-producing) isolate. However, a high imipenem dose yielded an appreciable effect against the VIM-1-producing isolates with a low imipenem MIC (2–4 mg/L), suggesting the possible usefulness of increased drug doses against VIM-producing carbapenem-susceptible isolates [7].

Another study evaluated the efficacy of either carbapenems or aztreonam, in a rabbit model of peritoneal abscess, by using an extended-spectrum  $\beta$ -lactamase-negative, carbapenem-susceptible, VIM-1-producing *Escherichia coli* clinical isolate (MICs of imipenem, meropenem, ertapenem and aztreonam of 1,  $\leq 0.25$ , 1.5, and  $\leq 0.25$ , respectively). Imipenem, meropenem, ertapenem or aztreonam showed a statistically significant reduction of viable colonies in treated animals as compared with controls. Among the three carbapenems, ertapenem produced the highest reduction in viable counts, probably due to a longer time above the MIC. Treatment with aztreonam resulted in a significantly lower mortality than in controls, and in 26.7% of the animals in the aztreonam group there were no viable colonies after treatment [8].

*In vitro* studies have shown a strong inoculum effect with carbapenems when the susceptibility of VIM-1-producing *K. pneumoniae* has been tested [7,9]. The killing activity of carbapenems against VIM-1-producing *K. pneumoniae* was evaluated in two studies. The first study considered seven *K. pneumoniae* isolates with variable susceptibilities to carbapenems (MIC range, 1–64 mg/L). Despite initial killing (at 2 h), regrowth was observed with all tested isolates after 24 h of incubation with 8–16 mg/L imipenem, meropenem or ertapenem, irrespective of the MIC levels. In the same study, aztreonam exhibited bactericidal activity against all susceptible isolates [9]. In contrast, in the second study, imipenem exhibited bactericidal activity against some of the tested VIM-1-producing *K. pneumoniae* clinical isolates with MICs  $\leq 4$  mg/L [10].

As far as newer carbapenem compounds are concerned, *in vitro* data suggest that doripenem does not offer any advantage over the older compounds in this class against many carbapenemase producers [11], with the notable exception of carbapenem-resistant *A. baumannii* producing OXA-58 [12].

Among the non-carbapenem compounds, both tigecycline and polymyxins have been carefully evaluated, because they lack cross-resistance with  $\beta$ -lactams.

The MIC<sub>90</sub> of tigecycline for 54 VIM-producing *K. pneumoniae* isolates [13] and for 109 *Enterobacteriaceae* isolates producing MBLs [14] proved to be 2 mg/L, whereas the MIC<sub>90</sub> for 104 *Enterobacteriaceae* isolates producing either MBLs or KPC-type enzymes [15] and for 95 KPC-producing *K. pneumoniae* isolates [16] was 1 mg/L. In the study of Bratu *et al.* [16], tigecycline proved to be bactericidal against two of 16 isolates investigated by time-kill analysis. Resistance to tigecycline is, regrettably, increasing in some geographical areas such as Greece, as only 65.4% of 50 KPC-producing *K. pneumoniae* strains isolated from Greece during the period 2007–2008 showed an MIC of  $\leq 2$  mg/L [17].

The *in vitro* activity of colistin for VIM-producing *K. pneumoniae* isolates from Greece has been studied, and the MIC<sub>90</sub> was 16 mg/L (Souli *et al.*, 47th IDSA, 2009, Abstract 218). In the aforementioned study of Bratu *et al.* [16], polymyxin B showed bactericidal activity at 2 and 4 mg/L against most KPC-producing *K. pneumoniae* isolates (MIC<sub>90</sub>, 2 mg/L). In the aforementioned study by Castanheira *et al.* [15], polymyxin B exhibited an MIC<sub>90</sub> of  $>4$  mg/L, and it was active against 88% of the tested isolates.

Given the scarcity of possible options for monotherapy and the uncertainty regarding animal data, the effects of antibiotic combinations have been studied using both MBL-producing and KPC-producing isolates.

The combination of imipenem and colistin has been studied with a time-kill methodology involving 42 genetically distinct VIM-1-producing *K. pneumoniae* isolates from Greek hospitals. This combination showed synergy against 50% of colistin-susceptible isolates and against 50% of isolates with low-level resistance to colistin (MIC, 3–4 mg/L), but it was antagonistic against 55.6% of colistin-susceptible isolates (MIC, 2 mg/L). The killing activity was significantly better against isolates susceptible to both agents and isolates non-susceptible to imipenem but susceptible to colistin [10].

The combination of polymyxin B and either rifampin or imipenem was tested against 16 KPC-producing *K. pneumoniae* isolates by time-kill analysis. A synergistic effect was noted for most of the tested isolates, whereas the combination of polymyxin B (0.5  $\times$  MIC) and imipenem was antagonistic for three isolates [16].

Fosfomycin was tested by time-kill methodology alone and in combination with either meropenem, gentamicin or colistin against eight clinical isolates of KPC-producing *K. pneumoniae*. Fosfomycin alone was not bactericidal. All combinations exhibited indifference, with the exception of the combination of fosfomycin and meropenem, which showed synergy against two of the tested isolates (fosfomycin MIC, 16 mg/L; meropenem MIC, 32–64 mg/L) (Souli *et al.*, 47th IDSA, 2009, Abstract 218).

A new non- $\beta$ -lactam  $\beta$ -lactamase inhibitor, NXL104, in combination with various  $\beta$ -lactam antibiotics, was shown to be active against KPC-producing *Enterobacteriaceae* but not against those producing IMP or VIM metalloenzymes [18–20]. Although *in vitro* data are promising, the clinical development of this compound is still in its early phases, and the clinical usefulness remains to be determined. Among the other promising agents that are also in the early phases of development are the new carbapenem compound tompenem [21] and various chelating agents, such as hLFI-11 [22].

#### Clinical data

Studies evaluating potential risk factors for colonization or infection due to MBL-producing non-fermenters have identified antibiotic use and prolonged hospital stay as independent predictors [23,24]. Previous use of either  $\beta$ -lactams [25,26] or quinolones [25,27] has been identified as a risk factor. Analysis of the antimicrobial chemotherapy received by patients before isolation of MBL-positive strains showed many of them to have been given non-carbapenem  $\beta$ -lactams. The frequent co-resistance to other classes of antibiotics observed in MBL producers, owing to the simultaneous presence of additional resistance determinants often carried on integrons, such as genes for aminoglycoside-modifying enzymes, and/or mutations that upregulate efflux systems, underlines the possibility that MBLs may also be co-selected by the clinical use of unrelated classes of antibiotics [28]. Nevertheless, it has been emphasized that early recognition of MBL-producing isolates and rigorous infection control precautions to prevent their transmission might both be more important than curbing antibiotic consumption for controlling MBLs in hospitals [26].

Studies evaluating the influence of MBL production on mortality in *P. aeruginosa* infections have revealed higher mortality rates (hazard ratio 1.55, 95% CI 1.06–2.27), most likely related to the severity of these infections and to delays in appropriate antimicrobial therapy [29]. The attributable mortality of MBL-producing *P. aeruginosa* bacteraemia in a Brazilian hospital was reported to be as high as 71.4%, as the same percentage of patients received inappropriate empirical therapy [30].

The reported mortality rates associated with infections caused by MBL-producing *Enterobacteriaceae* range from 18.8% [31] to 66.7% [32]. Daikos *et al.* [33] suggested that the mortality rate in bloodstream infections caused by VIM-1-producing *K. pneumoniae* isolates exhibiting an MIC of 4 mg/L was lower than that associated with isolates exhibiting an MIC of  $>4$  mg/L (13.3 vs. 53.8%), but not significantly different from that in the control group of patients infected with MBL-negative strains. In that report, resistance to

carbapenems and a high APACHE II score were independently associated with mortality.

KPC-producing *Enterobacteriaceae* infections are usually systemic and not site-specific. Risk factors for acquiring infections with these bacteria include prolonged hospitalization, intensive-care unit stay, invasive devices, immunosuppression, and previous use of various antibiotics, including, but not limited to, carbapenems [34–36]. Both in Israel and in the USA, a high mortality rate has been attributed to KPC-producing *K. pneumoniae* infections, ranging from 38% to 57% [37,38]. A lower attributable mortality (22.2% and 27.8%) was reported from Greece [17,39]. Mortality has been reported to be higher for patients infected with imipenem-resistant KPC-producing *Enterobacter* spp. than for those infected with imipenem-susceptible strains [40].

As CPGNs become increasingly prevalent and cause life-threatening infections, clinical data on optimal treatment are still surprisingly scarce. Although many studies have focused on the efficacy of either individual drugs or various combinations against multidrug-resistant (MDR) or even extended drug-resistant (XDR) non-fermenters, very few have investigated the underlying mechanisms of resistance. Thus, data on actual carbapenemase producers are limited.

Infections caused by either *P. aeruginosa* or *A. baumannii* producing a carbapenemase pose an enormous challenge, because these strains are very frequently multiresistant and are often only susceptible to colistin. In fact, polymyxins have emerged as the rational clinical option for these infections, and the successful treatment with colistin of maxillary sinusitis, orbital cellulitis and pneumonia due to MBL-producing *P. aeruginosa* in a neutropenic patient has been reported [41]. However, a recent report of the development of hetero-resistance to colistin in an *in vitro* pharmacokinetic/pharmacodynamic model seriously put into question the use of this compound alone as a sound therapeutic option [42].

As compared to monotherapy, combination therapy with several classes of antibiotics for the treatment of CPGN infections has shown greater activity and delayed development of resistance [43]. A number of recent studies have shown synergy between rifampin and either colistin or a carbapenem (including imipenem, meropenem and doripenem). These combinations achieved bactericidal activity at one-fourth of the MIC for *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, even in the presence of resistance to the carbapenems and rifampin alone [5] (Urban et al., 49th ICAAC, 2009, Abstract E-1447). On an anecdotal basis, the successful treatment with ciprofloxacin and gentamicin of a prosthetic valve endocarditis due to MBL-producing *P. aeruginosa* has been recently reported [44]. The *in vitro* findings of another recent study provide support for the idea that fosfomycin

might be a therapeutic option for the treatment of infections due to MDR strains of *P. aeruginosa* [45], but there is only one published case report of successful treatment of prostaticitis due to VIM-2-producing *P. aeruginosa* with the combination of fosfomycin and aztreonam [46]. Unfortunately, synergistic effects are strain-specific, and *ad hoc* combinations of drugs must be tested against each clinical isolate in order to obtain indications of potential usefulness in therapy.

Carbapenemase-producing *Enterobacteriaceae* often display an XDR phenotype and, in many cases, the therapeutic options are extremely limited. In a report from Australia, however, the majority of *Enterobacteriaceae* isolates producing IMP-4 were susceptible to quinolones, and four patients were successfully treated with this class of agent [47].

Recently, some experience has been gained with tigecycline in the treatment of these infections. Tigecycline has been used either alone or in combination with another agent in a limited number of patients. Among six patients with pneumonia or bloodstream infections, two patients treated with tigecycline combined with polymyxin B or E survived, another three had an unfavourable outcome, and one had microbiological failure and an uncertain clinical outcome [48]. In an observational retrospective study on the *in vivo* efficacy of tigecycline administered as the only active antimicrobial for the treatment of infections caused by MDR pathogens, a successful clinical response was noted in nine of 13 patients (69.2%) infected with MBL-producing *Enterobacteriaceae* (MIC range, 0.019–3 mg/L) [49].

In a case series including 17 seriously ill patients, 12 patients were treated successfully with colistin alone (four patients) or in combination with either a carbapenem (six patients) or an active aminoglycoside (two patients) [31]. In a recent observational study of 67 patients with bloodstream infection caused by VIM-producing *K. pneumoniae* [50], 49 (73.1%) received 'appropriate' empirical therapy (at least one agent had proved active *in vitro*) and 18 (26.9%) 'inappropriate' empirical therapy (none of the administered antibiotics had shown *in vitro* activity). Among those who received 'appropriate therapy', 12 received combination therapy with two active drugs (nine received meropenem and three imipenem in combination with either colistin (eight patients) or an active aminoglycoside (four patients)), and 37 received therapy with one active drug (nine meropenem, five imipenem, 15 colistin, and eight an active aminoglycoside). The lowest mortality rate (8.3%) was observed in the group of patients who had received combination therapy with two active drugs, one of which was a carbapenem and the other of which was either colistin or an active aminoglycoside, whereas therapy with one active drug (a carbapenem, colistin or an aminoglycoside) resulted in a mortality rate (27%)

similar to that observed in patients who had received therapy with no active drug (27.8%). Among 18 patients suffering from a severe infection caused by KPC-producing *K. pneumoniae*, 12 (66.7%) were successfully treated with colistin, either as the only active antimicrobial (six patients) or in combination with an active aminoglycoside (one patient), tigecycline (three patients), or a carbapenem in conjunction with catheter removal (one patient) [17].

The aforementioned study by Falagas *et al.* [45] has recently supported the idea of fosfomycin being a possible therapeutic option for MDR *K. pneumoniae*. Eleven adult intensive-care unit patients with infections caused by carbapenem-resistant *K. pneumoniae* received intravenous fosfomycin (2–4 g every 6 h) in combination with other antibiotics; all patients had good bacteriological and clinical outcome of infection, and none of them experienced fosfomycin-related adverse events [51].

Carbapenem MICs for carbapenemase-producing *Enterobacteriaceae* are often in the susceptible range, raising the critical question of whether carbapenems might be active *in vivo* for treating infections caused by such organisms. Anecdotal reports have claimed microbiological and clinical responses in patients infected with MBL-positive, but *in vitro* carbapenem-susceptible, *K. pneumoniae* strains after treatment with a carbapenem [52], although no details were given of either doses or administration schedule. On the contrary, the aforementioned data from Greece [50] showed that monotherapy with a carbapenem was not superior to inappropriate therapy, in terms of 14-day mortality. On the basis of this experience, it remains doubtful whether monotherapy with a carbapenem would be effective in the treatment of such infections. On the other hand, carbapenems in combination with another active agent (colistin or an aminoglycoside) may provide some therapeutic benefit in treating infections due to VIM-positive carbapenem-susceptible *Enterobacteriaceae*.

In this respect, the issue of either reporting such isolates as fully resistant to carbapenems or taking the respective MICs at face value should remain open. In a recent study from the USA [53], frequent (56%) clinical and microbiological failures were observed with both imipenem and meropenem when they were used to treat infections caused by *K. pneumoniae* isolates that yielded imipenem-susceptible results but were subsequently discovered to be KPC-positive by PCR. Three of the unsuccessfully treated patients had tracheobronchitis; thus, residual colonization after therapy could not be excluded. In seven of ten patients whose isolates were initially reported as carbapenem-resistant, treatment with either tigecycline or an aminoglycoside was successful. Meropenem monotherapy was successful in one

additional patient, giving a success rate of 80%. It is difficult to draw significant conclusions, owing to the small number of patients and to the retrospective nature of the study; however, the data are in overall accordance with previous reports.

## Infection Control

Experiences from different countries—with special reference to Greece [33] and Israel [54]—have shown that CPGN transmission and cross-infection can be responsible for increased adverse outcomes, with a substantial threat to the healthcare system. Unfortunately, the lack of adequate and timely reaction has also been commonly observed.

Suggested control measures differ substantially between settings with sporadic occurrence or complete absence of CPGNs and countries with ongoing CPGN outbreaks or endemic CPGNs. The suggested control plan is mostly generic, and can be applied to any threatening MDR bacterium associated with high case-fatality rates, and that has a high potential for spread in healthcare settings and then further into the community. The rationale for the plan is that early detection and intervention has a much higher likelihood of aborting the eminent epidemic; although this requires a preparedness plan and resource allocation, in the long term it is much less costly than confronting an epidemic. When endemicity has been established, a regionally coordinated effort is required in order to contain the epidemic.

### Settings with sporadic occurrence or complete absence of CPGNs

Efficient CPGN control mandates that a preparedness plan has been developed and implemented for different healthcare settings, so as to enable the initiation of urgent and rapid action when a first case has been detected and microbiologically confirmed. The overall focus of infection control measures in these settings should be complete eradication, according to the classic 'search and destroy strategy', which is based on concepts that have been previously described [55,56]. However, this approach needs to be tailored to the local CPGN epidemiology.

Reliable detection of the first index case in a given region or hospital is crucial for the implementation of timely interventions. Microbiological detection may be challenging [28]; thus, all microbiology laboratories should have a highly sensitive screening method in place. If this crucial step is overlooked, the detection of CPGN clusters will be delayed,

leading to difficult-to-eradicate outbreaks, as has already been observed in several countries (Wendt *et al.*, 19th Society of Healthcare Epidemiology of America Scientific Meeting, 2009, Abstract 365). A reference laboratory should provide back-up support, with rapid confirmation of suspected CPGN cases. Communication channels should be established in advance, in order to facilitate rapid notification and feedback.

Throughout Europe, active screening policies need to be clearly established, by defining patients at high risk of CPGN carriage or infection. At this moment, these risk factors mainly consist of previous contacts with medical facilities—such as hospitals, dialysis units, or long-term-care facilities [57]—with known ongoing outbreaks of CPGNs. For these patients, pre-emptive isolation while the screening results are awaited is highly recommended.

The primary surveillance screening site is the stool or rectal swab; limited data indicate that surveillance screening of stool specimens, rectal swabs or perirectal swabs might produce higher yields than testing of other body sites (e.g. nares or skin) [58]. In patients with indwelling devices, specimens from the related site should also be screened. Skin swabs, urine and sputum could also be checked in certain patient groups, e.g. patients with chronic wounds, indwelling urinary catheters, or endotracheal intubation.

An action plan for rapid implementation of infection control measures should include all items listed in Table 1.

Isolation precautions should be implemented and strictly applied, although, in several settings, simple contact isolation was not sufficient to stop local outbreaks (Schechner V, TASMIC Cre Group, 48th ICAAC and 46th IDSA, 2008, Abstract K-3508) [59], and cohorting of patients with a dedicated staff is warranted. Long-term follow-up should be provided, with re-admission alerts of identified CPGN carriers. Timely dissemination of information at both the local level and the national level is a cornerstone for early response. As things stand now, there is no strong evidence in favour of using either topical or systemic antibiotic decolonization treatment.

**TABLE 1. Suggested action plan for rapid implementation of infection control measures in settings with sporadic occurrence or complete absence of carbapenemase-producing Gram-negatives**

Screening of all patients in contact with an index case  
Epidemiological investigation with root cause analysis in cases of nosocomial cross-transmission events with more than two secondary cases  
Measures to keep staff and hospital administration informed  
Stringent infection control aimed at containment and ultimate eradication of nosocomial clusters  
Coordination and supervision by public health authorities

### Settings with endemic CPGNs or ongoing regional outbreaks

Once CPGNs have become prevalent in a given region or country, stringent control measures may become more cumbersome and expensive. Nevertheless, considering the huge clinical and public health problems related to CPGN transmission, a stringent and intensive control programme seems to be justified, even for regions or countries with ongoing CPGN outbreaks or where CPGNs are already endemic.

These control measures should aim for maximum containment of CPGNs, and must include a multifaceted approach with different components, as summarized in Table 2. Crucial to a successful CPGN control programme is a national task force coordinated and supported by a central public health authority with competence in hospital infection control. The aims of this task force are multifaceted and include top priority action items as listed in Table 3.

As suggested above for the sporadic occurrences, one or more reference laboratories should play a crucial role in containing large-scale outbreaks, by providing the services listed in Table 3.

At the local level, strong commitment on the part of the hospital administration is necessary to guarantee the success

**TABLE 2. Suggested control measures for countries with ongoing carbapenemase-producing Gram-negative (CPGN) outbreaks or endemic CPGNs**

At the national level  
Establishment of a national task force, supported by the Ministry of Health  
Isolation guidelines for carriers—required for all acute-care hospitals  
Monthly progress reports about CPGN control for concerned institutions  
Evaluation of concerned hospitals and identification of problem areas by a public health agency with competence in infection control  
At the hospital level  
Physical separation of carriers from non-carriers  
Dedicated staff  
Active surveillance of high-risk patients  
Training and measures to keep staff and hospital administration informed  
Ongoing CPGN surveillance with prospective data collection and daily census of CPGN carriers

**TABLE 3. National organizations and their priorities for action in countries with ongoing carbapenemase-producing Gram-negative (CPGN) outbreaks or endemic CPGNs**

National task force  
Policy-making and communication with hospital administrations  
Development of stringent and detailed CPGN control guidelines  
Preparation of intervention tools  
Supervision of control measures and preparation of corrective actions in the case of ongoing institutional outbreaks without adequate preventive measures  
Active surveillance with rapid feedback at a regional level and national level  
Reference laboratories  
Confirmation of suspected CPGN cases  
Evaluation of molecular epidemiology and establishment of clonality  
Detection of new resistance mechanisms  
Development of laboratory manuals with descriptions of adequate methods  
Quality assurance for clinical microbiology laboratories

of the aforementioned containment measures, by providing logistic and financial support in close cooperation with the national task force.

Importantly, infection control measures have to be adapted to local conditions and specific hospital sectors, by taking into account the prevalence and incidence of CPGN carriage and infection. Training and detailed information has to be provided to all concerned healthcare workers. Clinicians have to be made aware of the problem and should actively participate in the process, so that routine patient-care activities can continue without compromising infection control measures.

Close collaboration with the microbiology laboratory is crucial, given the need for highly specific and sensitive CPGN detection, from both clinical and epidemiological samples, and for rapid reporting. After an initial containment phase, each new hospital-acquired case should be investigated in depth. In order to identify previously known CPGN carriers and achieve long-term containment of CPGN spread, re-admission alerts based on electronic files or chart flagging are crucial. Information transfer between institutions should be standard procedure whenever a CPGN patient is transferred from one hospital to another.

Finally, it is worth underscoring the role of nursing homes as possible reservoirs of CPGNs [60]. Although data are still scarce, these institutions are likely to represent for carbapenemases the same problem recently pointed out for ESBLs [61]. Studies to assess the prevalence and risk factors for CPGN carriage are urgently needed, alongside public health measures addressing the specific needs of this reservoir population.

## Conclusions

Information on how to treat infections with MDR or XDR CPGNs and how to contain their spread is still surprisingly limited, in spite of the rapidly increasing prevalence of these organisms and of their isolation from patients with life-threatening infections.

Clinical data are still scarce, and they are obtained from either MBL-producing or KPC-producing isolates (and thus not applicable to both). The internal diversity of MBLs is not taken into consideration, as most studies obviously come from areas where VIM enzymes (with special reference to VIM-1) are prevalent. Also, data on *Enterobacteriaceae* refer almost exclusively to *Klebsiella* isolates, and, although they are likely to be similar (or, at least, not worse) in *E. coli*, they do not reflect the current diffusion of carbapenemases among many different *Enterobacteriaceae*

species. In particular, it is worth bearing in mind that neither tigecycline nor polymyxin has activity against *Proteaceae*, among which carbapenemases are being isolated at increasing frequency [62,63].

Limited clinical experience [31,50] and several *in vitro* synergy studies [10] (Souli *et al.*, 47th IDSA, 2009, Abstract 218) seem to support the view that antibiotic combinations should be preferred to monotherapies. But, in light of the data available to date, it is currently impossible to quantify the real advantage of drug combinations in the treatment of these infections.

Comprehensive clinical studies of the main therapeutic options, broken down by pathogen, enzyme and clinical syndrome, are definitely lacking and, as carbapenemases continue to spread, are urgently needed. Although any extrapolation of results from the animal model to humans calls for great caution, optimal management should include double or triple combinations of antimicrobials shown to be synergistic against a given isolate. The best combination is always strain-dependent, and could include colistin, rifampin, a carbapenem, or an aminoglycoside.

In the meantime, containment of the spread of CPGNs essentially relies on implementing strict infection control measures. Experiences from different countries—chiefly Greece and Israel—have shown that CPGN transmission and cross-infection can cause a substantial threat to the healthcare system, with increased adverse outcomes. Unfortunately, not all hospitals concerned have reacted adequately and early enough to counter this emerging threat to public health. Therefore, all European countries should be made aware of the problem and should have a preparedness plan ready for implementation at a national level.

For settings with sporadic occurrence or complete absence of CPGNs, a preparedness plan should ensure early detection of first cases on the basis of reliable screening methods, reference laboratories that are prepared for the confirmation of suspected cases, and active screening of high-risk patients transferred from countries or institutions with epidemic or endemic CPGN occurrence. Infection control measures in these settings should be based on the classic 'search and destroy strategy'.

For regions or countries in which CPGNs have become endemic, stringent control measures should aim for maximum containment of CPGNs. Although correct implementation of such measures is presumably cumbersome and expensive, the huge clinical and public health problems related to CPGN transmission, alongside the current scarcity of therapeutic options, seem to fully justify this choice.

## Acknowledgements

The authors would like to thank M. Schwaber, Tel Aviv, for his help in outlining the control strategies.

## Transparency Declaration

This article was written as a follow-up to an expert meeting on carbapenemases held in Athens, Greece, on 11–12 December 2008, which was solely funded by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and was organized through the ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS). The authors declare the absence of any commercial relationship or potential conflict of interest of any nature that might be related to the present article.

## References

- Bellais S, Mimos O, Léotard S et al. Efficacy of beta-lactams for treating experimentally induced pneumonia due to a carbapenem-hydrolyzing metallo-beta-lactamase-producing strain of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2002; 46: 2032–2034.
- Oie S, Fukui Y, Yamamoto M et al. In vitro antimicrobial effects of aztreonam, colistin, and the 3-drug combination of aztreonam, ceftazidime and amikacin on metallo  $\beta$ -lactamase-producing *Pseudomonas aeruginosa*. *BMC Infect Dis* 2009; 9: 123.
- Sader HS, Rhomberg PR, Jones RN. In vitro activity of beta-lactam antimicrobial agents in combination with aztreonam tested against metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Chemother* 2005; 17: 622–627.
- Miyajima Y, Hiramatsu K, Mizukami E et al. In vitro and in vivo potency of polymyxin B against IMP-type metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2008; 32: 437–440.
- Tripodi M-F et al. Comparative activities of colistin, rifampicin, imipenem and sulbactam/ampicillin alone or in combination against epidemic multidrug resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2007; 30: 537–540.
- Montero A, Ariza J, Corbella X et al. Antibiotic combinations for serious infections caused by carbapenem-resistant *Acinetobacter baumannii* in a mouse pneumonia model. *J Antimicrob Chemother* 2004; 54: 1085–1091.
- Daikos GL, Panagiotakopoulou A, Tzelepi E et al. Activity of imipenem against VIM-1 metallo- $\beta$ -lactamase-producing *Klebsiella pneumoniae* in the murine thigh infection model. *Clin Microbiol Infect* 2007; 13: 202–205.
- Souli M, Konstantinidou E, Tzepe I et al. Are carbapenems effective against carbapenem-susceptible, metallo-b-lactamase producing *Enterobacteriaceae*? Results from an animal model. *J Antimicrob Chemother* (submitted).
- Panagiotakopoulou A, Daikos GL, Miriagou V et al. Comparative in vitro killing of carbapenems and aztreonam against *Klebsiella pneumoniae* producing VIM-1 metallo- $\beta$ -lactamase. *Int J Antimicrob Agents* 2007; 29: 360–362.
- Souli M, Rekatsina P, Chryssouli Z et al. Does the combination of imipenem and colistin *in vitro* exceed the problem of resistance in metallo- $\beta$ -lactamase-producing *Klebsiella pneumoniae* isolates? *Antimicrob Agents Chemother* 2009; 53: 2133–2135.
- Mushtaq S, Ge Y, Livermore DL. Comparative activities of doripenem versus isolates, mutants, and transconjugants of *Enterobacteriaceae* and *Acinetobacter* spp. with characterized  $\beta$ -lactamases. *Antimicrob Agents Chemother* 2004; 48: 1113–1119.
- Marti S, Sánchez-Céspedes J, Alba V, Vila J. In vitro activity of doripenem against *Acinetobacter baumannii* clinical isolates. *Int J Antimicrob Agents* 2009; 33: 181–182.
- Souli M, Kontopidou FV, Koratzanis E et al. In vitro activity of tigecycline against multiple-drug-resistant, including pan-resistant, Gram-negative and Gram-positive clinical isolates from Greek hospitals. *Antimicrob Agents Chemother* 2006; 50: 3166–3169.
- Pliatsika V, Afkous Z, Protonotariou E, Sofianou D. In vitro activity of tigecycline against metallo-b-lactamase-producing *Enterobacteriaceae*. *J Antimicrob Chemother* 2007; 60: 1406–1407.
- Castanheira M, Sader HS, Deshpande LM et al. Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase- and metallo- $\beta$ -lactamase-producing *Enterobacteriaceae*: report from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother* 2008; 52: 570–573.
- Bratu S, Tolaney P, Karumudi U et al. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. *J Antimicrob Chemother* 2005; 56: 128–132.
- Souli M, Galani I, Antoniadou A et al. An outbreak of KPC-2-producing *Klebsiella pneumoniae* in a Greek university hospital: molecular characterization, epidemiology and outcomes. *Clin Infect Dis* 2009 (in press).
- Livermore DM, Mushtaq S, Warner M et al. NXL104 combinations versus *Enterobacteriaceae* with CTX-M extended-spectrum beta-lactamases and carbapenemases. *J Antimicrob Chemother* 2008; 62: 1053–1056.
- Endimiani A, Choudhary Y, Bonomo RA. In vitro activity of NXL104 in combination with beta-lactams against *Klebsiella pneumoniae* isolates producing KPC carbapenemases. *Antimicrob Agents Chemother* 2009; 53: 3599–3601.
- Stachyra T, Levasseur P, Péchereau MC et al. In vitro activity of the (beta)-lactamase inhibitor NXL104 against KPC-2 carbapenemase and *Enterobacteriaceae* expressing KPC carbapenemases. *J Antimicrob Chemother* 2009; 64: 326–329.
- Koga T, Sugihara C, Kakuta M et al. Affinity of tomopenem (CS-023) for penicillin-binding proteins in *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009; 53: 1238–1241.
- Dijkshoorn L, Brouwer CP, Bogaards SJ et al. The synthetic N-terminal peptide of human lactoferrin, hLF(1–11), is highly effective against experimental infection caused by multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2004; 48: 4919–4921.
- Zhang JF, Chen BL, Xin XY et al. Carbapenem resistance mechanism and risk factors of *Pseudomonas aeruginosa* clinical isolates from a university hospital in Xi'an, China. *Microb Drug Resist* 2009; 15: 41–45.
- Tam VH, Chang KT, LaRocco MT et al. Prevalence, mechanisms, and risk factors of carbapenem resistance in bloodstream isolates of *Pseudomonas aeruginosa*. *Diagn Microbiol Infect Dis* 2007; 58: 309–314.
- Cornaglia G, Akova M, Amicosante G et al. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. *Int J Antimicrob Agents* 2007; 29: 380–388.
- Zavacki AP, Barth AL, Gaspareto PB et al. Risk factors for nosocomial infections due to *Pseudomonas aeruginosa* producing metallo-



- beta-lactamase in two tertiary-care teaching hospitals. *J Antimicrob Chemother* 2006; 58: 882–885.
27. Nouér SA, Nucci M, de-Oliveira MP *et al.* Risk factors for acquisition of multidrug-resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamase. *Antimicrob Agents Chemother* 2005; 49: 3663–3667.
  28. Miriagou V, Cornaglia G, Edelstein M *et al.* Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. *Clin Microbiol Infect* 2010; 16: 112–122.
  29. Zavascki AP, Barth AL, Gonçalves AL *et al.* The influence of metallo-beta-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2006; 58: 387–392.
  30. Marra AR, Pereira CA, Gales AC *et al.* Bloodstream infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: epidemiology, microbiology, and clinical outcomes. *Antimicrob Agents Chemother* 2006; 50: 388–390.
  31. Souli M, Kontopidou FV, Papadomichelakis E *et al.* Clinical experience of serious infections caused by *Enterobacteriaceae* producing VIM-I metallo-beta-lactamase in a Greek university hospital. *Clin Infect Dis* 2008; 46: 847–854.
  32. Herbert S, Halvorsen DS, Leong T *et al.* Large outbreak of infection and colonization with gram-negative pathogens carrying the metallo-beta-lactamase gene blaIMP-4 at a 320-bed tertiary hospital in Australia. *Infect Control Hosp Epidemiol* 2007; 28: 98–101.
  33. Daikos GL, Karabinis A, Paramythiotou *et al.* VIM-I-producing *Klebsiella pneumoniae* bloodstream infections: analysis of 28 cases. *Int J Antimicrob Agents* 2007; 29: 471–473.
  34. Bratu S, Landman D, Haag R *et al.* Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. *Arch Intern Med* 2005; 165: 1430–1435.
  35. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009; 9: 228–236.
  36. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S *et al.* Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. *Antimicrob Agents Chemother* 2008; 52: 1028–1033.
  37. Patel G, Huprikar S, Factor SH *et al.* Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol* 2008; 29: 1099–1106.
  38. Woodford N, Tierno PM Jr, Young K *et al.* Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York medical center. *Antimicrob Agents Chemother* 2004; 48: 4793–4799.
  39. Maltezou HC, Giakkoupi P, Maragos A *et al.* Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). *J Infect* 2009; 58: 213–219.
  40. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 2008; 52: 1413–1418.
  41. Saito T, Takaori-Kondo A, Tashima M *et al.* Successful treatment with intravenous colistin for sinusitis, orbital cellulitis, and pneumonia caused by multidrug-resistant metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in a patient with acute myeloid leukemia. *Int J Hematol* 2009; 89: 689–692.
  42. Tan CH, Li J, Nation RL. Activity of colistin against heteroresistant *Acinetobacter baumannii* and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2007; 51: 3413–3415.
  43. Rahal JJ, Urban C, Segal-Maurer S. Nosocomial antibiotic resistance in multiple gram-negative species: experience at one hospital with squeezing the resistance balloon at multiple sites. *Clin Infect Dis* 2002; 34: 409–503.
  44. Kato Y, Ohashi H, Tsutsumi Y *et al.* Prosthetic valve endocarditis caused by metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *J Card Surg* 2009; 24: 347–349.
  45. Falagas ME, Kanellopoulou MD, Karageorgopoulos DE *et al.* Antimicrobial susceptibility of multidrug-resistant Gram-negative bacteria to fosfomicin. *Eur J Clin Microbiol Infect Dis* 2008; 27: 439–443.
  46. Guerin F, Henegar C, Spiridon G *et al.* Bacterial prostatitis due to *Pseudomonas aeruginosa* harbouring the blaVIM-2 metallo-beta-lactamase gene from Saudi Arabia. *J Antimicrob Chemother*, 2005; 56: 601–602.
  47. Peleg AY, Franklin C, Bell JM, Spelman DW. Dissemination of the metallo-beta-lactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. *Clin Infect Dis* 2005; 41: 1549–1556.
  48. Kelesidis T, Karageorgopoulos DE, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant *Enterobacteriaceae*: a systematic review of the evidence from microbiological and clinical studies. *J Antimicrob Chemother*, 2008; 62: 895–904.
  49. Poulakou G, Kontopidou FV, Paramythiotou *et al.* Tigecycline in the treatment of infections from multi-drug resistant gram-negative pathogens. *J Infect* 2009; 58: 273–284.
  50. Daikos GL, Petrikos P, Psichogiou M *et al.* Prospective observational study of the impact of VIM-I metallo-beta-lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. *Antimicrob Agents Chemother* 2009; 53: 1868–1873.
  51. Michalopoulos A, Virtzili S, Rafailidis P *et al.* Intravenous fosfomicin for the treatment of nosocomial infections caused by carbapenem-resistant *Klebsiella pneumoniae* in critically ill patients: a prospective evaluation. *Clin Microbiol Infect* 2010; 16: 184–186.
  52. Lee NY, Yan JJ, Lee HC *et al.* Clinical experiences of bacteremia caused by metallo-beta-lactamase-producing gram-negative organisms. *J Microbiol Immunol Infect* 2004; 37: 343–349.
  53. Weisenberg SA, Morgan DJ, Espinal-Witter R, Larone DH. Clinical outcomes of patients with *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* after treatment with imipenem or meropenem. *Diagn Microbiol Infect Dis* 2009; 64: 233–235.
  54. Schwaber MJ, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: a potential threat. *JAMA* 2008; 300: 2911–2913.
  55. Wertheim HF, Vos MC, Boelens HA *et al.* Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. *J Hosp Infect* 2004; 56: 321–325.
  56. Kluytmans-Vandenbergh MF, Kluytmans JA, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). *Infection* 2005; 33: 309–313.
  57. Urban C, Bradford PA, Tuckman M *et al.* Carbapenem-resistant *Escherichia coli* harboring *Klebsiella pneumoniae* carbapenemase beta-lactamases associated with long-term care facilities. *Clin Infect Dis* 2008; 46: 127–130.
  58. Lledo W, Hernandez M, Lopez E *et al.* Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. *MMWR* 2009; 58: 256–260.
  59. Kochar S, Sheard T, Sharma R *et al.* Success of an infection control program to reduce the spread of carbapenem-resistant *Klebsiella pneumoniae*. *Infect Control Hosp Epidemiol* 2009; 30: 447–452.
  60. Endimiani A, Depasquale JM, Forero S *et al.* Emergence of blaKPC-containing *Klebsiella pneumoniae* in a long-term acute care hospital: a new challenge to our healthcare system. *J Antimicrob Chemother* 2009; Sep 9 [Epub ahead of print].
  61. Rooney PJ, O'Leary MC, Loughrey AC *et al.* Nursing homes as a reservoir of extended-spectrum beta-lactamase (ESBL)-producing ciprofloxacin-resistant *Escherichia coli*. *J Antimicrob Chemother* 2009; 64: 635–641.

62. Tsakris A, Ikonomidis A, Poulou A et al. Transmission in the community of clonal *Proteus mirabilis* carrying VIM-I metallo-beta-lactamase. *J Antimicrob Chemother* 2007; 60: 136–139.
63. Tsakris A, Ikonomidis A, Spanakis N et al. Characterization of In3Mor, a new integron carrying VIM-I metallo-beta-lactamase and satI gene, from *Morganella morganii*. *J Antimicrob Chemother* 2007; 59: 739–741.