Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts

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Citation style for this article:
Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, Vatopoulos A, Gniadkowski M, Toth A, Pfeifer Y, Jarlier V, Carmeli Y, the CNSE Working Group. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 2010;15(46):pii=19711. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19711

Article published on 18 November 2010

The emergence and global spread of carbapenemase-producing *Enterobacteriaceae* is of great concern to health services worldwide. These bacteria are often resistant to all beta-lactam antibiotics and frequently co-resistant to most other antibiotics, leaving very few treatment options. The epidemiology is compounded by the diversity of carbapenem-hydrolysing enzymes and the ability of their genes to spread between different bacterial species. Difficulties are also encountered by laboratories when trying to detect carbapenemase production during routine diagnostic procedures due to an often heterogeneous expression of resistance. Some of the resistance genes are associated with successful clonal lineages which have a selective advantage in those hospitals where antimicrobial use is high and opportunities for transmission exist; others are more often associated with transmissible plasmids. A genetically distinct strain of *Klebsiella pneumoniae* sequence type (ST) 258 harbouring the *K. pneumoniae* carbapenemases (KPC) has been causing epidemics of national and international proportions. It follows the pathways of patient referrals, causing hospital outbreaks along the way. Simultaneously, diverse strains harbouring New Delhi metallo-beta-lactamase (NDM-1) are repeatedly being imported into Europe, commonly via patients with prior medical exposure in the Indian subcontinent. Since the nature and scale of carbapenem-non-susceptible Entrobacteriaceae as a threat to hospital patients in Europe remains unclear, a consultation of experts from 31 countries set out to identify the gaps in diagnostic and response capacity, to index the magnitude of carbapenem-non-susceptibility across Europe using a novel five-level staging system, and to provide elements of a strategy to combat this public health issue in a concerted manner.

Introduction

Enterobacteriaceae are among the most abundant commensal microorganisms in humans. They are also the most frequent cause of bacterial infections in patients of all ages [1]. Their ubiquity and frequent acquisition of mobile genetic elements means that their human hosts are regularly exposed to new strains with novel genetic repertoires – including antibiotic resistance – through food and water, or from other animate and inanimate sources in the community, hospitals and during travel.

Since the 1950s and 60s – when broad-spectrum antibiotics became available for the treatment of Gram-negative infections – *Enterobacteriaceae* have acquired a growing range of mechanisms to evade these agents. In particular, beta-lactam antibiotics such as penicillins and cephalosporins are vulnerable to hydrolysis by enzymes called beta-lactamases. In the mid 1970s two new beta-lactamase-stable cephalosporin compounds, cefamandole and cefuroxime were marketed [2,3], soon followed by related analogues such as cefotaxime and ceftriaxone [4]. However, novel extended-spectrum beta-lactamases (ESBL) soon emerged in *Enterobacteriaceae*, compromising these new compounds [5]. The first hospital outbreaks caused by ESBL producers occurred in France in the mid-1980s

[6], soon followed by large outbreaks in the United States (US) [7,8]. ESBL producers, are now widespread worldwide, and often are multidrug-resistant (MDR) also to fluoroquinolones and aminoglycosides [9].

A further class of beta-lactam antibiotics, the carbapenems, came into clinical use in 1985 [10]. These drugs combine exceptional intrinsic antibacterial activity with stability to most of the prevalent beta-lactamases, including ESBLs and have become the treatment of choice for infections due to the ESBL-producing strains, which are increasingly diagnosed in European hospitals. Regrettably, it has become clear that bacteria also can acquire carbapenem-hydrolysing beta-lactamases (carbapenemases). Such enzymes have emerged in various parts of the world, including Europe, the Indian subcontinent and the US [11]. In Europe and countries that were covered by the European Antimicrobial Resistance Surveillance System (EARSS, now EARS-Net) large nationwide outbreaks with carbapenemase-producing *Klebsiella pneumoniae* have occurred in Israel and Greece, and problems of variable scale are unfolding elsewhere in Europe [12-14]. Acknowledging the ineffectiveness of almost all alternative antibiotics and resistance even to those under development, there is a growing awareness that carbapenem-non-susceptible *Enterobacteriaceae* (CNSE) may thwart the ability to treat many life-threatening infections in the future.

The need for a European-wide consultation on this matter was recognised during the 2009 annual EARSS meeting, and thus a workshop of scientists involved in the surveillance of antibiotic resistance in *Enterobacteriaceae* from 31 European countries was hosted at the Netherlands' National Institute for Public Health and the Environment (RIVM) on 29 and 30 April 2010. These scientists already participated in the EARSS network and were selected on the basis of their expertise in the epidemiology of carbapenem resistance. This workshop aimed (i) to identify the gaps in diagnostic and response capacity, (ii) to index the magnitude of carbapenem-non-susceptibility across Europe using a novel five-level staging system, and (iii) to provide elements of a strategy to combat this public health issue in a concerted manner

This report summarises the discussion and outlines the complexity of the diagnostic issues and also provides information on the epidemiologic situation in European countries. The experts' conclusions aim to solidify diverse country-specific experiences into a coherent plan of action on surveillance and response to prevent the endemic establishment of carbapenemase-producing *Enterobacteriaceae* in European hospitals.

The emergence of carbapenem-non-susceptible *Enterobacteriaceae*

In contrast to the increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe [15], CNSE were extremely rare during the 1990s and early 2000s, and mostly comprised *K. pneumoniae*, and *Enterobacter* spp. with a permeability deficit that reduced drug uptake. This was associated with the inactivation of genes coding for outer membrane proteins (in *K. pneumoniae* OmpK35 and OmpK36) that function as major porins, allowing solutes to enter the bacterial cell [16,17]. This impermeability reduces carbapenem susceptibility when combined with ESBLs or AmpC beta-lactamases [18] which have trace carbapenem-hydrolysing activity. Ertapenem is the carbapenem most affected by this mechanism, and several cases of emerging ertapenem resistance during treatment have been reported [19]. Most isolates are unique, with limited clonal dissemination, perhaps because the impermeability is detrimental to the bacteria, making them less competitive in the absence of antibiotics.

Carbapenemases, which readily hydrolyse carbapenems, became an international health issue 15 years after the introduction of carbapenems, and pose a greater threat. They have been described in all four classes of beta-lactamases, but the epidemiologically most relevant carbapenemases fall into three of these [20]: Class B includes the metallo-beta-lactamases

Table 1

Clinical breakpoints defined by minimum inhibitory concentrations in mg/L for the categories S=susceptible and R=resistant according to recommendations of CLSI and EUCAST

Antibiotic compound	CLSI 2010		EUCAST 2010		
	S[a]	R	S	R	ECOFF for *E. coli* and *K. pneumoniae*[b]
Imipenem	≤1 (≤4)[c]	≥4 (≥16)	≤2	>8	≤0.5 for *E. coli* ≤1 for *K. pneumoniae*
Meropenem	≤1 (≤4)	≥4 (≥16)	≤2	>8	≤0.125
Ertapenem	≤0.25 (≤2)	≥1 (≥8)	≤0.5	>1	≤0.06
Doripenem	≤1 (ND)	≥4 (ND)	≤1	>4	≤0.12

CLSI: Clinical Laboratory Standards Institute; ECOFF: epidemiological cut-off values; EUCAST: European Committee on Antimicrobial Susceptibility Testing; MIC: minimum inhibitory concentration; ND: no data.

[a] I=intermediate is implied by the values between the S-breakpoint and the R-breakpoint.

[b] ECOFF for *E. coli* and *K. pneumoniae* define the top end of the wildtype distribution; bacteria with MICs ≥ ECOFF have acquired some mechanism of resistance.

[c] Values in parentheses indicate the CLSI breakpoints recommended before June 2010.

(MBLs) IMP (imipenemase)*, and VIM (Verona integron-encoded metallo-beta-lactamase) and the recently described New Delhi metallo-beta-lactamase (NDM-1). In class A, KPC (*K. pneumoniae* carbapenemases) is clinically and epidemiologically the most important enzyme, whereas SME (*Serratia marcescens* enzyme), NMC-A/IMI (not metalloenzyme carbapenemase/imipenem-hydrolysing beta-lactamase) and GES (Guiana extended spectrum) pose minor problems. Class D includes the OXA-type carbapenemases which are mostly found in *Acinetobacter* spp., although OXA-48 occurs in *Enterobacteriaceae*.

The first transferable carbapenemase identified in Gram-negative bacteria was an IMP-like MBL in the Far East [21], followed by VIM types in Europe [22]. In early 2003, VIM-producing *Enterobacteriaceae* began to spread in Greek hospitals [23]. *Enterobacteriaceae* with VIM MBLs have also caused some hospital outbreaks in Spain [24] and have been observed sporadically in other countries. In some cases, MBL-positive isolates were associated with travel, such as importation from Greece of *K. pneumoniae* producing VIM-1 and -2 carbapenemases [25]. Since 2008, *Enterobacteriaceae* producing NDM-1 metallo-beta-lactamases have been imported repeatedly into Europe from the Indian subcontinent [26], particularly into the United Kingdom (UK) [27] but also to Austria, Belgium, France, Germany, the Netherlands, Norway and Sweden. There were also importations to Australia, Canada, Japan and the US, again largely in patients with recent hospitalisation in India, Pakistan or Bangladesh [27,28]. Most were susceptible only to colistin and, more variably, tigecycline.

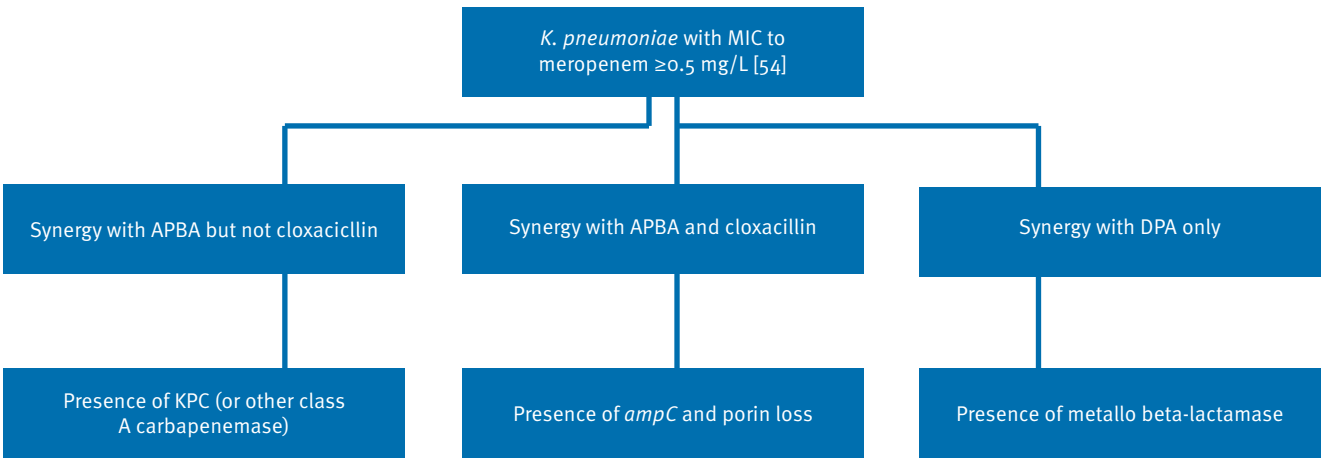
NDM-1 producers are mainly *K. pneumoniae* but also include Escherichia coli and *Enterobacter* spp. Most isolates with MBLs, particularly those with NDM types, also contain ESBL and acquired *amp*C genes, which makes them resistant to all antibiotics except polymyxins, tigecycline and, occasionally, certain aminoglycosides.

K. pneumoniae with KPC carbapenemase were first detected in 1996 in North Carolina, then spread along the east coast of the US [29,30], and finally across the whole country [31], posing a significant threat with 70% or higher mortality in bacteraemic patients [32,33]. Outside the US, *K. pneumoniae* with KPC have spread widely in Israel and Greece, with outbreaks or isolated cases in hospitals in other European countries (below). Spread is also occurring in China and Latin America [34]. Many *K. pneumoniae* isolates with KPC enzymes belong to a single clonal complex, CC11, and predominantly to a single sequence type, ST 258, containing different variants of the bla_{KPC} gene [13,31,35-37]. Apart from *K. pneumoniae*, KPC enzymes have been found in other species of *Enterobacteriaceae* (e. g. *K. oxytoca*, *Enterobacter* spp., *E. coli*) [38,39], and, more recently, also in *Pseudomonas* spp. and *Acinetobacter baumannii* [40,41]. As with the MBL producers, few treatment options remain, although some isolates remain susceptible to few aminoglycosides (gentamicin, isepamicin) as well as to polymyxins (such as colistin) or tigecycline.

OXA-48 was first described in Turkey during an outbreak of *K. pneumoniae* in Istanbul but has since attained international distribution not only among *K. pneumoniae* but also *E. coli* [42,43]. By 2009, strains with OXA-48 enzyme were being reported from the Middle East, India, Europe and North Africa [44-46], with 25 cases of OXA-48-producing *K. pneumoniae* in the UK alone. Strains with OXA-48 enzyme pose a problem for detection when using the existing expert rules embedded in automated diagnostic test systems as they often retain susceptibility to expanded-spectrum

Figure

Algorithm for interpretation of disk diffusion synergy tests and combined disk tests to detect carbapenem-non-susceptible *Enterobacteriaceae* isolates*



APBA: aminophenyl boronic acid (a beta-lactamase inhibitor); DPA: dipicolinic acid (a metal-chelating agent); KPC: *K. pneumoniae* carbapenemase.

cephalosporins and monobactams but express resistance or decreased susceptibility to carbapenems [47].

Carbapenem-non-susceptibility thus displays a very diverse picture, in geographical occurrence and enzyme types; it also challenges conventional diagnostic abilities because the presence of carbapenemase genes does not always translate into clinical resistance as defined by the current guidelines and breakpoints, as discussed below.

Identification of carbapenem-non-susceptible *Enterobacteriaceae* by routine susceptibility testing methods

The standard approach for the testing of the antimicrobial susceptibility of bacteria in routine diagnostic practice is based on measuring bacterial growth in the presence of the drugs; either by classical agar disk diffusion assays (Kirby Bauer technique) or with commercially available automated test systems that expose bacterial suspensions to a limited range of antimicrobial concentrations. The goal is to predict clinical outcomes by classifying bacterial isolates as susceptible (S), intermediate (I) or resistant (R) on the basis of agreed breakpoints. These breakpoints take into account (i) the range of antimicrobial susceptibility in a natural bacterial population in the absence of resistance mechanisms (the so-called wildtype distribution), (ii) the pharmacology with regards to the time course of the drug concentration in the human body (pharmacokinetics) and the biological effect of the drug at these concentrations on the bacteria (pharmacodynamics), and (iii), whenever available, information on clinical outcomes in relation to the minimum inhibitory concentration (MIC). Clinical results provide the most important information but are also the most difficult to acquire and to evaluate [48].

International breakpoint committees such as the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) in the US and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) set and modify breakpoints by well defined decision processes [49,50]. Importantly, breakpoints as defined by these committees are applied by automated diagnostic test systems provided by various manufacturers or, after conversion into inhibition zone diameters, by agar disk diffusion assays. EUCAST also provides crucial guidance for the European Medicines Agency (EMA, formerly EMEA) when approving the clinical indications for new antimicrobial agents.

The CLSI modified its clinical breakpoints for carbapenems after an expert consultation meeting in January 2010, reducing their previous values two-fold in order to better identify the carbapenemase-producing *K. pneumoniae* (mainly with KPC enzymes) that have attained considerable prevalence in US hospitals in the last 10 years. EUCAST had previously, in 2008, decided to set their breakpoints for the purpose of clinical, i.e. therapeutic decision-making and not for optimal detection of carbapenemase production *per se*. As a consequence, the clinical breakpoints adopted by EUCAST remain one dilution step higher than the modified CLSI values (Table 1). Since the updated CLSI breakpoints came into use in June 2010, laboratories using these standards have been defining susceptibility more conservatively than those using EUCAST [51].

Both committees recommend reporting susceptibility testing results at face value, and performing phenotypic tests for carbapenemase production only for epidemiological or infection control purposes. This will have consequences for clinical diagnosis, routine surveillance and public health.

Clinical laboratory diagnosis

On the basis of available evidence and simulated target attainment probabilities, EUCAST decided that *Enterobacteriaceae* should be regarded as clinically susceptible to imipenem at an MIC of ≤2 mg/L when treated with the standard recommended adult dose of 500 mg four times a day intravenous administration. However, the maximum dose of 1g four times a day for severe infections was taken into consideration in setting the I/R breakpoint >8 mg/L [52]. EUCAST adds a note to the breakpoint table that "some strains that produce carbapenemase are categorized as susceptible with these breakpoints and should be reported as tested, i.e. the presence or absence of a carbapenemase does not in itself influence the categorization of susceptibility. In many areas, carbapenemase detection and characterization is recommended or mandatory for infection control purposes" [53].

The more conservative modified CLSI breakpoint addresses the wide demand in the US for simplification of phenotypic characterisation of *K. pneumoniae* isolates in the wake of the KPC epidemic. The adoption of the new breakpoints is intended to render the modified Hodge test (see below) unnecessary, whereas this test was recommended in previous updates to the guidelines and had to be used frequently in many laboratories. The adoption of these breakpoints by the CLSI clearly improves the ability of microbiological laboratories to detect carbapenemase-producing *Enterobacteriaceae*, but with an unknown trade-off in specificity because more strains with combinations of impermeability and ESBL or AmpC are likely to be scored as resistant. Moreover, even these breakpoints will fail to detect carbapenemase-producing *Enterobacteriaceae* with very low MICs [54]. For clinical purposes, a breakpoint-guided therapeutic decision algorithm, as favoured by EUCAST, may be sufficient if additional molecular events such as mutational porin losses that reduce susceptibility during treatment are rare enough for bacteriological treatment failures to remain uncommon [16,55].

Routine surveillance and the role of the clinical laboratory in public health

The detection of carbapenem resistance within passive surveillance systems such as EARS-Net is complicated by breakpoint changes. Until recently most laboratories within EARS-Net have used the old CLSI breakpoint (S if MIC ≤4 mg/L imipenem or meropenem), but reported resistance rates will artificially rise when European countries/laboratories shift to use the lower EUCAST breakpoints routinely in 2010-11. Some laboratories may continue with CLSI methodology but by adopting the now lower breakpoint prescribed by that organisation will cause a resistance shift in the same direction. This will lead to some minor discrepancies and it remains to be seen if passive surveillance in its current format provides sufficient information for infection control practitioners and public health experts about the extent of the problem at national or international levels.

The contribution of diagnostic laboratories to infection control and public health is often underappreciated, underfunded and increasingly compromised by the streamlining of hospital budgets along tight service lines, which often results in the outsourcing of diagnostic services [56]. The conundrum of carbapenemase-producing *Enterobacteriaceae* with resistance below the radar of routine surveillance but relevant enough to cause concern, exposes the lack of consensus on the precise role of microbiological diagnostic laboratories in European countries. If isolates with lower-level resistance are worth monitoring for infection control and public health purposes then a simple laboratory tool is needed for detection - as simple as Etest, or double-disk synergy test (DDST), combination disk tests (CDTs) or an expert rule integrated into automated test systems.

Detection of carbapenemase-producing *Enterobacteriaceae*

Various selective agar media can be used for preliminary screening and are convenient, especially if they are chromogenic, allowing different species or resistance types to be recognised easily [57]. Different selective media may be necessary to detect carbapenemase-producers with very low carbapenem MICs. The addition of a selective agar that contains extended-spectrum cephalosporins would improve sensitivity in the detection of carbapenemases, but would also have lower specificity (regular ESBLs are also detected) [58]. Comparing growth on agars containing cephalosporins and carbapenems might also help in the detection of OXA-48 producers.

For confirmation of carbapenemase production, two inexpensive types of tests can be deployed in routine as well as reference laboratories:

The first are disk diffusion synergy tests, where the potential carbapenemase-producer is tested against a carbapenem antibiotic in the presence of carbapenemase-inhibiting compounds, including dipicolinic acid for MBLs and boronic acid for KPC enzymes (Figure). These tests may be performed in disk-approximation, i.e. DDST, or disk-combination, i.e. CDT formats [59]. Combination discs can be prepared locally by applying defined amounts of inhibitors to routine (meropenem 10 µg) antibiotic disks, or can be purchased [60]. Inhibition, and carbapenemase presence, is indicated by zone expansion. The respective inhibitors achieve reasonable specificity for MBLs and KPC enzymes [60,61] but no specific inhibitor of OXA-48 has been identified so far.

The other type of test exploits the leakage of carbapenemases from the producer into the surrounding agar and its ability to protect susceptible strains on the same plate. These tester-reporter assays consist of various modifications of the cloverleaf or Hodge test. They lack specificity, are difficult to standardise, labour-intensive and require a certain degree of experience to provide confident interpretation of results [61,62].

Molecular confirmation tests

Molecular confirmation tests are largely the realm of reference laboratories, but can be used for rapid screening under epidemic conditions, as demonstrated by experiences with faecal screening in Israel [63]. PCR assays can be designed to seek different target genes in single or multiplex formats with different modes of amplicon detection [64]. A commercial one-day test utilising ligase chain reaction and a microarray hybridisation format provides a versatile platform for the identification of ESBLs and KPCs [65-67], whilst a new test that also detects the genes for AmpC, VIM, IMP and NDM-1 enzymes is currently under clinical evaluation.

Carbapenem-non-susceptible *Enterobacteriaceae* in European countries

Although first seen sporadically in the Far East, CNSE are now established in Europe. *K. pneumoniae* is the species that most often hosts the resistance and, depending on the country, assumes various epidemiological patterns. The current knowledge of the epidemiology of carbapenem-non-susceptible *Enterobacteriaceae* in Europe is summarised below.

Greece

In Greece, the proportion of imipenem-resistant *K. pneumoniae* increased from less than 1% to 20% among isolates from hospital wards over five years, from 2001 to 2006, and to 50% among isolates from intensive care units. In 2002 this type of resistance was reported from only three hospitals but, by 2008, was present in at least 25 of the 40 hospitals participating in the Greek Antimicrobial Resistance Surveillance System. The situation was initially caused by the spread of the bla_{VIM-1} cassette among rapidly evolving plasmids conferring multiresistance or even

pan-resistance to many strains of *K. pneumoniae* and other species of *Enterobacteriaceae* [68]. The epidemic seemed to be polyclonal with no particular clone dominating [69]. In addition, there has been since 2007 a rapidly progressing nationwide epidemic of *K. pneumoniae* belonging to ST258 and harbouring KPC-2 and SHV-12 genes [70-72; Vatopoulos, unpublished results]. This rapid spread could be explained only in part by the movement of patients between hospitals. During the first few months of 2010 *K. pneumoniae* strains carrying both VIM and KPC enzymes have increasingly been identified in Greek hospitals [73].

Israel

In Israel, the first sporadic *Enterobacteriaceae* with KPC-2 carbapenemases were recognised in 2005, and comprised *Enterobacter* (three clones) and *E. coli* (polyclonal). When an increase in *K. pneumoniae* with carbapenemase production was noted in winter of 2005-06, patients were treated in isolation. Additional diagnostic support (PCR) was suggested but not regarded as a cost-effective measure at a time when isolates were sporadic and polyclonal. By spring 2006, *K. pneumoniae* with KPC-3 enzyme had become prevalent and were found to comprise a single clone (ST258). Towards the summer, the caseload had increased exponentially and, by the end of 2006, all Israeli hospitals recognised that this strain had reached epidemic proportions, as established during an *ad hoc* meeting of the Israeli Infection Control Group in early 2007. Following this meeting, a nationwide reporting system and control measures were agreed and enacted [Carmeli, unpublished results].

In March 2007 alone this system recorded 180 cases of infection with the ST258 strain but by 2010 its occurrence had been reduced and stabilised at about 30 new cases per month. Control measures included guidelines for screening, isolation and cohort nursing, as well as central reporting. Governmental commitment was crucial in supporting hospital management to enforce the necessary efforts.

Poland

In Poland, MBL-positive *Enterobacteriaceae* isolates (including *Serratia marcescens*, *Enterobacter cloacae*, *Klebsiella* spp.), mostly with VIM enzymes, have been submitted to the National Medicines Institute in Warsaw on 35 occasions since 2006 [Gniadkowski, unpublished data]. In May 2008, the first case *K. pneumoniae* ST258 producing KPC-2 and SHV-12 beta-lactamases was identified [36]. By the end of 2009, 10 hospitals in Warsaw and its surroundings were affected. Each reported between one and nine cases, and indicating continuing and widening spread. By April 2010, cases had been reported from more than 30 hospitals, and from six outpatient clinics in 16 cities. The situation is still dynamic, with some hospitals seemingly in control of the problem whilst others report newly emerging cases or have stopped reporting new cases altogether.

Italy

In Italy, various *Enterobacteriaceae* producing VIM enzymes have been reported from different regions since 2002. These isolates were mostly sporadic [74,75], although a single outbreak involving nine patients with bloodstream infections arose due to the clonal spread of a VIM-1-positive *Klebsiella* [76]. VIM carbapenemases were also found in 36 of 5,500 routinely collected *Enterobacteriaceae* from acute care hospitals and longterm-care facilities in Bolzano (Alto Adige region). These isolates also had various other resistance traits (*qnrS*, bla_{SHV}, bla_{CTX-M}) [77]. In late 2008, the first patient with KPC-3-positive *K. pneumoniae* ST258 was identified in Tuscany [78], but by early 2010, indistinguishable strains had already been reported from at least 11 locations in seven regions, and in some hospital settings they have already reached remarkable levels [Rossolini, unpublished results]. Part of this spread was associated with patient transfers between hospitals.

Germany

In Germany, the first outbreak of KPC-2-producing *K. pneumoniae* was reported in 2008 [79,80]. In 2009 and 2010 two outbreaks with KPC-3-producing *K. pneumoniae* (ST 258) and more than 40 single cases of KPC-2/3 in *E. coli* and *K. pneumoniae* were observed. Identified index patients came from Greece or Israel. Regional spread of two distinct multidrug-resistant clones over two years due to movement of colonised patients between hospitals was shown in Bavaria. Moreover several small regional clusters of OXA-48 producing *K. pneumoniae* were identified in 2009 [unpublished results]. Currently, OXA-48 is the most frequent carbapenemase in Germany, occasionally in patients with connection to Turkey. NDM-1 MBL was identified in three *E. coli* and one *Acinetobacter baumannii* isolates from epidemiologically unrelated patients.

France

In France, five monoclonal (single hospital or regional) outbreaks with carbapenemase-producing *Enterobacteriaceae* have been reported to health authorities since 2004 through the national early warning system set up at the beginning of the 2000s. In three of these outbreaks, involving strains with VIM-1 or KPC-2 enzymes, the index patient had been transferred from a Greek hospital [25]. A national programme initially designed to contain the spread of vancomycin-resistant *Enterococcus* spp. (VRE) was applied to each outbreak. This consisted of the rapid implementation of a step-by-step containment plan within the affected hospital, constant support by local infection control teams, regional experts and health authorities, and feedback to the medical community at the national level. The hospital containment strategy has the following components: (i) stopping transfer of cases and contacts within and between hospitals, (ii) cohorting separately case and contact patients with dedicated healthcare workers, (iii) screening all contact patients, and (iv) continuous vigilance through surveillance.

Hungary

In Hungary, nine *K. pneumoniae* isolates with non-susceptibility to carbapenems carrying the KPC-2 carbapenemase and SHV-12 ESBL were isolated in three centres in the North Eastern Region between October 2008 and April 2009. All belonged to the ST258 international clone, were indistinguishable by pulsed-field gel electrophoresis (PFGE), and were extensively drug-resistant. Eight were resistant even to colistin although none of the source patients had received this drug, which had never been used in any of the affected hospitals in that period. All infected patients died. The index patient had a history of hospitalisation in Greece [37]. Since then, only one further carbapenemase producer has been recorded, a *K. pneumoniae* ST11 strain with VIM-4 enzyme. Interestingly, ST11 is a single-locus variant of ST258 and, in Hungary, is commonly seen producing the CTX-M-15 ESBL but normally lacking VIM genes [81].

Spain

In Spain, VIM-positive *Enterobacteriaceae* have, as of April 2010, been reported from 15 different hospitals, IMP-positive strains from another two, and KPC-positive *Enterobacteriaceae* (*K. pneumoniae* and *Citrobacter freundii*) from two university hospitals in Madrid [24]. The KPC-positive *K. pneumoniae* strains did not belong to the ST258 epidemic clone, but to the ST384 and ST388 clones. ST388 *K. pneumoniae* had previously persisted as a carbapenem-susceptible CTX-M-10 beta-lactamase-producing clone [82]. A structured survey in 2008 covering 38 hospitals across Spain, collected 100,132 isolates of *Enterobacteriaceae* but only identified 43 with carbapenemases, mainly VIM types (76%), whilst none had KPC types [83]. However, 45 of 245 carbapenem-non-susceptible *Enterobacteriaceae* isolates submitted for reference testing between January 2009 and April 2010 harboured VIM (18%), 15 IMP (6%) and 15 were non-carbapenemase-producing strains (6%). Two outbreaks involving VIM-1 enzyme-producing *K. pneumoniae* were reported at two hospitals in Madrid [84] and three small outbreaks were reported with carbapenemase-producing *Enterobacter* spp. [85].

United Kingdom

In the United Kingdom NDM seems to de the dominant carbapenemase in *Enterobacteriaceae*, although producers of KPC (increasingly), VIM and OXA-48 carbapenemases are also recorded. Patients infected with producers of this NDM-1 enzyme have a history of hospitalisation on the Indian subcontinent, where producer strains of K. pneumoniae, *E. coli* and other *Enterobacteriaceae* appear to be in wide circulation [27]. The dominance of this enzyme in the UK may reflect the country's historic links with India, and the consequent population flows to and from the subcontinent.

Other countries

Import of CNSE by travel has also been detected in other countries such as Sweden, Denmark, Norway, Finland, and Belgium but the spread was limited, most likely thanks to infection control [35,70,86,87].

Proposing a staging scheme for the epidemiology of carbapenem resistance in European countries

The experiences reported above suggest that the epidemiology of CNSE and especially carbapenemase-producing *K. pneumoniae* in European countries follows a pattern typical for hospital-acquired pathogens. Initially there is sporadic occurrence and stochastic extinction, followed by single hospital outbreaks and then spread along the regional and national hospital patient referral routes. This also means that hospitals that share the same patients are at a high risk of importing colonised or infected individuals, providing the sources for the next outbreaks. An intuitive way of assessing the degree to which carbapenemase-producing *Enterobacteriaceae* have become established in national hospital networks is by indexing these stages, and we therefore suggest a simple numerical staging system (Table 2).

Applying this staging scheme and data provided by 31 reference laboratories for the period up to July 2010 allowed us to categorise the European countries (Table 3). Most countries that reported early stage events mentioned documented introduction by travel and many were concerned about likely underreporting owing to a lack of detection or lack of communication (not shown). In clonal outbreak situations, KPC is the dominant resistance mechanism, mainly linked to the spread of *K. pneumoniae* international clone ST258.

Conclusions

Care of hospitalised patients throughout Europe is threatened by the spread of carbapenem-non-susceptible *Enterobacteriaceae*. There are very few therapeutic options left to treat these patients, and invasive infections are associated with disturbingly high mortality rates. Little is known about the patient-related risk factors other than hospitalisation abroad, but the description of outbreaks indicates that producer strains seem to benefit from selective advantages in hospitals where antimicrobial use is much higher and opportunities for transmission more frequent than in the community [88]. The association of KPC (and occasionally VIM) enzymes with an internationally successful clonal lineage of *K. pneumoniae* indicate that hospital outbreaks are local expansions following long transmission chains. This is also supported by the frequent international introduction of sporadic or primary cases. Consistent with the spread of hospital-adapted lineages is the repeated observation that outbreaks, especially of KPC-positive ST258 *K. pneumoniae*, follow patient referral patterns, with initial local spread and occasional regional and nationwide dissemination. The fact that transmission of these clones is mainly confined to healthcare settings provides an opportunity for targeted prevention and control. Israel has shown that

national consensus approaches with agreed screening protocols and mandatory reporting can reduce the incidence of resistance during a nationwide Klebsiella ST258 epidemic.

Table 2

Epidemiological scale and stages of nationwide expansion of healthcare-associated carbapenem-non-susceptible *Enterobacteriaceae**

Epidemiological scale	Description	Stage
No cases reported	No cases reported	0
Sporadic occurrence	Single cases, epidemiologically unrelated	1
Single hospital outbreak	Outbreak defined as two or more epidemiologically related cases in a single institution	2a
Sporadic hospital outbreaks	Unrelated hospital outbreaks with independent, i.e. epidemiologically unrelated introduction or different strains, no autochthonous inter-institutional transmission reported	2b
Regional spread	More than one epidemiologically related outbreak confined to hospitals that are part of a regional referral network, suggestive of regional autochthonous inter-institutional transmission	3
Inter-regional spread	Multiple epidemiologically related outbreaks occurring in different health districts, suggesting inter-regional autochthonous inter-institutional transmission	4
Endemic situation	Most hospitals in a country are repeatedly seeing cases admitted from autochthonous sources	5

Table 3

Expansion of healthcare-associated carbapenem-non-susceptible *Enterobacteriaceae* in Europe: epidemiological scale and stages by country, as of July 2010

Country	Stage	Epidemiological scale	Documented introduction from abroad	Dominant class	Underreporting
Greece	5	Endemic	Yes	KPC/VIM	
Israel[a]				KPC	
Italy	4	Interregional spread	Yes	KPC	
Poland					Likely
France	3	Regional spread	Yes	KPC	
Germany				OXA-48/VIM	Likely
Hungary				KPC	Likely
Belgium	2b	Independent hospital outbreaks	Yes	VIM	Likely
Spain				KPC/VIM/IMP	Likely
England and Wales				NDM	
Cyprus	2a	Single hospital outbreak		VIM	
Netherlands			Yes	KPC	
Norway			Yes	KPC	
Scotland				KPC	
Sweden			Yes	KPC	
Bosnia Herzegovina	1	Sporadic occurrence	Yes	KPC	
Denmark				KPC/VIM	
Finland			Yes	KPC	
Croatia				VIM	
Czech Republic			Yes	VIM/KPC	
Ireland				KPC	Likely
Lithuania				?	Likely
Latvia				?	Likely
Malta				?	
Portugal				KPC	Likely
Romania				?	Likely
Switzerland				KPC	
Austria	0	Not reported		-	
Bulgaria					Likely
Estonia					Likely
Iceland					
Slovenia					

[a] Likelihood of acquisition of CNSE for hospitalised patients low due to containment measures.
Luxembourg and Slovakia were invited to the meeting but did not participate.

Plasmid-borne spread within and between species also can occur for bla_{KPC} and is the dominant mode of dissemination for NDM and VIM genes. This epidemiology is more complex and harder to interrupt, complicating national intervention strategies.

Regardless of whether it is the spread of the strain or the gene that is dominant, the key to success in preventing the establishment of carbapenemase-producing *Enterobacteriaceae* is early detection and good diagnostic practice. Recent decisions by international breakpoint committees are taking into account the fact that MICs for carbapenemase-producing *Enterobacteriaceae* may represent a continuum close to or even overlapping with the top end of the wildtype distribution. Even the most conservative breakpoints will not assign all carbapenemase-producing *Enterobacteriaceae* into the non-susceptible class and may also classify strains with porin loss and presence of ESBL or AmpC lactamases as resistant. The epidemiological consequences of underdetection of carbapenemase producers with MICs in the sub-breakpoint range are still unclear. Current MIC breakpoints are set to guide treatment, but more clinical studies on the effectiveness of carbapenems against carbapenemase-producing *Enterobacteriaceae* with relatively low MICs are needed, as are simple, inexpensive, phenotypic tests to recognise producers, irrespective of MIC, with adequate specificity and sensitivity. Molecular confirmation tests are useful for reference purposes and are conveniently rapid when screening faecal samples during outbreaks.

Hospital outbreaks, defined as more than two epidemiologically related cases, need to be brought to the attention of regional health authorities as well as to all hospitals receiving referred patients. A close collaboration between the microbiological laboratory and the local and regional infection control team(s) is decisive for the prevention and control at local hospital level.

Areas for improvement

The workshop identified ten areas for improvement, displayed in the list below. Areas 1 to 6 recognise the need for better laboratory-based detection and surveillance, whereas areas 7 to 10 address infection control and clinical research needs.

Area 1: *Ad hoc* case ascertainment with existing laboratory capacity

- All routine diagnostic laboratories must test the susceptibility of all isolated *Enterobacteriaceae*, from all anatomical sites in each patient, with at least one carbapenem, specifically meropenem or imipenem*. Resistance to ertapenem is more prone to arise through combinations of impermeability and AmpC or ESBL activity, especially in *Enterobacter* spp., reducing specificity.
- Laboratories should inform their local infection control teams of their tentative findings and report non-susceptibility of blood culture isolates to the national EARS-Net data manager.
- They should forward isolates to national reference centres for confirmation and molecular testing.

Area 2: Standardisation of detection and reporting

- Agreement needs to be achieved on the minimum test requirements for detection and data reporting of CNSE within the national and international reporting structures.
- A panel of highly characterised CNSE isolates should be made available to all laboratories for test calibration.
- For laboratories that wish to participate in national or international surveillance initiatives (EARS-Net) participation in regular external quality assessment exercises should be mandatory, and should include carbapenemases.
- An improvement to EARS-Net would be a recommendation to report carbapenem-non-susceptibility rather than resistance, similar to the reporting of susceptibility for penicillin in *Streptococcus pneumoniae*.
- A list of national reference and other centres with the required skills to identify molecular mechanisms of carbapenem resistance should be available to all clinical laboratories in each country.

Area 3: Need for consistent capacity building of reference diagnostics

- Workshops should be organised to train reference laboratory personnel on a set of phenotypic and genetic test methods to allow exhaustive characterisation of carbapenemase-producing isolates submitted by routine diagnostic laboratories.
- The workshops shall follow a 'training the trainers approach' in order to provide European reference laboratories with the means to train peripheral laboratories.
- This will increase the coherence of reporting and strengthen national surveillance and diagnostic capacity.
- The training should ideally take place in an endemic country to provide course participants with hands-on experience of the workload, and to appraise the challenges in task management, procurement and costing.

Area 4: Need for structured surveys to determine sensitivity and specificity of defined breakpoints or other inclusion criteria

- A group of experts shall develop the protocol for a structured survey aiming to optimise a diagnostic algorithm to identify CNSE with a high degree of accuracy and a minimum number of false positives.
- This will require agreement on a set of selection criteria (e.g. overall resistance profile and a meropenem MIC ≥ 0.5 mg/L) [54].
- Furthermore the sampling frame needs to be defined, a sample size estimated, and a design for roll-out to all European countries developed.
- The results should not only provide the best sensitivity and specificity but also reveal the true

prevalence of CNSE in a representative cross-sectional sample of the population.

Area 5: Need for a harmonised typing tool/initiative

- Molecular typing of CNSE is complex due to the multifaceted nature of their spread. Plasmid spread occurs among different species, and repeated introduction to Europe via travel may challenge typing laboratories with a near-random sample of strains circulating in other countries.
- Nevertheless, the potential for rapid spread of single lineages, as seen with *K. pneumoniae* ST258 in Greece, Israel and recently in Poland and Italy, underscores the need for rapid assessment of the spread of such clones, as these are of particular public health importance.
- It is therefore highly desirable to invest in the development of typing systems with a better resolution for strains and plasmids.
- Only sequence-based data will provide the robustness and portability required for modern decentralised approaches.
- In any case the practicability and applicability of typing methods must be considered.

Area 6: Need for central data collection on the dissemination and introduction of strains with particular public health importance

- With harmonised test methods, detection, typing and reporting criteria comes the ability to network the data collected at local and national levels into international databases that would be freely accessible and searchable for hospitals and public health agencies.
- This will allow for early recognition of temporal-spatial tends, outbreaks and importation by travel. Systems of this kind have been developed for infections caused by *Legionella pneumophila* and *Staphylococcus aureus* [89,90].
- We believe that the spread of CNSE and the consequent treatment problems create an urgent need for the construction of a similar IT platform to prevent these traits from becoming endemic in the European region.

Area 7: Need for guidelines for graded approaches to infection control

- Appropriate infection control measures need to be guided by epidemiological staging, which can be defined at national level as described above (See Tables 2 and 3).
- If CNSE have not yet been reported, highly sensitive detection criteria coupled with an early warning system and preparedness should be in place.
- For countries with sporadic outbreaks, infection control teams should be trained to implement measures to contain spread at the local level following a ready-to-use stepwise approach.
- Reporting the occurrence and the outcome of outbreaks will inform health authorities on the epidemiology and success of national strategies.
- Countries with advanced-stage epidemiology should resort to screening and isolation in accordance with epidemiological and geographic extent of the cases reported. Such policies were successful in Israel.
- In such settings, national health authorities should inform other EU Member States on the prevailing epidemiology, so that safe policies for patient transfer from their countries can be established.

Area 8: Antibiotic policy

- Antibiotic overuse and misuse are the main factors that select multidrug-resistant organisms such as CNSE from the commensal flora.
- Diversification and de-escalation of antibiotic treatment, particularly carbapenems, fluoroquinolones but also third-generation cephalosporins, are key to the response to the CNSE emergence.
- This should include guidelines on antibiotic use for non-severe infections (e.g. urinary tract infections) and an intensified dialogue with prescribers across Europe.

Area 9: Treatment and clinical research

- Clinical trials about the effectiveness of remaining alternative treatment strategies for CNSE infections are urgently needed.
- Incentives need to be provided for the development of new antibiotics active against CNSE.

Area 10: Political commitment

- Importantly, the political commitment at national governmental as well as European level is critical.
- The European Centre for Disease Prevention and Control should play a role in harmonising European surveillance, detection and identification strategies.
- The World Health Organization (WHO) should address this issue in a proactive manner globally, possibly through the International Health Regulations which are an international legal instrument that is binding for 194 countries across the globe, including all the WHO member states.
- Their aim is to help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide [91].

Funding
The consultation and the work leading up to it was funded by the European Commission Directorate for Research and Development through the collaborative project Translational Research on Combating Antimicrobial Resistance (TROCAR) project number 223031.

***Authors' correction:**
At the request of the authors, the following changes were made on 25 November 2010.

- In the section entitled 'The emergence of carbapenem-non-susceptible Enterobacteriaceae', 'IMP (active on imipenem)' was changed to 'IMP (imipenemase)'.
- In the the top box of the Figure, 'imipenem ≥1 mg/L (non-wildtypea)' was changed to 'meropenem ≥0.5 mg/L [54]', and footnote a was deleted.
- In the first bullet point of Area 1 in the section entitled 'Areas for improvement', 'specifically imipenem or meropenem' was changed to 'specifically meropenem or imipenem'

In addition, on 10 December 2010, the following change was made in Table 2 at the request of the authors: 'Outbreak defined as more than two epidemiologically related cases in a single institution' should read 'Outbreak defined as two or more epidemiologically related cases in a single institution'.

References

1. Donnenberg MS. Enterobacteriaceae. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. New York: Elsevier, Churchill, Livingstone, 2005:2567–2586.a
2. Moellering, RC Jr. Cefamandole-a new member of the cephalosporin family. J Infect Dis. 1978;137 Suppl:S2-S9.
3. O'Callaghan CH, Sykes RB, Griffiths A, Thornton JE. Cefuroxime, a new cephalosporin antibiotic: activity in vitro. Antimicrob Agents Chemother. 1976;9(3):511-9.
4. Garzone P, Lyon J, Yu VL. Third-generation and investigational cephalosporins: I. Structure-activity relationships and pharmacokinetic review. Drug Intell Clin Pharm. 1983;17(7-8):507-15.
5. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection. 1983;11(6):315-7.
6. Buré A, Legrand P, Arlet G, Jarlier V, Paul G, Philippon A. Dissemination in five French hospitals of Klebsiella pneumoniae serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam. Eur J Clin Microbiol Infect Dis. 1988;7(6):780-2.
7. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of Klebsiella infection resistant to late-generation cephalosporins. Ann Intern Med. 1993;119(5):353-8.
8. Medeiros AA. Nosocomial outbreaks of multiresistant bacteria: extended-spectrum beta-lactamases have arrived in North America. Ann Intern Med. 1993;119(5):428-30.
9. Cullik A, Pfeifer Y, Prager R, von Baum H, Witte W. A novel IS26 structure surrounds blaCTX-M genes in different plasmids from German clinical Escherichia coli isolates. J Med Microbiol. 2010;59(Pt5): 580-7.
10. Lyon JA. Imipenem/cilastatin: the first carbapenem antibiotic. Drug Intell Clin Pharm. 1985;19(12):895-9.
11. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:i1-9.
12. Samra Z, Ofir O, Lishtzinsky Y, Madar-Shapiro L, Bishara J. Outbreak of carbapenem-resistant Klebsiella pneumoniae producing KPC-3 in a tertiary medical centre in Israel. Int J Antimicrob Agents. 2007;30(6):525-9.
13. Navon-Venezia S, Leavitt A, Schwaber MJ, Rasheed JK, Srinivasan A, Patel JB, et al. First report on a hyperepidemic clone of KPC-3-producing Klebsiella pneumoniae in Israel genetically related to a strain causing outbreaks in the United States. Antimicrob Agents Chemother. 2009;53(2):818-20.
14. Giakkoupi P, Maltezou H, Polemis M, Pappa O, Saroglou G, Vatopoulos A, et al. KPC-2-producing Klebsiella pneumoniae infections in Greek hospitals are mainly due to a hyperepidemic clone. Euro Surveill. 2009;14(21):pii=19218. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19218
15. Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill. 2008;13(47):pii=19044. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19044
16. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. J Antimicrob Chemother. 2009;63(4): 659-67.
17. Woodford N, Dallow JW, Hill RL, Palepou MF, Pike R, Ward ME, et al. Ertapenem resistance among Klebsiella and Enterobacter submitted in the UK to a reference laboratory. Int J Antimicrob Agents. 2007;29(4): 456-9.
18. Chia JH, Su LH, Lee MH, Kuo AJ, Shih NY, Siu LK, et al. Development of high-level carbapenem resistance in Klebsiella pneumoniae among patients with prolonged hospitalization and carbapenem exposure. Microb Drug Resist; 2010 Jun 6. [Epub ahead of print].
19. Skurnik D, Lasocki S, Bremont S, Muller-Serieys C, Kitzis MD, Courvalin P, et al. Development of ertapenem resistance in a patient with mediastinitis caused by Klebsiella pneumoniae producing an extended-spectrum beta-lactamase. J Med Microbiol. 2010;59(Pt 1)115-9.
20. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect. 2002;8:(6)321-31.
21. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1991;35(1):147-51.
22. Lauretti L, Riccio ML, Mazzariol A, Cornaglia G, Amicosante G, Fontana R, et al. Cloning and characterization of blaVIM, a new integron-borne metallo-beta-lactamase gene from a Pseudomonas aeruginosa clinical isolate. Antimicrob Agents Chemother. 1999;43(7):1584-90.
23. Vatopoulos A. High rates of metallo-beta-lactamase-producing Klebsiella pneumoniae in Greece - a review of the current evidence. Euro Surveill. 2008;13(4):pii=8023. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8023
24. Tato M, Coque TM, Ruíz-Garbajosa P, Pintado V, Cobo J, Sader HS, et al. Complex clonal and plasmid eepidemiology in the first outbreak of Enterobacteriaceae infection involving VIM-1 metallo-beta-lactamase in Spain: toward endemicity? Clin Infect Dis. 2007;45(9):1171-8.
25. Kassis-Chikhani N, Decré D, Gautier V, Burghoffer B, Saliba F, Mathieu D, et al. First outbreak of multidrug-resistant Klebsiella pneumoniae carrying blaVIM-1 and blaSHV-5 in a French university hospital. J Antimicrob Chemother. 2006;57(1):142-5.
26. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046-54.
27. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597-602.
28. Centers for Disease Control and Prevention (CDC). Detection of Enterobacteriaceae isolates carrying metallo-beta-lactamase - United States, 2010. MMWR Morb Mortal Wkly Rep. 2010;59(24):750.
29. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother. 2001;45(4):1151-61.
30. Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J, et al. Characterization of blaKPC-containing Klebsiella pneumoniae isolates detected in different institutions in the Eastern USA. J Antimicrob Chemother. 2009;63(3):427-37.
31. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53(8):3365-70.
32. Endimiani A, Depasquale JM, Forero S, Perez F, Hujer AM, Roberts-Pollack D, 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;64(5):1102-10.
33. Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol. 2009;30(10);972-6.
34. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):228-36.
35. Samuelsen Ø, Naseer U, Tofteland S, Skutlaberg DH, Onken A, Hjetland R, et al. Emergence of clonally related Klebsiella pneumoniae isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. J Antimicrob Chemother. 2009;63(4):654-8.
36. Baraniak A, Izdebski R, Herda M, Fiett J, Hryniewicz W, Gniadkowski M, et al. Emergence of Klebsiella pneumoniae ST258 with KPC-2 in Poland. Antimicrob Agents Chemother. 2009;53(10):4565-7.

37. Tóth A, Damjanova I, Puskás E, Jánvári L, Farkas M, Dobák A, et al. Emergence of a colistin-resistant KPC-2-producing Klebsiella pneumoniae ST258 clone in Hungary. Eur J Clin Microbiol Infect Dis. 2010;29(7):765-9.
38. Rasheed JK, Biddle JW, Anderson KF, Washer L, Chenoweth C, Perrin J et al. Detection of the Klebsiella pneumoniae carbapenemase type 2 Carbapenem-hydrolyzing enzyme in clinical isolates of Citrobacter freundii and K. oxytoca carrying a common plasmid. J Clin Microbiol. 2008;46(6):2066-9.
39. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the beta-lactamase bla KPC gene. Antimicrob Agents Chemother. 2008;52(4):1257-63.
40. Wolter DJ, Schmidtke AJ, Hanson ND, Lister PD. Increased expression of ampC in Pseudomonas aeruginosa mutants selected with ciprofloxacin. Antimicrob Agents Chemother. 2007;51(8):2997-3000.
41. Robledo IE, Aquino EE, Santé MI, Santana JL, Otero DM, León CF, et al. Detection of KPC in Acinetobacter spp. in Puerto Rico. Antimicrob Agents Chemother. 2010;54(3):1354-7.
42. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant Klebsiella pneumoniae isolates in Istanbul, Turkey. Antimicrob Agents Chemother. 2008;52(8): 2950-4.
43. Gülmez D, Woodford N, Palepou MF, Mushtaq S, Metan G, Yakupogullari Y, et al. Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss. Int J Antimicrob Agents. 2008;31(6):523-6.
44. Cuzon G, Naas T, Lesenne A, Benhamou M, Nordmann P. Plasmid-mediated carbapenem-hydrolysing OXA-48 beta-lactamase in Klebsiella pneumoniae from Tunisia. Int J Antimicrob Agents. 2010;36(1):91-3.
45. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Huang TD, Nordmann P. Plasmid-encoded carbapenem-hydrolyzing beta-lactamase OXA-48 in an imipenem-susceptible Klebsiella pneumoniae strain from Belgium. Antimicrob Agents Chemother. 2008;52(9):3463-4.
46. Matar GM, Dandache I, Carrër A, Khairallah MT, Nordmann P, Sabra A, et al. Spread of OXA-48-mediated resistance to carbapenems in Lebanese Klebsiella pneumoniae and Escherichia coli that produce extended spectrum beta-lactamase. Ann Trop Med Parasitol. 2010;104(3):271-4.
47. Woodford N, Eastaway AT, Ford M, Leanord A, Keane C, Quayle RM, et al. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mechanisms responsible for carbapenem resistance in Enterobacteriaceae. J Clin Microbiol. 2010;48(8):2999-3002.
48. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, et al. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. Clin Microbiol Infect. 2006;12(6):501-3.
49. Clinical and Laboratory Standards Institute (CLSI) [Internet]. Wayne:CLSI. [Accessed 10 Oct 2010]. Available from: http://www.clsi.org/
50. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST Procedure for Harmonising and Defining Breakpoints. The Swedish Reference Group for Antibiotics. [Accessed:10/10/2010]. Available from: http://www.srga.org/eucastwt/bpsetting.htm
51. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twentieth informational supplement (June 2010 update). M100-S20-U. 2010;30(15). Available from: http://www.clsi.org/source/orders/free/m100-s20-u.pdf
52. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Imipenem. Rationale for the EUCAST clinical breakpoints, version 1.3. 1 Jun 2009. [Accessed 10 Oct 2010]. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Imipenem_EUCAST_Rationale_Document_1.3_090601.pdf
53. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST Clinical Breakpoint Table v. 1.1 2010-04-27, Enterobacteriaceae. EUCAST: 2010. [Accessed 10 Oct 2010]. Available from: http://eucast.org./fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.1.xls
54. Vading M, Samuelsen O, Haldorsen B, Sundsfjord AS, Giske CG. Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing Klebsiella pneumoniae with EUCAST and CLSI breakpoint systems. Clin Microbiol Infect. 2010. [Epub ahead of print].
55. Song W, Suh B, Choi JY, Jeong SH, Jeon EH, Lee YK, et al. In vivo selection of carbapenem-resistant Klebsiella pneumoniae by OmpK36 loss during meropenem treatment. Diagn Microbiol Infect Dis. 2009;65(4):447-9.
56. Humphreys H, Nagy E, Kahlmeter G, Ruijs GJ. The need for European professional standards and the challenges facing clinical microbiology. Eur J Clin Microbiol Infect Dis. 2010;29(6):617-21.
57. Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. J Clin Microbiol. 2008;46(9):3110-1.
58. Carrer A, Fortineau N, Nordmann P. Use of ChromID extended-spectrum beta-lactamase medium for detecting carbapenemase-producing Enterobacteriaceae. J Clin Microbiol. 2010; 48(5):1913-4.
59. Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, et al. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect. 2010;16(2):112-22.
60. Giske CG, Gezelius L, Samuelsen O, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo-beta-lactamases and KPC in Klebsiella pneumoniae with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect. 2010 Jun 28. [Epub ahead of print].
61. Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, et al. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J Antimicrob Chemother. 2010;65(8):1664-71.
62. Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling false-positive results obtained with the Hodge and Masuda assays for detection of class a carbapenemase in species of Enterobacteriaceae by incorporating boronic Acid. J Clin Microbiol. 2010;48(4):1323-32.
63. Schechner V, Straus-Robinson K, Schwartz D, Pfeffer I, Tarabeia J, Moskovich R, et al. Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the Enterobacteriaceae family. J Clin Microbiol. 2009;47(10):3261-5.
64. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65(3):490-5.
65. Cohen Stuart J, Dierikx C, Al Naiemi N, Karczmarek A, Van Hoek AH, Vos P, et al. Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemother. 2010;65(7):1377-81.
66. Endimiani A, Hujer AM, Hujer KM, Gatta JA, Schriver AC, Jacobs MR, et al. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M-, and KPC-type beta-lactamase genes in Gram-negative isolates. J Clin Microbiol. 2010;48(7):2618-22.
67. Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P. Evaluation of a DNA microarray, the check-points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob Agents Chemother. 2010;54(8):3086-92.
68. Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, et al. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerg Infect Dis. 2006;12(7):1145-8.
69. Psichogiou M, Tassios PT, Avlamis A, Stefanou I, Kosmidis C, Platsouka E, et al. Ongoing epidemic of blaVIM-1-positive Klebsiella pneumoniae in Athens, Greece: a prospective survey. J Antimicrob Chemother. 2008;61(1):59-63.
70. Tegmark-Wisell K, Hæggman S, Gezelius L, Thompson O, Gustafsson I, Ripa T, et al. Identification of Klebsiella pneumoniae carbapenemase in Sweden. Euro Surveill. 2007;12(51):pii=3333. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3333
71. Cuzon G, Naas T, Demachy MC, Nordmann P. Plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC-2 in Klebsiella pneumoniae isolate from Greece. Antimicrob Agents Chemother. 2008;52(2):796-7.
72. Maltezou HC, Giakkoupi P, Maragos A, Bolikas M, Raftopoulos V, Papahatzaki H, et al. Outbreak of infections due to KPC-2-producing Klebsiella pneumoniae in a hospital in Crete (Greece). J Infect. 2009;58(3):213-9.
73. Giakkoupi P, Pappa O, Polemis M, Vatopoulos AC, Miriagou V, Zioga A, et al. Emerging Klebsiella pneumoniae isolates coproducing KPC-2 and VIM-1 carbapenemases. Antimicrob Agents Chemother. 2009;53(9):4048-50.

74. Luzzaro F, Docquier JD, Colinon C, Endimiani A, Lombardi G, Amicosante G, et al. Emergence in Klebsiella pneumoniae and Enterobacter cloacae clinical isolates of the VIM-4 metallo-beta-lactamase encoded by a conjugative plasmid. Antimicrob Agents Chemother. 2004;48(2):648-50.
75. Rossolini GM, Luzzaro F, Migliavacca R, Mugnaioli C, Pini B, De Luca F, et al. First countrywide survey of acquired metallo-beta-lactamases in gram-negative pathogens in Italy. Antimicrob Agents Chemother. 2008;52(11):4023-9.
76. Cagnacci S, Gualco L, Roveta S, Mannelli S, Borgianni L, Docquier JD, et al. Bloodstream infections caused by multidrug-resistant Klebsiella pneumoniae producing the carbapenem-hydrolysing VIM-1 metallo-beta-lactamase: first Italian outbreak. J Antimicrob Chemother. 2008;61(2):296-300.
77. Aschbacher R, Doumith M, Livermore DM, Larcher C, Woodford N et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-beta-lactamase (blaVIM-1) genes in multiple species of Enterobacteriaceae from Bolzano, Italy. J Antimicrob Chemother. 2008;61(3):515-23.
78. Giani T, D'Andrea MM, Pecile P, Borgianni L, Nicoletti P, Tonelli F, et al. Emergence in Italy of Klebsiella pneumoniae sequence type 258 producing KPC-3 Carbapenemase. J Clin Microbiol. 2009;47(11):3793-4.
79. Gröbner S, Linke D, Schütz W, Fladerer C, Madlung J, Autenrieth IB, et al. Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates at the university hospital of Tubingen, Germany. J Med Microbiol. 2009;58(Pt 7):912-22.
80. Wendt C, Schütt S, Dalpke AH, Konrad M, Mieth M, Trierweiler-Hauke B, et al. First outbreak of Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae in Germany. Eur J Clin Microbiol Infect Dis. 2010;29(5):563-70.
81. Kristóf K, Tóth A, Damjanova I, Jánvári L, Konkoly-Thege M, Kocsis B, et al. Identification of a blaVIM-4 gene in the internationally successful Klebsiella pneumoniae ST11 clone and in a Klebsiella oxytoca strain in Hungary. J Antimicrob Chemother. 2010;65(6):1303-5.
82. Curiao T, Morosini MI, Ruiz-Garbajosa P, Robustillo A, Baquero F, Coque TM, et al. Emergence of bla KPC-3-Tn4401a associated with a pKPN3/4-like plasmid within ST384 and ST388 Klebsiella pneumoniae clones in Spain. J Antimicrob Chemother. 2010;65(8):1608-14.
83. Miró E. Prevalence of AmpC plasmidic beta-lactamases and carbapenemases in Enterobacteriaceae in Spain. Abstract No 706. XIV Congress of Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). 2010: Barcelona (Spain).
84. Sánchez-Romero I. Description of a VIM-1-producing Klebsiella pneumoniae outbreak in a third level hospital. Abstract Nº 473., in XIV Congress of Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). 2010: Barcelona (Spain).
85. Oteo J, Hernández-Almaraz JL, Gil-Antón J, Vindel A, Fernández S, Bautista V, et al. Outbreak of Vim-1-Carbapenemase-Producing Enterobacter cloacae in a Pediatric Intensive Care Unit. Pediatr Infect Dis J. 2010 Aug 3. [Epub ahead of print].
86. Österblad M, Kirveskari J, Koskela S, Tissari P, Vuorenoja K, Hakanen AJ, et al. First isolations of KPC-2-carrying ST258 Klebsiella pneumoniae strains in Finland, June and August 2009. Euro Surveill. 2009;14(40):pii=19349. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19349
87. Hammerum AM, Hansen F, Lester CH, Jensen KT, Hansen DS, Dessau RB. Detection of the first two Klebsiella pneumoniae isolates with sequence type 258 producing KPC-2 carbapenemase in Denmark. Int J Antimicrob Agents. 2010;35(6):610-2.
88. Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, et al. Carriage rate of carbapenem-resistant Klebsiella pneumoniae in hospitalised patients during a national outbreak. J Hosp Infect. 2010;74(4):344-9.
89. European Centre for Disease Prevention and Control (ECDC). European Legionnaires' Disease Surveillance Network (ELDSNet). Stockholm:ECDC;2010. Available from: http://www.ecdc.europa.eu/en/activities/surveillance/ELDSNet/Pages/Index.aspx
90. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, et al. Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med.2010;7(1): e1000215.
91. World Health Organization (WHO). What are the International Health Regulations? Geneva:WHO; [Accessed 28 Aug 2010]. Available from: http://www.who.int/features/qa/39/en/index.html

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* We deeply regret the untimely loss of our dear friend and colleague Helmut Mittermayer.