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Europe's journal on infectious disease epidemiology, prevention and control

Special edition:

Antimicrobial resistance

18 November 2010

- To tie in with the European Antibiotic Awareness Day 2010, a Eurosurveillance special issue presents a series of articles on antimicrobial resistance with a particular focus on the emergence of resistant *Klebsiella pneumoniae* and reviews the spread of the New Delhi metallo-beta-lactamase 1 in European countries

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EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Editorial team

Based at the European Centre for
Disease Prevention and Control (ECDC),
171 83 Stockholm, Sweden

Telephone number

+46 (0)8 58 60 11 38 or +46 (0)8 58 60 11 36

Fax number

+46 (0)8 58 60 12 94

E-mail

Eurosurveillance@ecdc.europa.eu

Editor-in-Chief

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Managing Editor

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Design / Layout

Fabrice Donguy / Martin Wincent

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Antimicrobial resistance 2010: global attention on carbapenemase-producing bacteria

ECDC Antimicrobial Resistance and Healthcare-Associated Infections Programme[1,2]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Members of the programme are listed at the end of the editorial

Citation style for this article:
ECDC Antimicrobial Resistance and Healthcare-Associated Infections Programme. Antimicrobial resistance 2010: global attention on carbapenemase-producing bacteria . Euro Surveill. 2010;15(46):pii=19719. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19719

Article published on 18 November 2010

A year ago in November 2009, a study in this journal highlighted the emergence of infections with totally or almost totally resistant bacteria in European intensive care units [1]. Most of them were Gram-negative bacilli that showed resistance to a class of antibiotics considered last-line therapy: the carbapenems. Already in 2008, Souli *et al*. had reviewed the emergence of extensively drug-resistant (XDR) bacteria in Europe [2] and pointed out the high proportion of isolates that were resistant to carbapenems, through production of a carbapenemase enzyme. Indeed, an increasing number of reports on carbapenemases and infections with carbapenemase-producing bacteria have been published in recent years indicating the rising importance of these bacteria. A PubMed search with the keyword 'carbapenemase' and excluding review articles, yielded 35 articles for the year 2007, 48 articles for 2008, 80 articles for 2009 and 109 articles for 2010 (as of 14 November).

The year 2010 will certainly be remembered as the year when carbapenemase-producing, XDR bacteria attracted global attention. Significant media attention and increasing awareness of these bacteria followed the publication by Kumarasamy *et al*. on 11 August on the spread to the United Kingdom (UK) of a new type of carbapenemase, the New Delhi metallo-beta-lactamase 1 (NDM-1), often associated with travel to India or Pakistan [3]. In this issue of *Eurosurveillance*, Struelens *et al*. review the spread of NDM-1 in the European Union (EU), Iceland and Norway and show that, in addition to the UK, 11 other EU countries plus Norway have identified patients infected or colonised with NDM-1-producing *Enterobacteriaceae* [4]. Similar to the cases described in the UK, the majority of these NDM-1 cases had previously travelled or been admitted to a hospital in India or Pakistan. In addition, a few cases had been hospitalised in the Balkan region [4].

Several other types of carbapenemases have been described since the 1990s such as *Klebsiella pneumoniae* carbapenemase (KPC), Verona integron-encoded metallo-beta-lactamase (VIM) and the oxacillinase-type beta-lactamase OXA-48 [5]. All these have in common that they are able to rapidly hydrolyse most of the beta-lactams including the carbapenems, thus conferring resistance to these antibiotics. In addition, they are in most cases encoded by a gene located on transferable elements which allows transfer of the gene among species of *Enterobacteriaceae*. This issue of *Eurosurveillance* highlights the challenges represented by carbapenemase-producing, XDR bacteria, but also offers examples from EU countries on how the spread of such bacteria can be contained.

Although NDM-1 has been the focus of media attention concerning antimicrobial resistance during the past months, it is neither the most frequently identified carbapenemase in Europe, nor the only carbapenemase associated with transfer of patients between countries. In this issue of the journal, a group of European experts report on carbapenemase-producing *Enterobacteriaceae* in Europe and show that carbapenemases other than NDM-1 are the dominant types in all European countries except the UK [5]. As an example, Decré *et al*. describe the likely importation from Morocco to France of an OXA-48-producing *K. pneumoniae* strain with subsequent cross-transmission to another patient [6], a pattern similar to that described for previous OXA-48 cases from other countries. As for NDM-1, the spread of KPC- and OXA-48-producing bacteria has been associated with transfer of patients from hospitals in countries where they are frequently found, to hospitals in other countries [7,8].

Accurate laboratory detection, control of patient-to-patient transmission and prudent use of antibiotics are cornerstones of containment

Identification of carbapenemase-producing bacteria remains a challenge. According to the survey by Grundmann *et al*. there is likely underreporting of such isolates in more than one third of European countries [5]. Struelens *et al*. found that less than half of the countries reported having national guidance on surveillance and detection methods for carbapenemase-producing bacteria and, with two exceptions, countries that reported NDM-1 cases also reported having such

national guidance [4]. Availability of guidance and sufficient capacity of laboratories to routinely detect and confirm carbapenemase-producing isolates throughout and beyond Europe, are of paramount importance for their containment. Active surveillance and isolation of patients who are infected or colonised are essential for controlling the spread of these bacteria. Struelens *et al*. indicate that 11 European countries have developed infection control guidelines which in some countries, e.g. France, recommend the pre-emptive isolation and screening of patients transferred from hospitals in other countries [4].

To address the issues above, the United States (US) Centers for Disease Control and Prevention (CDC) developed a guidance document for the detection of metallo-beta-lactamases such as NDM-1 [9] and produced guidance for control of these infections in acute care facilities [10]. In Europe, the European Centre for Disease Prevention and Control (ECDC) is preparing evidence-based guidance on screening and confirmation of carbapenemase-producing bacteria and conducts a systematic review of the published evidence on interventions to control carbapenemase-producing *Enterobacteriaceae*. A group of European experts convened by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) reviewed detection and surveillance issues [11]. Another expert group under the auspices of ESCMID, suggested implementation of different control measures for countries with sporadic occurrence of these bacteria and for countries where they are endemic [12].

Early warning and sharing information between countries facilitates prevention and control

In this issue, Kassis-Chikhani *et al*. [13] show that it is possible to contain outbreaks of carbapenemase-producing bacteria if rapid control measures are implemented. National and international early warning and response systems allow for the timely sharing of information that is necessary to investigate possible inter-hospital transmission. The EU Early Warning and Response System (EWRS) is a tool to rapidly share confidential information between countries, with the assistance of the European Commission, to improve prevention and control of communicable diseases. However, the EWRS has rarely been used for communication about resistant bacteria in the past. In addition to rapid exchange of information, discussion between risk assessment entities and experts in EU countries is crucial to prevent the spread of resistant bacteria including the ones discussed in this editorial. To support such discussions, ECDC is developing a specific module of its Epidemic Intelligence Information System (EPIS).

Antimicrobial resistance and consumption in EU Member States

Data on antimicrobial resistance are available from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS) (http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx). They show increasing resistance to third-generation cephalosporins and multidrug resistance in invasive infections due to *K. pneumoniae* and *Escherichia coli* in many EU countries. For this reason, hospital physicians have increasingly used carbapenems, in particular to treat infections in the most severely ill patients, e.g. in intensive care units. In a point prevalence survey on antimicrobial consumption in a sample of 75 European hospitals, the European Surveillance of Antimicrobial Consumption (ESAC) project showed that on average 11%, and up to 50%, of patients in intensive care units were receiving a carbapenem [14]. Since the introduction of antibiotics into medical practice, prescribers have mostly relied on the constant availability of new antibiotics to effectively treat patients infected with resistant bacteria. However, this forward escape strategy now looks like a leap of faith since innovative antibiotics active against these bacteria are unlikely to be developed in the very near future [15], leaving therapeutic options for carbapenemase-producing, XDR bacteria limited. These consist mainly of the polymyxins and tigecycline, but experts agree that neither of them are ideal because of the toxicity of polymyxins and the variable clinical efficacy of tigecycline [8,12]. Avoiding unnecessary use of antibiotics and reserving them for appropriate indications, starting with carbapenems, is therefore essential to preserve options for therapy of infections in hospitalised patients.

Point prevalence surveys have been developed to ascertain the appropriateness of antibiotic prescription practices in hospitals and other healthcare facilities. In the ESAC point prevalence survey, 57% of antibiotic courses for surgical prophylaxis lasted more than one day, thus highlighting short duration of prophylaxis as an obvious target for improvement of antibiotic prescribing practices in hospitals [14]. Even in a country with a history of prudent use of antibiotics such as the Netherlands, Willemsen *et al*. showed that, in their prevalence survey in 19 hospitals, 16% patients were receiving antimicrobial therapy that they judged inappropriate [16]. The Eurobarometer survey on antimicrobial resistance performed in November-December 2009 showed that almost half of Europeans still believed that 'antibiotics are active against colds and flu' and these results point towards a challenge for prudent use of antibiotics outside of hospitals [17].

Meticillin-resistant *Staphylococcus aureus*

Recent data from EARS-Net show that six countries reported decreasing trends in the proportion of meticillin-resistant *Staphylococcus aureus* (MRSA) among *S. aureus* isolates from invasive infections for the period 2006 to 2009. This is likely due to sustained efforts to contain the spread of MRSA in hospitals and other healthcare facilities [18]. MRSA remains a public health threat with a proportion of MRSA above 25% in more than one third of countries participating in EARS-Net. In addition, new strains of MRSA are emerging

from other environments such as human infections in the community, food animals and foods [19]. In this issue, De Jonge *et al*. add to our knowledge about MRSA with a study suggesting that, although present in some meat samples in the Netherlands, the risk to humans of being colonised by MRSA through handling of contaminated meat is low [20].

MRSA emerged in hospitals in the 1960s and, with the exception of the Scandinavian countries and the Netherlands, other European countries did not seriously consider its prevention and control before the 1990s. In countries with a low MRSA prevalence, MRSA control relies heavily on the so-called 'search-and-destroy' strategy which includes the pre-emptive isolation and screening of patients who have been in contact with healthcare facilities in countries with high prevalence of MRSA [18].

International efforts to tackle antimicrobial resistance - joining forces is essential

Europe is reacting much faster to contain the spread of carbapenemase-producing, extensively drug-resistant bacteria when compared with MRSA. It follows the path of a few leading countries which are taking measures similar to those for MRSA prevention and control in low prevalence countries. Contemporary life-style, however, poses an additional challenge with ever increasing international travel and patients seeking healthcare abroad, which means that containment of carbapenemase-producing, XDR bacteria can only be addressed internationally.

The European Commission has reported this year that EU countries have made significant progress toward implementing the Council Recommendation of 15 November 2001 on the prudent use of antimicrobial agents in human medicine. However, there are still several areas where improvement is needed, including education and awareness of healthcare personnel and the general public [21]. On 18 November 2010, 36 European countries will participate in the third European Antibiotic Awareness Day (http://antibiotic.ecdc.europa.eu). The focus of this year's European Antibiotic Awareness Day is to raise awareness about prudent use of antibiotics among hospital prescribers. Key messages have been developed to help hospitals and hospital prescribers in their efforts to reach this goal. Evidence suggests that multifaceted hospital strategies may improve antibiotic prescribing practices and decrease antibiotic resistance. In addition, specific strategies may help prescribers optimise antibiotic therapy and reduce unnecessary use.

Worldwide attention on antimicrobial resistance allows for many stakeholders and countries to be involved. In planning for next year, the World Health Organization has declared antimicrobial resistance and its global spread as the topic for the next World Health Day on 7 April 2011 [22]. Already this year, antibiotic awareness campaigns are taking place at the same time on both sides of the Atlantic. The United States' Get Smart About Antibiotics Week (http://www.cdc.gov/getsmart/) takes place on 15-21 November [23] and Canada's first Antibiotic Awareness Day (http://antibioticawareness.ca/) will take place on the same day as European Antibiotic Awareness Day, 18 November 2010. Joining forces is essential for tackling a global issue such as antimicrobial resistance.

Members of the ECDC Antimicrobial Resistance and Healthcare-Associated Infections Programme
Dominique L. Monnet (Coordinator), Carl Suetens (Deputy Coordinator), Sarah Earnshaw, Carlo Gagliotti, Jolanta Griškevičienė, Ole E. Heuer, Elmira Khazeeva, Niels Kleinkauf, Eva Liljestedt, Anna-Pelagia Magiorakos, Francisco Santos, Marc J. Struelens, J. Todd Weber, Klaus Weist.

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Possible importation and subsequent cross-transmission of OXA-48-producing *Klebsiella pneumoniae*, France, 2010

D Decre (dominique.decre@sat.aphp.fr)[1,2], G Birgand[3], D Geneste[2], E Maury[4], J C Petit[1], F Barbut[3], G Arlet[2]
1. Assistance Publique-Hôpitaux de Paris, Microbiology, Saint-Antoine Hospital, Paris, France
2. University Pierre and Marie Curie, ER8 Antibiotiques et Flore digestive, Paris VI, France
3. Assistance Publique-Hôpitaux de Paris, Unité d'Hygiène et de Lutte contre les Infections Nosocomiales, Saint-Antoine Hospital, Paris, France
4. Assistance Publique-Hôpitaux de Paris, Medical Intensive Care Unit, Saint-Antoine Hospital, Paris, France

Citation style for this article:
Decre D, Birgand G, Geneste D, Maury E, Petit JC, Barbut F, Arlet G. Possible importation and subsequent cross-transmission of OXA-48-producing Klebsiella pneumoniae, France, 2010. Euro Surveill. 2010;15(46):pii=19718. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19718

Article published on 18 November 2010

We report the possible first patient-to-patient transmission of *Klebsiella pneumoniae* with decreased susceptibility to imipenem and producing OXA-48, CTX-M15, TEM-1 and OXA-1 in a French hospital.

Background

The class D beta-lactamase OXA-48 conferring decreased susceptibility to carbapenems in *Klebsiella pneumoniae* was firstly identified in sporadic isolates from Turkey. Subsequently, large outbreaks have been described in Istanbul and other cities in Turkey [1]. OXA-48-producing *K. pneumoniae* isolates have since been reported from the United Kingdom, Israel, Lebanon, Argentina, France, and recently from Germany, Tunisia and Morocco [2-6]. It has been shown that bla_{OXA-48} was located on *Tn1999*, a composite transposon made of two copies of *IS1999* [2].

We report the possible first occurrence of patient-to-patient transmission of OXA-48-producing *K. pneumoniae* in a French hospital following the likely importation of the index isolate from Morocco.

Case 1

The index patient lived in Morocco most of the year, who had an underlying chronic condition and had been hospitalised for an operation in Morocco in March 2009. The patient had been in France for a few weeks when, after having fallen, was admitted to a University hospital in Paris, in early March 2010. The case was hospitalised and underwent surgery on the day following admission. Almost one week later, the patient was transferred to the medical intensive care unit (ICU) for an acute respiratory failure. In this ICU, screening of patients for faecal carriage of extended-spectrum-beta-lactamase (ESBL)-producing *Enterobacteriaceae* is implemented since September 2009 and must be performed at admission and weekly.

Laboratory results

An ESBL-producing *K. pneumoniae* isolate was recovered from the rectal swab sampled from this patient at admission in the ICU. By using the disc diffusion method on Mueller-Hinton agar, the isolate showed resistance to fluoroquinolones, tobramycin, gentamicin and cotrimoxazole, and intermediate resistance level to imipenem. It has been determined through the same method that the isolate was only susceptible to colistin and amikacin. The zone diameter for imipenem was of 20 mm and the minimum inhibitory concentration (MIC) determined by Etest (Bio-Rad Laboratoires) was 2 mg/L. The modified Hodge test was positive. PCR using a panel of primers specific for the detection of *Klebsiella pneumoniae* carbapenemase (KPC) acquired AmpC-type beta-lactamases, OXA-type carbapenemases, metallo-beta-lactamases (MBLs) and ESBLs [7] and sequencing revealed the presence of bla_{TEM-1}, bla_{OXA-1}, $blaC_{TX-M-15}$ and bla_{OXA-48} beta-lactamase genes.

Control measures

On 19 March, barrier precautions were implemented, and promotion of hand hygiene using alcohol-based products and room cleaning were reinforced. In addition, screening for faecal carriage of OXA-48-producing *K. pneumoniae* was performed. Rectal swabs were recovered from all patients who had been hospitalised in the medical ICU or in the orthopaedic ward during the same period as the index patient.

Case 2

At the end of March 2010, a second patient colonised with OXA-48 producing *K. pneumoniae* was notified by the laboratory. This second patient had initially been admitted in the medical ICU with pneumonia. Upon admission, following screening, the case was found negative for ESBL and then transferred to the geriatric ward where weekly screening was continued. The epidemiological analysis showed that this patient had stayed in the same area of the medical ICU as the index patient for six days, and that both patients had been managed by the same team of healthcare workers, suggesting potential cross-transmission. Both patients had previously been receiving broad-spectrum antibi-

otics such as third generation cephalosporins but not carbapenems.

Laboratory results

The two isolates showed the same resistance phenotype to beta-lactams, aminoglycosides and quinolones. Enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis showed the same profile compared with non-epidemiologically related *K. pneumoniae* strains (data not shown). As reported for the strain isolated in Tunisia [5], plasmid analysis indicated the presence of three plasmids and the plasmid of approximatively 70-80 Kb carried bla_{OXA-48}.

Control measures

Following identification of OXA-48-producing *K. pneumoniae* in the second patient, a set of infection control measures were implemented. This set included (i) cohorting of the two colonised patients in the geriatric ward, (ii) stopping transfers of the two colonised patients and of patients who had previously been in contact with them, (iii) performing a systematic search for additional patients who could have been in contact with the colonised patients, (iv) screening of all contact patients by rectal swabbing once a week, or twice a week for contact patients receiving antibiotics. No other OXA-48-producing- *K. pneumoniae* has been isolated in the hospital since 31 March 2010.

Discussion and conclusion

The global spread of ESBLs, particularly CTX-M enzymes, in clinical isolates of *Escherichia coli* and *K. pneumoniae* has driven therapeutic choice towards carbapenems and lead to emergence of carbapenem resistance mechanisms. Carbapenemases now represent a public health challenge. In *Enterobacteriaceae*, carbapenemases are diverse; these belong to beta-lactamases molecular class A (KPC), class B (IMP, VIM) and class D (OXA-48), are involved in outbreaks in various geographical regions and are increasingly reported in sporadic cases worldwide [8,9]. Until recently, OXA-48 seemed to be limited to few countries, but has begun to spread, in particular in countries from the eastern and southern Mediterranean region [2,5,6]. In Germany, OXA-48 is the most frequent carbapenemase [5]. Since 2005, all beta-lactamases from multidrug-resistant *Enterobacteriaceae* isolates in our institution have been characterised and this is the first strain identified as producing OXA-48. The Moroccan origin of the strain is suggested by the fact that the index patient mostly lived in Morocco where OXA-48 has recently been reported [6], that she had not previously been hospitalised in France and that none of the contact patients from the orthopaedic ward was colonised.

Since OXA-48 remains difficult to detect, especially when it is not associated with an ESBL [10], enhanced surveillance and rapid identification are essential. In addition, once OXA-48 is identified, adequate infection control measures should rapidly be implemented to prevent cross-transmission.

Acknowledgements

We are grateful to Bruno Coignard (Institut de Veille Sanitaire) for helpful discussion.

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New Delhi metallo-beta-lactamase 1–producing *Enterobacteriaceae*: emergence and response in Europe

M J Struelens (marc.struelens@ecdc.europa.eu)[1], D L Monnet[1], A P Magiorakos[1], F Santos O'Connor[1], J Giesecke[1], the European NDM-1 Survey Participants[2]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. The European NDM-1 Survey Participants are listed at the end of the article

Citation style for this article:
Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, Giesecke J, the European NDM-1 Survey Participants. New Delhi metallo-beta-lactamase 1–producing Enterobacteriaceae: emergence and response in Europe. Euro Surveill. 2010;15(46):pii=19716. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19716

Article published on 18 November 2010

Acquired carbapenemases confer extensive antibiotic resistance to *Enterobacteriaceae* and represent a public health threat. A novel acquired carbapenemase, New Delhi metallo-beta-lactamase 1 (NDM-1), has recently been described in the United Kingdom and Sweden, mostly in patients who had received care on the Indian subcontinent. We conducted a survey among 29 European countries (the European Union Member States, Iceland and Norway) to gather information on the spread of NDM-1-producing *Enterobacteriaceae* in Europe, on public health responses and on available national guidance on detection, surveillance and control. A total of 77 cases were reported from 13 countries from 2008 to 2010. *Klebsiella pneumoniae* was the most frequently reported species with 54%. Among 55 cases with recorded travel history, 31 had previously travelled or been admitted to a hospital in India or Pakistan and five had been hospitalised in the Balkan region. Possible nosocomial acquisition accounted for 13 of 77 cases. National guidance on NDM-1 detection was available in 14 countries and on NDM-1 control in 11 countries. In conclusion, NDM-1 is spreading across Europe, where it is frequently linked to a history of healthcare abroad, but also to emerging nosocomial transmission. National guidance in response to the threat of carbapenemase-producing *Enterobacteriaceae* is available in approximately half of the surveyed European countries. Surveillance of carbapenemase- producing *Enterobacteriaceae* must be enhanced in Europe and effective control measures identified and implemented.

Introduction

New Delhi metallo-beta-lactamase 1 (NDM-1) is a newly-described metallo-beta-lactamase (MBL), first identified in 2008 in single isolates of *Klebsiella pneumoniae* and *Escherichia coli*, both recovered from a patient repatriated to Sweden after treatment in a hospital in New Delhi, India [1]. Like other acquired MBLs, NDM-1 hydrolyses all beta-lactam antibiotics except for aztreonam, which is usually inactivated by co-produced extended-spectrum or AmpC beta-lactamases. An association with other resistance mechanisms makes a majority of *Enterobacteriaceae* with bla_{NDM-1} extensively resistant to antibiotics and susceptible only to colistin and, less consistently, tigecycline [1,2].

Acquired carbapenemases are a large group of beta-lactamases of high structural diversity that, in most instances, hydrolyse not only carbapenems, but also oxyimino-cephalosporins and cephamycins [3]. For over a decade, different types of acquired carbapenemases have gradually begun to appear in clinical isolates of *Enterobacteriaceae* and other Gram-negative bacteria. In Europe, VIM-type MBLs and the so-called *K. pneumoniae* carbapenemases (KPC) are the most frequently isolated carbapenemases, although *K. pneumoniae* producing the class D OXA-48 carbapenemase is prevalent in Turkey ([3,4]. Overall, carbapenem-resistant *Enterobacteriaceae* are still rare causes of human infections in most parts of Europe, except for Greece and Cyprus [3-5]. According to the 2009 data from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS) [5] the rates of carbapenem-resistance among invasive *K. pneumoniae* infections were: 43.5% in Greece, 17.0% in Cyprus, 1.3% in Italy, 1.2% in Belgium and below 1% in the other 23 reporting countries [5]. Despite these generally low rates, carbapenemase-producing strains of *K. pneumoniae* harbouring either bla_{VIM} or bla_{KPC} have been the cause of country-wide epidemics of healthcare-associated infections in Greece, Israel, the United States (US), several Latin American countries and China, and of local outbreaks in Poland and Italy [3,4,6]. These epidemic strains, plasmids, and transposons bearing carbapenemases have been shown to spread when carried by patients who are transferred between hospitals [3,6]. Such introductions into healthcare systems across country borders have led to international epidemics by secondary local or regional transmission [1-3,6].

After the initial report of NDM-1 from Sweden in 2008 [1], the Health Protection Agency (HPA) in the United Kingdom (UK), concerned over the rapid increase in the number of cases of human colonisation and infection

with NDM-1 and other carbapenemase-producing *Enterobacteriaceae* in hospitals across the country, issued a national alert in July 2009 [7]. Similarly to the first case of NDM-1 reported by Yong *et al.* [1], the majority of the patients with NDM-1-positive bacteria in the UK had a history of travel to India or Pakistan, where many of them had been hospitalised with various indications, including elective surgery and renal dialysis [2,7].

These reports indicate that the majority of the bacteria carry bla_{NDM-1} on conjugative plasmids of variable size [1,2]. Among *Enterobacteriaceae*, *E. coli* and *K. pneumoniae* are the most frequent host species but NDM-1 has already been recorded in K*lebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter cloacae, Morganella morganii, Proteus* spp. and *Providencia* spp. [1,2].

The worldwide spread of *Enterobacteriaceae* that carry carbapenemase-producing genes, including bla_{NDM-1}, is a significant threat to human health: Firstly, the production of carbapenemase, in association with other resistance determinants, confers extensive drug resistance, leaving few or no therapeutic options. Secondly, the association with travel underscores the risk of healthcare in countries where antibiotic-resistant bacteria are endemic [2]. Lastly, studies of patients infected with *Enterobacteriaceae* producing KPC are at increased risk of complications and death [8,9].

On 23 August 2010, following publication of the UK cases [2], the French National Public Health Surveillance Institute (InVS) posted an alert on the European Early Warning and Response System (EWRS), to share with other European Union (EU) Member States information on two cases infected with NDM-1-producing bacteria related to hospitalisation on the Indian subcontinent. On 27 August, the European Centre for Disease Prevention and Control (ECDC) produced and shared with the Commission and all EU Member States a threat assessment on "New Delhi metallo-beta-lactamase (NDM-1) carbapenemase-producing *Enterobacteriaceae* from the Indian subcontinent", based on a preliminary review of all NDM-1-producing bacteria reported at that time in six EU member states (Belgium, France, Germany, the Netherlands, Sweden and the UK). The present study is an update of this initial assessment and describes the geographical distribution and epidemiological features of NDM-1-producing bacteria detected to date in the EU Member States, Iceland and Norway. It also reports on the availability of national guidance on detection, surveillance, notification and control of NDM-1- and other carbapenemase-producing *Enterobacteriaceae* in these countries.

Methods

We searched the medical literature for articles on "New Delhi metallo-beta-lactamase" OR "NDM-1" published until 15 November 2010 to identify cases reported from Europe and also included relevant references provided by consulted experts. To expand this search beyond published cases, an electronic questionnaire survey was sent out on 20 September 2010 to the ECDC National Antimicrobial Resistance Focal Points and the European Antimicrobial Resistance Surveillance Network (EARS-Net) Contact Points from all EU Member States, Iceland and Norway. The purpose of this survey was to register, by country, all cases of infection with NDM-1-producing bacteria. An NDM-1 case was defined as a patient from whom one or more *Enterobacteriaceae* had been isolated that expressed the NDM-1 enzyme as confirmed by an expert laboratory. The survey also collected the following clinical and microbiological data: the bacterial species producing the NDM-1 enzyme and the date of detection, the type of infection, the sex and age of the patient, the patient's clinical status at hospital discharge or at the last follow-up, any recent travel history (within the 30 days before detection of NDM-1) or contact with healthcare facilities abroad (also stating in which country), and any local transmission events with known contact with a travel-associated case.

The questionnaire also included queries about whether there were published national guidelines, recommendations or guidance documents addressing the following issues in the context of carbapenemase-producing *Enterobacteriaceae*: methods of microbiological detection, referral of isolates to reference microbiology laboratories, notification of public health authorities, and infection control measures to limit spread.

Results

We identified 19 peer-reviewed publications on NDM-1 enzyme [1,2,10-26], of which 12 reported primary data. NDM-1 cases reported from Europe (n=38) included two cases from Austria [20], two from Belgium [25], one from Denmark [23], one from France [26], two from the Netherlands [16,24], one from Sweden [1] and 29 from the UK [2]. Cases were also reported from Australia [18], Canada [19], Singapore [21] and the US [10]. Infections with NDM-1-producing *Acinetobacter baumannii* were reported from Germany, India and the UK [2,17,22]. Investigations showed that the NDM-1 enzyme was frequently detected among clinical *Enterobacteriaceae* isolates in Chennai, Haryana, Mumbai and other Indian cities [2,10].

All 29 questionnaires mailed to the countries were completed and returned. Table 1 summarises the epidemiological characteristics of the 77 patients with one or more isolates of *Enterobacteriaceae* with an NDM-1 enzyme (referred to as 'NDM-1 cases') reported in 13 European countries between 1 January 2008 and 7 October 2010, grouped by country of diagnosis. The total number of cases increased by year from eight in 2008, 30 in 2009 and 39 in the first nine months of 2010.

Table 1

Demographic characteristics and travel history of patients colonised or infected with *Enterobacteriaceae* producing New Delhi metallo-beta-lactamase 1 in the European Union, Iceland and Norway, 2008-2010 (N=77)

Country	Number of patients	Year of detection: first case/last case	NDM-1-producing bacterial species (number of isolates)	Sex (male:female)	Age range (years)	
Austria	3	2009/2010	*Escherichia coli* (1), *Klebsiella pneumoniae* (2)	3:0	14-56	
Belgium	3	2010	*E. coli* (2), *K. pneumoniae* (1), *Morganella morganii* (1)	2:1	46-53	
Bulgaria	0	NA	NA	NA	NA	
Cyprus	0	NA	NA	NA	NA	
Czech Republic	0	NA	NA	NA	NA	
Denmark	1	2010	*K. pneumoniae* (1)	0:1	57	
Estonia	0	NA	NA	NA	NA	
Finland	1	2010	*K. pneumoniae* (1)	1:0	46	
France	4	2009/2010	*Citrobacter freundii* (1), *E. coli* (1), *K. pneumoniae* (1), *Proteus mirabilis* (1)	2:2	18-63	
Germany	3[c]	2009/2010	*E. coli* (2), *K. pneumoniae* (1)	2:1	22-70	
Greece	0	NA	NA	NA	NA	
Hungary	0	NA	NA	NA	NA	
Iceland	0	NA	NA	NA	NA	
Ireland	0	NA	NA	NA	NA	
Italy	2	2009	*E. coli* (2)	2:0	NR	
Latvia	0	NA	NA	NA	NA	
Lithuania	0	NA	NA	NA	NA	
Luxembourg	0	NA	NA	NA	NA	
Malta	0	NA	NA	NA	NA	
Nether-lands	2	2008/2009	*K. pneumoniae* (2)	1:1	30-66	
Norway	2	2010	*E. coli* (1), *K. pneumoniae* (1)	1:1	65-70	
Poland	0	NA	NA	NA	NA	
Portugal	0	NA	NA	NA	NA	
Romania	0	NA	NA	NA	NA	
Slovakia	0	NA	NA	NA	NA	
Slovenia	2	2009/2010	*K.pneumoniae* (2)	1:1	59-79	
Spain	1	2010	*K.pneumoniae* (1)	1:0	36	
Sweden	2	2008/2010	*E. coli* (2), *K. pneumoniae* (1)	2:0	59-72	
United Kingdom (data set 1)	29[c]	2008/2009	*C. freundii* (2), *Enterobacter* spp.(4), *E. coli* (5), *K. pneumoniae* (17), *M. morganii* (1)	15:13	2-87	
United Kingdom (data set 2)	22	2010	NR	NR	NR	

NA: not applicable; NR: data not reported

[a] History of travel or contact with healthcare facilities in a foreign country within 30 days prior to NDM-1 detection.

[b] Under United Nations Security Council Resolution 1244.

[c] Additional cases of *Acinetobacter baumannii* with an NDM-1 enzyme: Germany (n=1), United Kingdom (n=9).

[d] Autochthonous cases staying at the same hospital unit at the same time as a patient who had previously travelled in India.

[e] The first case was admitted to a hospital in India with longer time interval (eight months) prior to NDM-1 detection.

[f] Specimen type: urine (n=19), wound (n=4), sputum (n=4), blood (n=3), other (n=7).

[g] Link to foreign country defined as having travelled within the year before NDM-1 detection in India or Pakistan or having been born there (n=17).

[h] Healthcare link to foreign country defined as having been admitted to hospital in the previous three years (n=14): India (n=8), Pakistan (n=4), India and Dubai (n=1) and Spain (n=1).

[i] Two clusters, each comprising one index travel-associated case and one secondary case, who were both admitted to the same hospital unit during the same period.

[j] Cluster of nine cases associated with a contaminated endoscope, caused by the same clonal type that had been found in a travel-associated case six months earlier.

	Clinical presentation		Fatal cases	Recent[a] travel to country (number of cases)	Recent[a] healthcare in country (number of cases)	Possible secondary cases	Reference
	Cases of colonisation	Cases of infection					
	1	Abdominal sepsis (1), Necrotising fasciitis (1)	0	India (1), Kosovo[b] (1), Pakistan (1)	India (1), Kosovo[b] (1), Pakistan (1)	0	[20]
	2	Sepsis from necrotic wound (1)	1	Kosovo[b] and Serbia (1), Montenegro (1), Pakistan (1)	Kosovo[b] and Serbia (1), Montenegro (1), Pakistan (1)	0	[25]
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	1	NA	0	Bosnia and Herzegovina (1)	Bosnia and Herzegovina (1)	0	[23]
	NA	NA	NA	NA	NA	NA	
	1	NA	0	India (1)	India (1)	0	
	2	Skin and soft tissue infection (1), Urinary tract infection (1)	0	India (4)	India (3)	0	[26]
	1	Urinary tract infection (2)	NR[e]	India (1), Pakistan (1)	India (1), Pakistan (1)	0	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	2	0	0	0	0	2[d]	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	2	0	0	India (2)	0	0	[24]
	1	Urinary tract infection and secondary bacteraemia (1)	0	India (1)[e]	India (1)[e]	0	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	NA	NA	NA	NA	NA	NA	
	0	Pneumonia (1) Urinary tract infection (1)	0	Serbia (1)	Serbia (1)	0	
	0	Abdominal abscess (1)	0	India (1)	India (1)	0	
	1	Urinary tract infection (1)	1	India (2)	India (2)	0	[1]
	NR	NRf	5	NR[g]	NR[h]	2[i]	[2]
	NR	NR	NR	NR	NR	9[lj]	

Of the 77 cases reported in the questionnaires, 51 originated from the UK. The patients' age ranged from 2 to 87 years and the male:female ratio was 0.62. Species information was available for 57 isolates (from 53 patients) producing an NDM-1 enzyme. They were distributed among six species: *K. pneumoniae* (n=31), *E. coli* (n=16), *Enterobacter* spp. (n=4), *C. freundii* (n=3), *M. morganii* (n=2) and *Proteus mirabilis* (n=1). In addition, the UK and Germany have recorded NDM-1 enzyme in *Acinetobacter* spp. (Table 1).

Among the 26 cases reported in European countries other than the UK, 14 were thought to be colonised with NDM-1-enzyme-producing organisms, whereas 12 presented with infections affecting the urinary tract (n=6), skin and soft tissues (n=3), intra-abdominal cavity (n=2), and lung (n=1). Among all 77 cases reported, including from the UK, four patients developed a bloodstream infection. Seven of the 77 patients died in hospital: In a 51-year old diabetic patient, death was attributed to septic shock from a necrotic leg wound infected with an NDM-1-positive *E. coli*. In another case the fatal outcome was unrelated to NDM-1. For the remaining five fatal cases, information on the cause of death was not available.

Thirty-eight of the 55 cases with a travel history had a link either to the Indian subcontinent (n=33) or to the Balkan region (n=5). Different temporal criteria were applied to travel history by the UK and the other survey participants to define this link. Among the 29 cases in the UK, 17 had travelled to India or Pakistan in the year before detection of NDM-1. Among the 26 cases from other EU countries, 22 had travelled in the month before diagnosis to a foreign country: 13 to India, three to Pakistan, two to Kosovo, two to Serbia, one to Montenegro and one to Bosnia and Herzegovina (Table 1).

Most patients with recent travel had been hospitalised in a foreign country during the 30 days prior to the detection of NDM-1 (Table 1). In the UK, 14 of 29 patients had been admitted within the three years before detection of NDM-1 to a foreign hospital in India (n=8), Pakistan (n=4), India and Dubai (n=1) and Spain (n=1). In the other reporting countries, 18 of 26 patients been admitted in the month before detection to a foreign hospital in India (n=10), the Balkans (n=5) or Pakistan (n=3) (Table 1). It appears that the majority of these cases were admitted to foreign hospitals due to an illness or accident that occurred during the journey, although a minority were travelling for medical tourism.

Preliminary evidence suggests that 13 of 77 patients from Italy and the UK were possible secondary cases linked to other hospitalised patients who had returned from India (Table 1). In Italy, two cases with no travel history had stayed in a hospital unit to which a patient returning from India had previously been admitted. In the UK, 11 patients were involved in three clusters of possible cross-transmission. The hospital stay of two UK patients with no link to foreign countries overlapped with a travel-associated case. In another UK hospital, an endoscope-related outbreak affected nine patients six month after a travel-associated case.

National guidance was available in 14 European countries in the form of online or peer-reviewed publications, addressing the management of *Enterobacteriaceae* producing NDM-1 or other carbapenemases (Table 2). They all recommended clinical laboratory methods for resistance detection and required that the resistance gene is confirmed by a reference laboratory. Cyprus and Latvia reported that reference microbiological methods were under development. It is of note that NDM-1 cases were reported in 10 of 14 countries with laboratory detection guidance, compared with three of 15 countries without such guidance (Tables 1 and 2). The majority of guidance documents also outlined the procedure for notification of health authorities and recommended infection control measures in healthcare facilities. Eight countries had a full set of guidance documents. Estonia, Ireland, and Slovakia indicated that such guidance was in development.

Guidance on control measures for patients with carbapenemase-producing *Enterobacteriaceae* was frequently part of broader recommendations on multidrug-resistant microorganisms. Finland stated that such guidance was under development in their country. Austria and Denmark indicated that infection control guidelines were developed by public health professionals at regional or hospital level. In France, guidance on the screening of patients transferred directly from foreign healthcare facilities was under revision to be extended to all patients exposed to such care facilities in the year preceding admission to a French hospital.

Discussion

Incidence and geographical distribution

Current data indicates an increase in the spread not only of NDM-1, but also of other carbapenemase-producing *Enterobacteriaceae* in Europe and worldwide [2-6]. We found here 77 NDM-1 cases in 13 countries in Europe over the last three years, with the majority of cases in the UK. It is likely that the number of cases reported is underestimated, because, in most countries, infections with highly-resistant *Enterobacteriaceae* are not notifiable, nor do they have to be laboratory-confirmed. Moreover, microbiological guidance on the detection and identification of acquired carbapenemases in *Enterobacteriaceae* is available in only a minority of European countries. These countries were more likely to identify cases. Cases have also been reported in 2010 from Australia [18], Canada [19], Singapore [21], the US [10], and, according to recent media reports, from China, Israel, Japan, Kenya, Malaysia, Oman and Taiwan. The majority of cases described in our survey, as in other reports, had a history of recent travel and hospital admission on the Indian subcontinent, but there was also a smaller proportion of cases who

Table 2

Available national guidance documents on the management of carbapenemase-producing *Enterobacteriaceae*, in 14 European countries, as of 7 October 2010

Country	National guidance on NDM-1-producing or, more generally, carbapenemase-producing *Enterobacteriaceae*				Comment	Reference or URL
	Detection and surveillance methods	Referral to reference laboratory	Notification to health authorities	Infection control measures		
Austria	•	•	•		Infection control guidelines at hospital level	http://www.referenzzentrum.at
Belgium	•	•	•	•		http://www.nsih.be/surv_carba/carbapenemase_fr.asp http://www.nsih.be/surv_carba/carbapenemase_nl.asp
Czech Republic	•	•	•			http://www.szu.cz/uploads/documents/CeM/Zpravy_EM/18_2009/3_brezen/100_betal.pdf
Finland	•	•			Infection control guidelines under development	http://www.ktl.fi/portal/17160
France	•	•	•	•	Infection control guidelines under revision	http://www.hcsp.fr/explore.cgi/hcspr20100518_bmrimportees.pdf
Germany	•	•		•		http://www.rki.de/cln_169/nn_205760/DE/Content/Infekt/Krankenhaushygiene/Erreger__ausgewaehlt/ESBL/ESBL__LIT__03,templateId=raw,property=publicationFile.pdf/ESBL_LIT_03.pdf
Greece	•	•	•	•		http://www.keelpno.gr/images/stories/keelpno/prokroustis/prokroustis2010.doc
Netherlands	•	•	•	•		[31,33]
Norway	•	•		•		http://www.unn.no/k-res/metoder-for-paavisning-av-karbapenemase-produserende-esbl-carba-enterobacteriaceae-article77546-21588.html http://www.fhi.no/dokumenter/96331178b9.pdf
Poland	•	•	•	•		http://www.antybiotyki.edu.pl http://www.korld.edu.pl
Portugal	•	•	•	•		Orientação nº 006/2010 de 04/10/2010 (Available at: http://www.dgs.pt)
Slovenia	•	•		•		http://www.mz.gov.si/fileadmin/mz.gov.si/pageuploads/mz_dokumenti/delovna_podrocja/zdravstveno_varstvo/zdravstveno_varstvo_v_posebnih/NAKOBO_oktober_2010/PRIPOROCILA_ESBL_18.10.2010.doc
Sweden	•	•	•	•		http://soapimg.icecube.snowfall.se/strama/ESBLdokument%20inkl%20bakgrund.pdf http://soapimg.icecube.snowfall.se/strama/supplement%202%20ESBL%20definition.pdf
United Kingdom	•	•	•	•		http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1248854046470 http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1248854045473

NDM-1: New Delhi metallo-beta-lactamase-1.

had received hospital care in Balkan countries. Further studies should determine the risk of healthcare-associated acquisition of NDM-1 and other carbapenemase-producing *Enterobacteriaceae* in different parts of the world.

Laboratory detection and identification

Carbapenem resistance mediated by NDM-1 enzyme has been detected by clinical laboratories with routine phenotypic testing methods, including disc diffusion testing [1,2,10,11,18-26]. Any *Enterobacteriaceae* isolate that exhibits a minimum inhibitory concentration (MIC) above the epidemiological cut-off or with clinical resistance to ertapenem, imipenem or meropenem should trigger further testing [12,27] Carbapenemase activity can be screened by using the modified Hodge test [2,10-12] and, as with other metallo-beta-lactamases, synergy can be detected by EDTA-imipenem disc or Etest [1,2,10-12,]. Widely used automated susceptibility systems show good sensitivity but poor specificity for detection of carbapenem resistance mediated by NDM-1 and other carbapenemases [13]. Further evaluation of in-house and commercial test systems with larger numbers of NDM-1-producing strains are desirable, given the variable phenotypic expression of carbapenemase activity, as observed with strains producing KPC- and VIM-like enzymes [3,6,11,14,15,27]. Confirmation of the NDM-1 enzyme requires molecular analysis, typically PCR or DNA sequencing, by a reference laboratory [1,2,7,11]. A limitation of our study was the absence of a molecular case definition or a description of molecular NDM-1 identification methods. However, 38 of 77 cases described here have been published elsewhere with details of molecular identification (Table 1) and the majority of the remainder were reported by the same expert laboratories according to published national standards (Table 2).

Epidemic risk assessment

What is the epidemic potential of NDM-1? The $bla_{\text{NDM-1}}$ determinant was located on conjugative plasmids in the majority of the producer *E. coli* and *K. pneumoniae* clinical isolates [2]. In a few isolates, $bla_{\text{NDM-1}}$ was located on the bacterial chromosome, indicating intragenomic recombination [2]. NDM-1 was produced both by a *K. pneumoniae* isolate from urine and a faecal *E. coli* isolate from the same patient, suggesting in vivo transfer [1]. These characteristics indicate a potent capacity for horizontal dissemination, as further evidenced by detection of $bla_{\text{NDM-1}}$ in multiple genera of *Enterobacteriaceae* and in *A. baumannii* [2,11].

Many NDM-1 cases had co-morbidities and/or had undergone an invasive care procedure [1,2,19-26]. The clinical spectrum and severity of illness appears similar to that expected for *Enterobacteriaceae* infections in this patient population. There is a paucity of information on the extent and mode of transmission of NDM-1-producing bacteria in the community and in healthcare settings. By analogy with the epidemiology of the bacterial host, indirect faecal-oral inter-human transmission is likely to play a major role, via contaminated hands, food or water, particularly in countries with limited access to adequate sanitary infrastructure. In India, the majority of NDM-1-producing *Enterobacteriaceae* were community-acquired [2,7]. In the present survey, travel-associated cases who had had no contact with healthcare systems presumably acquired NDM-1 in the community [16,24].

Control interventions

Screening of colonisation with multidrug-resistant organisms upon admission to hospitals has been advocated in patients who have received healthcare in endemic countries or epidemic facilities [28-31]. Further interventions include preemptive isolation of these patients in single bedrooms and barrier precautions for the period while the screening results are pending, and continued for colonised patients [28-31]. So far, evidence of secondary nosocomial transmission of bacteria with the NDM-1 enzyme in Europe is limited, possibly as the result of such proactive infection control measures. Evidence-based control measures should be identified for all carbapenemase-positive bacteria and implemented in patient care.

Public health response

Public health preparedness for the control of carbapenemase-producing *Enterobacteriaceae*, including those producing NDM-1 enzyme, is progressing across Europe as evidenced by our survey (Table 2). Key components of current public health practices include (i) dissemination of national guidelines for microbiological laboratory detection, and (ii) recommendations for active surveillance and additional infection control precautions for patients who have received cross-border healthcare. Laboratory and epidemiological support should be readily available for the investigation of imported or indigenous cases and for the control of secondary transmission. Recent experiences with large epidemics of KPC and VIM carbapenemase-producing *Enterobacteriaceae* associated with significant mortality in the US, Greece and Israel, have highlighted the need for strengthening public health and the response capacity of healthcare systems, notably by dedicated national task forces and public health laboratory networks [28-30]. Member States could also consider providing information to their citizens seeking healthcare in foreign countries about the risk of acquiring NDM-1 and other extensively antibiotic-resistant bacterial pathogens.

EU-wide surveillance should be strengthened to enable monitoring of extensively antibiotic-resistant pathogens such as carbapenemase-producing *Enterobacteriaceae*. Linking national reference laboratories and public health institutes through antimicrobial resistance surveillance networks such as the EARS-Net, implementing national generic communicable diseases reporting and early warning systems to ensure rapid communication and a timely response, could be further studied as best practice to be shared

among countries for effective containment of these extensively resistant pathogens. At the EU level, rapid information exchange by means of electronic communication platforms such as the Epidemic Intelligence System (EPIS) [32] or the Early Warning and Response System (EWRS) would result in an integrated European approach.

European NDM-1 Survey Participants:
Austria - A. Grisold, G. Zarfel; Belgium - B. Jans; Bulgaria - T. Velinov, T. Kantardjiev; Cyprus - M. Alexandrou; Czech Republic - H. Zemlickova, J. Hrabak; Denmark - N. Frimodt-Møller, A.M. Hammerum; Estonia - M. Maimets, M. Ivanova; Finland - J. Jalava, M. Rummukainen; France - RAISIN (French Healthcare-Associated Infection Alert, Investigation and Surveillance Network); Germany - T. Eckmanns, M. Kaase; Greece - X. Dedoukou, A. Vatopoulos; Hungary - K. Böröcz; Iceland - K.G. Kristinsson, O. Gudlaugsson; Ireland - R. Cunney; Italy - G.M. Rossolini, A. Pantosti; Latvia - U. Dumpis, A. Balode; Lithuania - R. Valinteliene; Luxembourg - P. Weicherding; Malta - M. Borg; Netherlands -M.A. Leverstein-van Hall, X. Huijsdens; Norway - Ø. Samuelsen, G.S. Simonsen; Poland - W. Hryniewicz, M. Gniadkowski; Portugal - A.C. Costa, M. Caniça; Romania - I. Codita, R. Serban; Slovakia - L. Siegfried, M. Stefkovicova; Slovenia - J. Kolman, M. Pirš; Spain - J. Oteo, J. Campos; Sweden - K. Tegmark-Wisell, P. Edquist; United Kingdom - D. Livermore, N. Woodford

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Prevalence of meticillin-resistant *Staphylococcus aureus* amongst professional meat handlers in the Netherlands, March–July 2008

R de Jonge (rob.de.jonge@rivm.nl)[1], J E Verdier[1,2], A H Havelaar[1,3]
1. National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2. Erasmus Medical Center, Rotterdam, the Netherlands
3. Utrecht University, Utrecht, the Netherlands

Citation style for this article:
de Jonge R, Verdier JE, Havelaar AH. Prevalence of meticillin-resistant Staphylococcus aureus amongst professional meat handlers in the Netherlands, March–July 2008. Euro Surveill. 2010;15(46):pii=19712. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19712

Article published on 18 November 2010

In the Netherlands, meticillin-resistant *Staphylococcus aureus* (MRSA) is detected on pork and veal farms, and hence farmers working with MRSA-positive animals are at an increased risk of being colonised. Recently retail meat products have been found positive for MRSA. Therefore, we tested the prevalence of MRSA among employees who work in the cold meat processing industry and in institutional kitchens. Nasal swabs and samples from the employees' hands as well as the handled meat were tested quantitatively and qualitatively for the presence of MRSA. Typical colonies were confirmed by PCR and typed using multi-locus sequence typing and *spa*–typing. All samples taken from 95 employees tested negative for MRSA, but 31 carried MSSA. From meat, five of 35 samples were positive for MRSA, containing between 0.01 and more than 10 bacteria per gram. The risk for professionals of MRSA colonisation from handling raw meat was therefore low in our setting, suggesting that the general population is at an even lower risk of being infected through meat handling.

Introduction

Staphylococcus aureus is a Gram-positive, catalase-positive commensal bacterium colonising both humans and animals. *S. aureus* is known for causing food poisoning through the production of enterotoxins [1,2]. Worldwide, strains have emerged that are resistant to a wide range of antibiotics. In the Netherlands, 0.6% of all *S. aureus* strains isolated from hospitals between 1999 and 2003 [3], were resistant to meticillin. From 2004 till 2007, this number increased to 1.1%, which is still well below the average for Europe: 23.7%, according to the European Antimicrobial Resistance Surveillance System (EARSS-network, now EARS-Net; [4]).

In 2004, Wertheim *et al*. [5] measured persons without predisposing risk to MRSA carriage before hospital admission and established that the prevalence of MRSA in the Dutch population was 0.03%. Between 2002 and 2006, newly recognised infections in the Netherlands were found to be caused by a zoonotic, originally PFGE non-typable, strain of MRSA. Surveillance studies have shown that this strain, not detected before 2002, accounted for up to 5.5% of all human MRSA isolates in the first half of 2006 and up to 21% in the second half of the same year [6]. A partial explanation for this steep increase is the fact that screening of risk groups, including persons frequently in contact with pigs or calves, for MRSA carriage at hospital admission was implemented in the Netherlands in 2006. Later, the same strain was typed by multi-locus sequence typing (MLST), and it was shown that the vast majority of isolates of this strain belonged to sequence type (ST) 398 [6]. Another typing method, staphylococcal protein A (*spa*) typing, is frequently used to determine the number and sequence of repeats in the *spa* gene. MRSA ST398 from livestock animals frequently contains *spa* types t011, t034, t108 and t567 [9,10]. The virulence potential of MRSA is associated with its ability to produce the Panton–Valentine leukocidin (PVL) cytotoxin [9].

The first links between farming and MRSA were made in 2004 and 2005, when a farmer and his family were infected, treated for eradication of *S. aureus*, then re-infected. Later, the farmer's pigs were proven to be colonised with a genotypically indistinguishable MRSA strain [10,11]. In 2006, 31 pig farms were screened in the Netherlands. On seven farms, pigs were colonised with MRSA. Eleven of the 22 farmers who had undergone voluntary screening were also colonised with MRSA. All isolates were negative for PVL and not typable by PFGE (hence at the time likely to be ST398) [7]. Another notable finding from this study was that a few pigs that were negative for MRSA became MRSA carriers after treatment with oxytetracycline for respiratory problems.

Other evidence that contact with animals can lead to higher *S. aureus* carriage was provided by a French study in which farmers were compared to non-farmers [12]. Farmers proved to have a significantly higher

S. aureus colonisation ratio (44.6%) compared to non-farmers (24.1%). In a Dutch [6] and in a Danish case-control study [13], it was demonstrated that pigs were indeed a source of the rapidly emerging ST398 MRSA.

In the Netherlands, the prevalence of MRSA in animals was also estimated in slaughterhouses. A total of 540 pigs were screened, a randomised selection of ten animals from 54 batches from nine slaughterhouses. Of those, 209 animals (39%) were positive for MRSA, distributed over 44 (81%) of the batches [14]. All samples were ST398 and negative for PVL.

Given that farm animals, farmers and slaughterhouses have been found positive for MRSA, the prevalence of MRSA in meat in the Netherlands was subsequently assessed. Meat samples of various species from retail suppliers were checked for the presence of MRSA, with 11.2% testing positive. Highest prevalences were found in meat from turkeys (31.3%), chicken (27.3%), veal (16.8%) and pigs (10.4%). Of all MRSA isolates, 84% (116 of 138) belonged to ST398 [15].

Although food can be a vehicle for MRSA, the consumption of MRSA-colonised meat is thought to carry only a small risk since heating is likely to kill all bacteria and *S. aureus* is assumed to be present only on the surface of the meat. However, there might be a risk of direct transmission from raw meat [16], especially for people who work with meat intensively. Transmission has already been assessed for *Micrococcus luteus*, and transmission from a hamburger to hands was shown to occur, albeit at the low rate of 0.06% [17].

In this study, we assessed the risk of colonisation with both MRSA and meticillin-sensitive *S. aureus* (MSSA) for professionals who work intensively with raw meat products.

Methods

Target population

The study was conducted between March and July 2008. The selected population consisted of professionals who worked with raw meat on a daily basis, but were not in contact with live farm animals as part of their work. It included institutional kitchen staff (from two hospitals) and staff working at three facilities processing cold meat, where carcass parts were cut into portions for consumption. Every person in the study population had to sign an informed consent form before the start of the sampling. A questionnaire was used to assess background risks and exposure [18].

Sampling of humans

A single nasal swab (Transwab, Medical Wire and Equipment, England) was collected from each participant and stored in an ice box until further analysis on the same day for MRSA and MSSA.

To test for the presence of MRSA and MSSA on hands, we used a rinse method [19]. Participants were asked to put on a sterile nitrile glove. Sterile Mueller Hinton broth (BD, United States) with 6.5% NaCl (MH+; 30ml) was then poured into the glove, and after 30 seconds of soaking, gloves and MH+ were collected in a sterile stomacher bag. If the participants already wore gloves, these were collected into a stomacher bag and 30 ml MH+ were added. All bags were stored in an ice box for a maximum of three hours at temperatures at 2–5 °C, until further analysis at the end of the day.

Sampling of meat

Meat samples were taken from a single randomly chosen piece of meat that was being prepared. Participants working with meat were asked to deposit a piece of it in a sterile bag. The samples were kept in separate containers at low temperatures (ice box, 2–5 °C) during transport and storage until further analysis at the end of the day.

Microbiological analysis

Nasal swabs

Nasal swabs were analysed for the presence of MRSA and MSSA in two ways. To detect high numbers, indicative for colonisation, the swabs were streaked directly onto a MRSA screening plate (MRSA brilliance, Oxoid) [20] and on a Baird Parker agar plate (Oxoid) supplemented with rabbit plasma fibrinogen (Oxoid). To detect low numbers, indicative for transmission, the swabs were then incubated in 10 ml MH+ for 18 hours at 37 °C. For a second enrichment step, 1 ml was transferred to 9 ml phenol red mannitol solution with 75 µg/ml aztreonam (MP Biomedicals, United States) and 0.4 µg/ml ceftizoxime (PRM tube, Biomerieux) and incubated for

Table 1

Primer sequences used for typing of meticillin-resistant *Staphylococcus aureus*

Gene	Primer	Sequence
MecA [22]	MecA-1	5'GTTGTAGTTGTCGGGTTTGG3'
	MecA-C3	5'CTTCCACATACCATCTTCTTTAAC3'
PVL [23]	SaPVL-1	5'ATCATTAGGTAAAATGTCTGGACATGATCCA3'
	SaPVL-2	5'GCATCAA(GC)TGTATTGGATAGCAAAAGC3'
Martineau [24]	Sa442-1	5'AATCTTTG-TCGGTACACGATATTCTTCACG3'
	Sa442-2	5'CGTAATGAGATTTCAGTAGATAATACAACA3'

Table 2

Prevalence of meticillin-resistant *Staphylococcus aureus* on different types of meat, the Netherlands, March–July 2008 (n=35)

Origin	MRSA present		Total
	Yes	No	
Veal	1	15	16
Pork	2	8	10
Chicken	2	4	6
Turkey	0	2	2
Fish	0	1	1
Total	**5**	**30**	**35**

a further 18 hours at 37 °C. A loopful (approximately 1µL) of each tube was streaked on a MRSA screening plate and a Colombia agar base plate with 5% sheep blood (CAB-sb; Oxoid) and incubated for 20 to 24 hours at 37°C [20]. Isolates were finally confirmed by PCR and typing.

Samples collected from hands and meat samples

Samples were analysed as described for low numbers in nasal swabs (above) following an MPN-approach. Meat samples plus MH+ were homogenised in a pulsifier (Microgen Bioproducts) prior to incubation.

MSSA screening

After enrichment of samples in MH+, material from the lowest dilution of any sample was tested for MSSA as described [21].

PCR testing for MRSA

All MRSA isolates were genetically characterised by PCR specific for *S. aureus* (Martineau), the *mecA* gene and the PVL toxin genes (Table 1). A selection of isolates was further typed by MLST and *spa*–typing.

Data analysis

Prevalence data of MRSA in humans were analysed with the BINOMDIST function and Solver add-in in Microsoft Excel. Most Probable Numbers (MPN) of MRSA (and 95% confidence intervals) on food were estimated using an Excel spreadsheet based on the MPN method originally described by De Man [23,24].

Results

In this study, we examined persons working in either an institutional kitchen or at a meat processing facility, as well as meat samples. The target population originally contained 101 persons (randomly chosen). For 89 persons the results from sampling and questionnaire were available. Twelve people were excluded because they were either not present at the day of sampling (n=2), or they did not come into contact with meat (n=4). Six people did not provide information for the questionnaire or could not read Dutch or English.

Human samples

The male:female ratio in the study population was uneven (80% males). The age ranged between 26 and 56 years. Sixty-eight respondents were born in the Netherlands, while 13 were from other European countries and eight were of non-European origin. Forty-one participants kept pets, while living on a farm or keeping farm animals was rare (n=4). All wore an overall when entering the butchery or kitchen. During their daily activities, 80 respondents stated they were wearing protective clothing (hairnet, gloves and overall). Nine used or had used antibiotics during the six months preceding the study. Only one of them recalled the antibiotic: amoxicillin. Eleven of the participants had been admitted to a hospital during the six months preceding the study, three of them more than once. Another person was hospitalised abroad. Ten persons in the study population suffered from a chronic disease.

All samples from hands and noses were negative for MRSA, but 31 participants were colonised with MSSA. Given the number of samples taken, these results imply that, with 95% confidence, the prevalence of MRSA colonisation among professional meat handlers is less than 3%.

Meat samples

The results of MRSA screening of the meat samples are shown in Table 2. Of 35 meat samples, five were contaminated with MRSA: pork (n=2), veal (n=1) and chicken (n=2). MPNs of MRSA as determined in the samples varied between 0.06 and more than 10 bacteria per gram of meat (Figure).

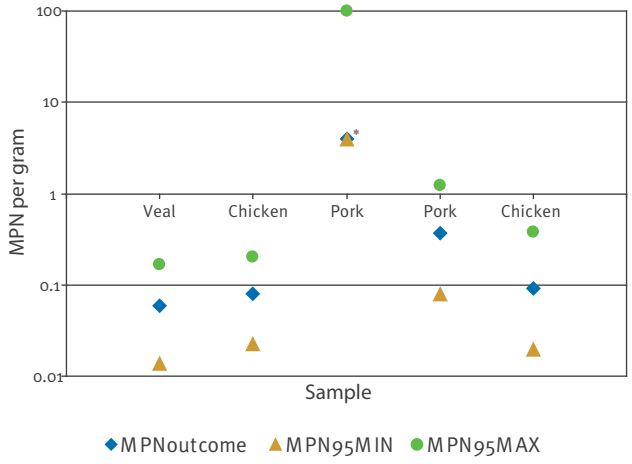
After PCR confirmation, MRSA-positive samples were submitted to the Dutch Reference Centre for Staphylococci for *spa* and MLST typing. Four isolates (one veal, two pork and one chicken) were typed as ST398 and *spa*-type t011, and one isolate from a chicken meat sample belonged to ST9/t1430.

Discussion

Hands and noses of all meat-handling professionals tested negative for MRSA. Among these negatively tested participants were those with a predisposing risk due to the use of antibiotics or hospital admission, as well as six persons that did not provide information for the questionnaire. Samples from these people were analysed before the results of the questionnaire were available. As all samples were negative, we did not

Figure

Most Probable Numbers of meticillin-resistant *Staphylococcus aureus* in meat samples, the Netherlands, March–July 2008 (n=35)



Lines indicate 95% confidence intervals, with the marker representing the Most Probable Number (MPN) per gram of sample.

*All MPN series tested positive for MRSA. The MPN of this sample was at least 110 bacteria per examined sample (approximately 25 g), or four bacteria per gram. The upper 95% confidence interval limit was infinite.

consider it necessary to exclude them from the analysis at a later stage. This result implies an upper putative colonisation rate of 3%. However, the observed prevalence of *S. aureus* (MSSA) in our study, 33%, was somewhat higher than the 24% reported for the general population in the Netherlands [5]. These results indicate that the susceptibility of our study population to *S. aureus* is at least as high as that of the general population and suggest that the observed absence of MRSA was not biased by a particular resistance of this group to *S. aureus* in general.

MRSA was found in five meat samples, with four isolates belonging to the MRSA strain ST398/t011 that has been associated with livestock [8], and one (a chicken meat sample) showing a type similar to those recently detected in chicken meat [15]. The prevalence of 14.3%, MRSA in the meat samples in this study was slightly higher compared with previously published data that reported a prevalence of 11.2% [15].

In a Swiss study done in 2009, MRSA could not be detected in pig farmers and slaughterhouse employees [27]. In contrast, a Dutch study from 2010 [18] found a high prevalence of nasal MRSA carriage (5.6%) in pig-slaughterhouse workers. The difference between the two studies correlated with a difference in the prevalence of MRSA in pigs between Switzerland and the Netherlands. In the present study, we investigated the prevalence of MRSA in meat-handling professionals, as it had been reported that the prevalence of MRSA in meat was high [15]. However, we were unable to detect MRSA in our test population. This might be due to the fact that the chosen test group was too small, or to different routes transmission of MRSA in slaughterhouses and meat processing facilities. Additionally, rather than a difference in prevalence, there may have been a difference in the concentrations of MRSA in MRSA-positive samples, which would result in different levels of exposure. We determined that the MPN of MRSA present in our meat samples varied between 0.01 and more than 10 per gram. For one sample, no accurate MPN could be determined as all tested dilution series were positive. Unfortunately, most studies on the prevalence of MRSA lack information on the concentration of MRSA.

This study showed that high-frequency exposure in the tested population did not result in a measurable risk of colonisation with MRSA. While the number of sampled persons, and hence the power of the study was limited, we believe that these findings imply that the risk of colonisation by contact with raw meat for the general Dutch population should be at most equal, if not several orders of magnitude lower. Professional meat handlers come into contact with raw meat many times every day, whereas the general public would come into contact with raw meat once a day or more rarely. The upper limit of colonisation prevalence of professionals (3%) would therefore correspond to a much lower estimate for the general population, which is in agreement with a prevalence of 0.03% in the Dutch population as reported by Wertheim *et al*. [5].

Acknowledgements

We like to thank Arjen van de Giessen and Xander Huijsdens for helpful suggestions and critically reading the manuscript, and Paul Hengeveld and Thijs Bosch for technical assistance.

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

H Grundmann (Hajo.Grundmann@rivm.nl)[1,2], D M Livermore[3], C G Giske[4], R Canton[5,6], G M Rossolini[7], J Campos[8], A Vatopoulos[9], M Gniadkowski[10], A Toth[11], Y Pfeifer[12], V Jarlier[13], Y Carmeli[14], the CNSE Working Group[15]

1. Department of Medical Microbiology, University Medical Centre, Groningen, the Netherlands
2. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
3. Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, London, United Kingdom
4. Microbiology and Tumour Biology Centre, Karolinska Institute, Stockholm, Sweden
5. Microbiology Service, CIBER Epidemiology and Public Health (CIBERESP), University hospital Cajal and Institute Ramón and Cajal for Health Research (IRYCIS), Madrid, Spain
6. Associated Resistance Unit National Research Council (Unidad de Resistencia Asociada al Consejo Superior de Investigaciones Científicas; CSIC), Madrid, Spain
7. Department of Molecular Biology, Section Microbiology, University of Siena, Siena, Italy
8. Antibiotic Laboratory, National Centre of Microbiology, Instituto de Salud Carlos III, Madrid, Spain
9. Department of Microbiology, National School of Public Health, Athens, Greece
10. National Medicines Institute, Warsaw, Poland
11. Department of Bacteriology, National Centre for Epidemiology, Budapest, Hungary
12. Robert Koch Institute, Nosocomial Infections, Wernigerode, Germany
13. University Pierre and Marie Curie–Hospital Pitié-Salpêtrière, Assistance Publique–Hôpitaux de Paris, Paris, France
14. Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
15. The members of the group are listed at the end of the article

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.

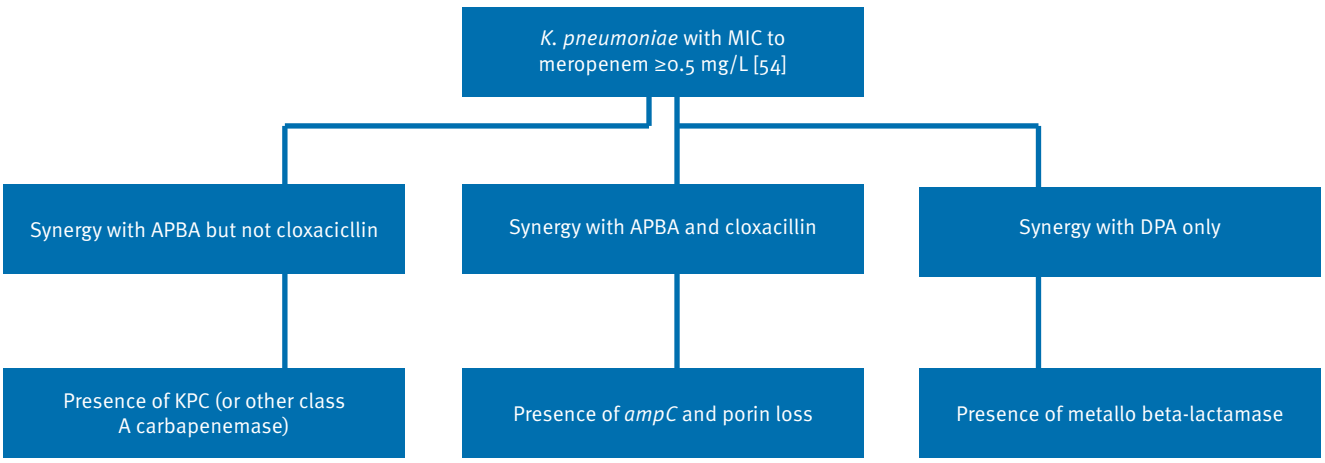
NDM-1 producers are mainly *K. pneumoniae* but also include *Escherichia coli* and *Enterobacter* spp. Most isolates with MBLs, particularly those with NDM types, also contain ESBL and acquired *amp*C genes, which makes them resistant to all antibiotics except polymyxins, tigecycline and, occasionally,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

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.

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

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'.

On 17 January 2011, the names of Aurora Garcia-Fernandez and Maurine A. Leverstein - van Hall were corrected in the members' list of the CNSE Working Group.

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CNSE Working Group
Helmut Mittermayer*, Institute of Hygiene, Microbiology and Tropical Medicine, Elisabethienen Hospital, Linz, Austria
Mathijs Goossens, Scientific Institute of Public Health, Brussels, Belgium
Nijaz Tihic, Department of Microbiology, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina
Yuliha Proevska, Alexander University Hospital, Medical University, Sofia, Bulgaria
Arjana Tambic, Hospital for Infectious Diseases, Zagreb, Croatia
Despo Pieridou-Bagatzouni, Microbiology Department, Nicosia General Hospital, Nicosia, Cyprus
Helena Zemlickova, National Reference Laboratory for Antibiotics, National Institute of Public Health, Prague, Czech Republic
Jaroslav Hrabak, Department of Microbiology, Faculty of Medicine, Plzen, Czech Republic
Anette M. Hammerum, Department of Microbiological Surveillance and Research, Statens Serum Institute, Copenhagen, Denmark
Marina Ivanova, Microbiology Department of the Central Laboratory, East-Tallinn Central Hospital, Tallinn, Estonia
Jari Jalavi, Department of Infectious Disease Surveillance and Control, National Institute for Health and Welfare, Turku, Finland
Vincent Jarlier, University Pierre and Marie Curie–Hospital Pitié-Salpêtrière, Assistance Publique–Hôpitaux de Paris, Paris, France
Yvonne Pfeifer, Robert Koch Institut, Wernigerode, Germany
Alkis Vatopoulos, Department of Microbiology, National School of Public Health, Athens, Greece
Akos Toth Department of Bacteriology, National Centre for Epidemiology, Budapest, Hungary
Hordur Hardarson, National University Hospital, Reykjavik, Iceland
Teck Wee Boo, Discipline of Bacteriology, National University of Ireland Galway, Ireland and Division of Clinical Microbiology, Galway University Hospitals, Ireland
Raul Colodner, Laboratory Medicine Department, Ha'Emek Medical Center, Afula, Israel
Yehuda Carmeli, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
Monica Monaco, Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy
Aurora Garcia-Fernandez, Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy
Gianni Rossolini, Department of Molecular Biology, Section Microbiology, University of Siena, Siena, Italy
Arta Balode, Paul Stradina Clinical University Hospital, Riga, Latvia
Jolanta Miciuleviciene, National Public Health Laboratory Center, Vilnius, Lithuania
Paul Caruana, Mater Dei Hospital, Valetta, Malta
Gunnar Skov, Simonsen Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
Orjan Samuelsen, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
Waleria Hryniewicz, National Medicines Institute, Warsaw, Poland
Marek Gniadkowski, National Medicines Institute, Warsaw, Poland
Manuela Caniça, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
Vera Manageiro, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
Irina Codita, Devison for Microbiology and Immunology, "Cantacuzino" National Institute of Research, Bucharest, Romania
Manica Mueller-Premru, Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia
Jana Kolman, University Medical Centre Ljubljana, Slovenia
Jose Campos, Antibiotic Laboratory, National Centre of Microbiology, Instituto de Salud Carlos III, Madrid, Spain
Jesus Oteo Iglesias, Instituto de Salud Carlos III, Madrid, Spain
Rafael Cantón, Microbiology Service, CIBER Epidemiology and Public Health (CIBERESP), University hospital Cajal and Institute Ramón and Cajal for Health Research (IRYCIS), Madrid, Spain
Petra J. Edquist, Department of Bacteriology, Division of Antibiotic Resistance and Infection Control, Swedish Institute for Infectious Disease Control, Stockholm, Sweden
Christian Giske, Microbiology and Tumour Biology Centre, Karolinska Institute, Stockholm, Sweden
Sara Droz, Institute for Infectious Diseases, University of Bern, Bern, Switzerland
David Livermore, Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, London, United Kingdom
Maurine A. Leverstein - van Hall, Department Medical Microbiology, University Medical Centre Utrecht, Netherlands
Xander Huijsdens, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands
Hajo Grundmann, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands
* We deeply regret the untimely loss of our dear friend and colleague Helmut Mittermayer.

Extented measures for controlling an outbreak of VIM-1 producing imipenem-resistant *Klebsiella pneumoniae* in a liver transplant centre in France, 2003–2004

N Kassis-Chikhani (najiby.kassis-chikhani@pbr.aphp.fr)[1,2], F Saliba[3], A Carbonne[4], S Neuville[5], D Decre[6,7,8], C Sengelin[1,2], C Guerin[1], N Gastiaburu[3], A Lavigne-Kriaa[3], C Boutelier[1], G Arlet[7,8,9], D Samuel[3,10], D Castaing[3,10], E Dussaix[2,10], V Jarlier[5,7,11]

1. Assistance Publique–Hôpitaux de Paris, hospital Paul Brousse, Infection Control Unit, Villejuif, France
2. Assistance Publique–Hôpitaux de Paris, hospital Paul Brousse, Microbiology Laboratory, Villejuif, France
3. Assistance Publique–Hôpitaux de Paris, hospital Paul Brousse, Centre Hépato-Biliaire, Villejuif, France
4. Centre de Coordination de la Lutte contre les Infections Nosocomiales (CCLIN, Coordinating centre in the fight against nosocomial infections) Paris-Nord, France
5. Assistance Publique–Hôpitaux de Paris, Central Infection Control Team, Paris, France
6. Assistance Publique–Hôpitaux de Paris, hospital Saint-Antoine, Bacteriology, Paris, France
7. Université Pierre et Marie Curie, Paris 6, France
8. ER8 Antibiotiques et Flore digestive, Université Paris 6, Paris, France
9. Assistance Publique–Hôpitaux de Paris, hospital Tenon, Bacteriology, Paris, France
10. Université Paris-Sud, UMR 785, Villejuif, France
11. Assistance Publique–Hôpitaux de Paris, hospital Pitié-Salpétrière, Paris, France

Citation style for this article:
Kassis-Chikhani N, Saliba F, Carbonne A, Neuville S, Decre D, Sengelin C, Guerin C, Gastiaburu N, Lavigne-Kriaa A, Boutelier C, Arlet G, Samuel D, Castaing D, Dussaix E, Jarlier V. Extented measures for controlling an outbreak of VIM-1 producing imipenem-resistant Klebsiella pneumoniae in a liver transplant centre in France, 2003–2004. Euro Surveill. 2010;15(46):pii=19713. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19713

Article published on 18 November 2010

We report the successful control of an outbreak caused by imipenem-resistant VIM-1-producing *Klebsiella pneumoniae* (IR-Kp) in France. This outbreak occurred in a care centre for abdominal surgery that includes a 15-bed liver intensive care unit and performs more than 130 liver transplantations per year. The index case was a patient with acute liver failure transferred from a hospital in Greece for urgent liver transplantation who was carrying IR-Kp at admission as revealed by routine culture of a rectal swab. Infection control measures were undertaken and included contact isolation and promotion of hand hygiene with alcohol-based hand rub solution. Nevertheless, secondary IR-Kp cases were identified during the six following months from 3 December 2003 to 2 June 2004. From 2 June to 21 October, extended infection control measures were set up, such as cohorting IR-Kp carriers, contact patients and new patients in distinct sections with dedicated staff, limiting ward admission, and strict control of patient transfer. They led to a rapid control of the outbreak. The global attack rate of the IR-Kp outbreak was 2.5%, 13% in liver transplant patients and 0.4% in the other patients in the care centre ($p<0.005$). Systematic screening for IR-Kp of all patients admitted to the care centre is still maintained to date and no secondary IR-Kp case has been detected since 2 June 2004.

Introduction

Klebsiella pneumoniae has been a prominent cause of nosocomial infections and outbreaks, particularly in intensive care units. Metallo-beta-lactamases (MBLs) hydrolyse all beta-lactam antibiotics including carbapenems (except aztreonam) and are reported increasingly in *Enterobacteriaceae*. Among MBLs, the first member of the VIM-family of enzymes, VIM-1, was identified in a clinical isolate of *Pseudomonas aeruginosa* in Verona, Italy [1]. During the last decade, VIM-type MBLs have spread in *Enterobacteriaceae* [2-10] and outbreaks of such strains have been reported in Greece [11] and Italy [12]. *Klebsiella pneumoniae* carbapenemase (KPC) is one of the most prevalent carbapenemases in *Enterobacteriaceae* especially in Asia, Israel, southern Europe, the United Kingdom (UK), and the United States (US) [13]. In 2009, a novel MBL named NDM-1 (New Delhi metallo-beta-lactamase) was identified by Yong D *et al*. in *K. pneumoniae* and *Escherichia coli* isolates recovered from a Swedish patient transferred from India to a hospital in Sweden [14]. A recent study reported NDM-1 MBLs in various *Enterobacteriaceae* in the UK [15]. Many of the NDM-1-positive patients had travelled in India or Pakistan in the year preceding their infection or had links with these countries [15]. Population mobility is known to be a major factor in the spread of multidrug-resistant organisms [16]. Prevention of the spread of carbapenemases, especially into northern and western European countries where these enzymes are not yet endemic, is vital. The European Union is facing the threat of multiple outbreaks involving carbapenemase-producing *Enterobacteriaceae* and thus needs to establish guidelines to control such outbreaks rapidly and efficiently.

We report on the first control of an outbreak involving a single strain of VIM-1-producing *K. pneumoniae* that occurred in 2004 at Paul Brousse hospital, a 716-bed

tertiary-care teaching hospital of Assistance Publique–Hôpitaux de Paris (AP-HP), the largest public health institution in France with 23,000 beds. The outbreak occurred in the abdominal surgery care centre that comprises 81 single bedrooms including 15 beds on the first floor dedicated to intensive care and an acute care facility of 66 beds located on the third and fourth floors of the building. The intensive care unit admits more than 800 patients per year. The abdominal surgery care centre performs more than 130 liver transplantations per year, 15% of them in patients from foreign countries, and 200 hepatectomies.

We have previously described the bacteriological characteristics of the strain involved in this outbreak [17]. We describe here the characteristics of the IR-Kp cases and the specific measures that led to the control of the outbreak.

Outbreak description

The index case (Case 1) was a patient with acute fulminant hepatic failure admitted on 2 December 2003 to the intensive care unit for an urgent liver transplantation. He was transferred from Athens, Greece, where he had been hospitalised for an acute hepatitis due to hepatitis B virus infection. It was the first time that an IR-Kp has been identified in our hospital, since systematic screening for extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* was introduced in 2002. We learnt later that the Greek hospital this

Table 1

Clinical characteristics of patients colonised or infected with imipenem-resistant *Klebsiella pneumoniae* in an abdominal surgery care centre, France, 2003–2004 (n=8)

Case	Age (years)	Date of admission	Clinical events	First isolate: site	Interval between admission and IR-Kp isolation (days)	Antibiotic treatment	Outcome	Suspected place of acquisition
1	in their 20s	December 2003	Liver transplantation	rectal swab	0	No treatment	Discharged	Index case transferred from Greece
2	in their 50s	December 2004	Liver transplantation	blood culture	13	No treatment	Discharged	Acute care facility
3	in their 60s	September 2003	Liver transplantation	tracheal fluid	175	Piperacillin/ tazobactama Ciprofloxacin[a]	Deceased (due to IR-Kp infection)	Acute care facility
4	in their 60s	February 2004	Liver transplantation	urine culture	48	No treatment (asymptomatic)	Deceased	Intensive care unit
5	in their 70s	April 2004	Liver cirrhosis	urine culture	31	No treatment (asymptomatic)	Deceased	Intensive care unit
6	in their 50s	January 2004	Liver transplantation	blood culture	173	Colistin	Deceased (due to IR-Kp infection)	Intensive care unit
7	in their 50s	March 2004	Liver transplantation	rectal swab	101	No treatment	Discharged	Intensive care unit
8	in their 40s	January 2004	Liver transplantation	rectal swab	206	No treatment	Discharged	Acute care facility

[a] Two antibiotics to which the stain was resistant. IR-Kp was identified post mortem.

Table 2

Organisation of distinct sections in intensive care unit and acute care facility during Period 2 of the control measures, abdominal surgery care centre, France, 2 June to 21 October 2004

Separation with plastic wall

First floor intensive care unit	IR-Kp patients section	IR-Kp-free – long stay intensive care unit section
	IR-Kp patients who stayed in intensive care until discharge Number of beds[a]: 3	Patients requiring intensive care for an expected duration of more than 48 hours Number of beds[a]: 12
Third floor acute care facility	IR-Kp free patients non-intensive care unit section	IR-Kp-free – short stay intensive care unit section
	Patients directly admitted to the acute care facility Number of beds[a]: 25	Patients having major surgery but requiring monitoring for less than 48 hours Number of beds[a]: 5
Fourth floor acute care facility	Contact acute care section	
	Patients hospitalised on the same floor and at the same time as an IR-Kp patient Number of beds[a]: 36	

IR-Kp: imipenem-resistant VIM-1-producing Klebsiella pneumoniae.

[a] Only single bedrooms.

patient had been transferred from was at that time experiencing an outbreak with IR-Kp [8].

A secondary IR-Kp case was defined as a patient with IR-Kp isolated from any clinical sample (if infected) and/or from a rectal swab (if colonised). In the context of this outbreak, five further cases of IR-Kp infection and two cases of IR-Kp colonisation occurred in our hospital. The characteristics of the IR-Kp cases are listed in Table 1. Six of the seven secondary cases had a liver transplantation and one had end-stage liver cirrhosis.

The interval between admission and the first IR-Kp-positive specimen ranged between 13 and 206 days (median: 101 days). Considering only the seven cases who acquired IR-Kp in the abdominal surgery care centre (Table 1), patient age ranged between 46 and 69 years (median 62 years).

Figure 1

Synoptic curve of imipenem-resistant *Klebsiella pneumoniae* cases (infection and colonisation) in an abdominal surgery care centre, France, December 2003 to October 2004 (n=8)

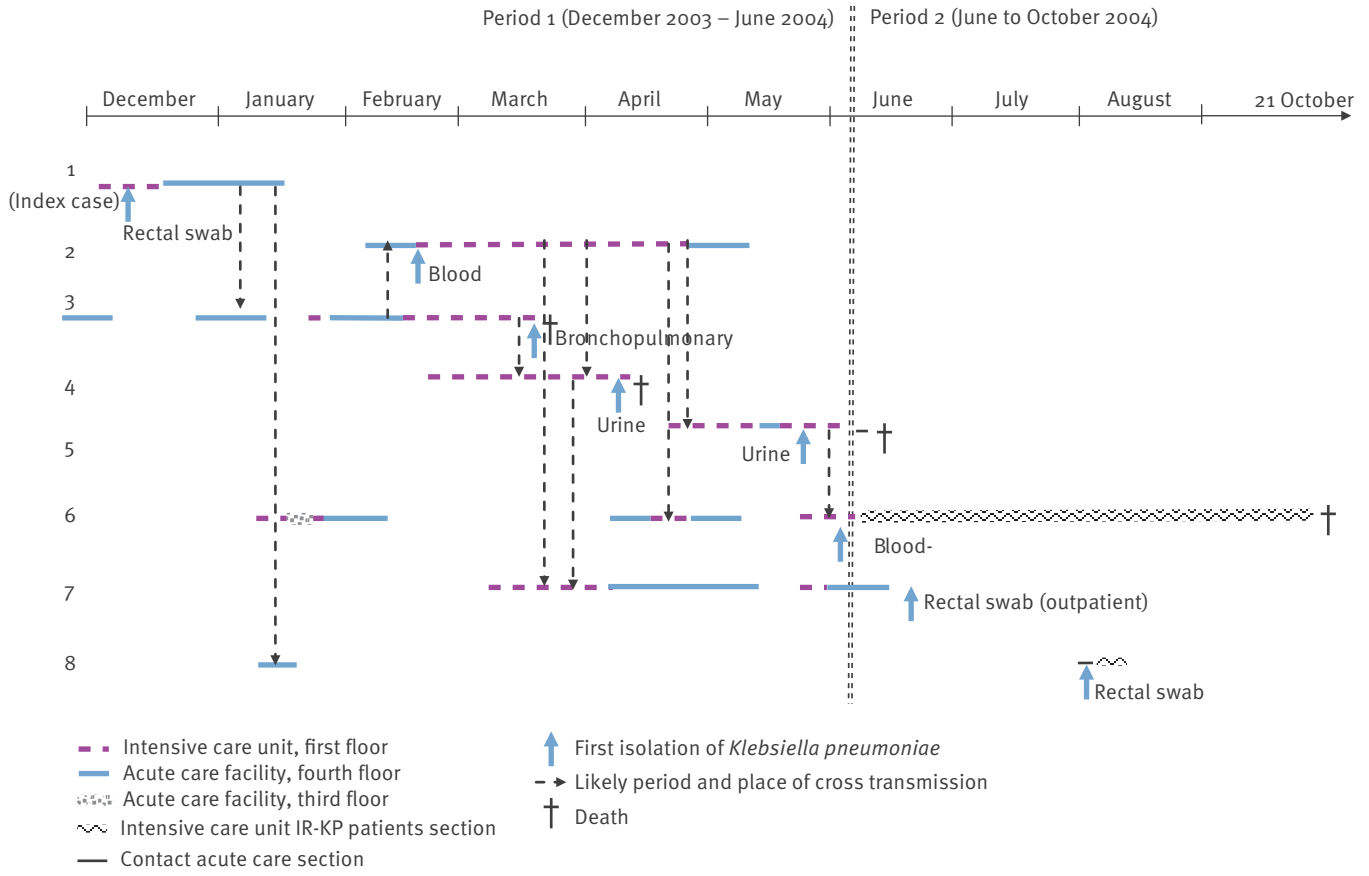
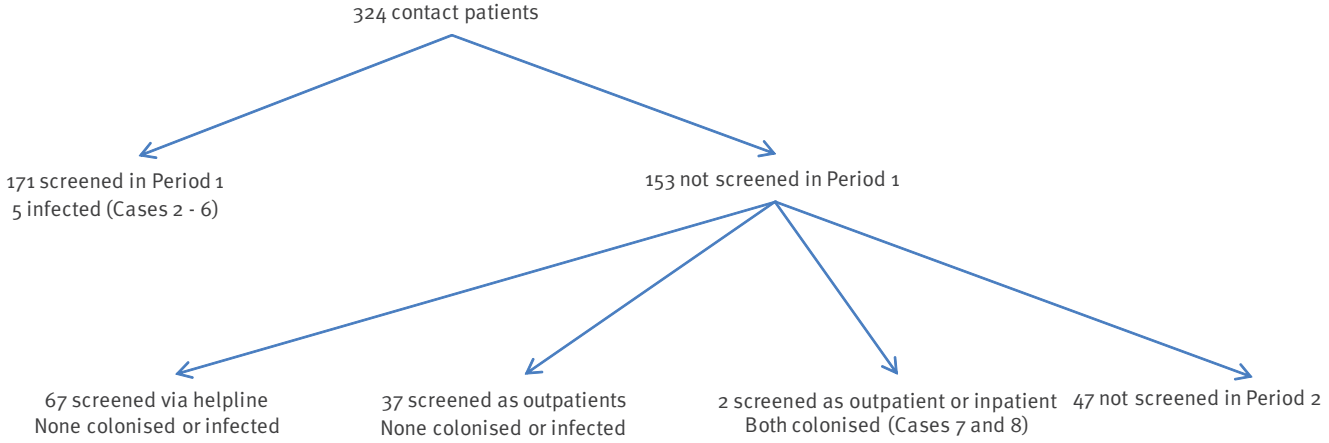


Figure 2

Patients screened in connection with an outbreak of imipenem-resistant *Klebsiella pneumoniae* in an abdominal surgery care centre, France, 2 December 2003 to 21 October 2004



Treatment and outcome

IR-Kp was isolated from a rectal swab of the index case sampled on the day of admission to the intensive care unit. This case did not receive any antibiotic therapy during his stay and remained colonised until he was discharged in January 2004 and returned to Greece.

The five secondary IR-Kp cases (Cases 2 to 6) were treated as follows: Case 2 had a central venous catheter-related bloodstream infection that resolved after removal of the catheter, without antibiotic treatment. Case 3 received piperacillin/tazobactam and ciprofloxacin for bacteraemia (two antibiotics to which the strain was resistant, see chapter on Microbiological diagnostic) and died of Gram-negative septic shock, the IR-Kp having been identified only after the patient's death. Cases 4 and 5 had an asymptomatic urinary tract infection with IR-Kp and did not receive any antibiotic therapy. Case 6 had a liver abscess caused by IR-Kp and was treated with colistin. Four months after an apparent cure, the patient had a relapse and died deceased from IR-Kp bacteraemia associated with end-stage liver failure.

The two secondary cases with IR-Kp colonisation (Cases 7 and 8) were not treated.

Cases 1, 2, 7 and 8 were discharged, while cases 3 to 6 died either in direct relation to their IR-Kp infection (Case 3 and 6) or from other reasons related to pathology. Interestingly, Cases 2 and 7 became IR-Kp-negative 15 months and two months, respectively, after initial diagnosis, whereas Case 8 was still carrying IR-Kp in July 2008, four years after initial diagnosis.

Control measures

Period 1 (2 December 2003 to 2 June 2004)

Immediately after identification of the index case, measures were implemented according to the French recommendations for controlling multidrug-resistant bacteria in hospital [18]: isolation of positive patients in a single bed room, barrier precautions (e.g. gowns and gloves for staff when entering the patient's room), hand disinfection with alcohol-based hand-rub solution before entering and after leaving the room, and environmental disinfection of the room [18]. Training of staff was organised to insure stringent application of these measures. All patients hospitalised in the intensive care unit were screened for IR-Kp once a week. This screening was maintained when these patients were transferred to the acute care facility.

Despite these measures, new IR-Kp carriers were identified in the intensive care unit during this period (see Table 1) which led to the implementation in June 2004 of extended infection control measures to stop cross-transmission.

Period 2 (2 June to 21 October 2004)

The additional control measures were implemented with the help of the central infection control team of AP–HP. Firstly, we defined five distinct sections in the intensive care unit and acute care facility (Table 2) in order to separate IR-Kp-carrying patients, contact patients and newly admitted patients who were neither IR-Kp-carrying nor contact patients (IR-Kp-free patients). Contact patients were defined as patients who had stayed on the same floor at the same time as an IR-Kp patient (even if this time was less than 24 hours) before the implementation of the reinforced control measures on 2 June 2004.

Secondly, we thoroughly modified the organisation of care in the abdominal surgery care centre. Nursing staff was assigned exclusively to one of the five sections. Admissions of new patients were limited to emergencies and liver transplants. In order to prevent spread of the outbreak to other care centres, transfers were allowed only for IR-Kp-free patients. Transfer of IR-Kp patients within our hospital or to another hospital was allowed only for specific investigations (e.g. computed axial tomography or magnetic resonance imaging) and organised by the local infection control team to ensure that barrier precautions were respected. If patients carrying IR-Kp were readmitted after discharge, they were directly hospitalised in the 'IR-Kp patients section'. If they presented as outpatients for consultation, the local infection control team was informed, control measures were taken and the patients were screened again for IR-Kp. Daily meetings between the medical and nursing staff at the abdominal surgery care centre and the local infection control team helped to strengthen compliance with the control measures. The local infection control team inspected the implementation of the measures at the bedside every day.

Thirdly, it was recommended that clinicians should optimise and restrict the use of antibiotics, in particular of imipenem/cilastatin. An information letter explaining the measures was posted at the information desk of each floor of the abdominal surgery care centre, given to all patients hospitalised in the centre and to their families and also distributed to the other units of the hospital. IR-Kp patients were informed of their status and received instructions when discharged: they were invited to come back preferentially to our hospital in case of medical problem and, if hospitalised in another hospital, to inform the medical staff there about their IR-Kp status.

Finally, the French health authorities organised a screening campaign targeting the contact patients discharged from the abdominal surgery care centre during Period 1 who had not been screened. They were invited to call a free helpline where two senior physicians and a resident physician were available from 26 July to 6 August 2004 and explained how to be screened either in our hospital or, alternatively, in any laboratory of the patients' choice. In the latter case, the procedure to detect IR-Kp was mailed to the chosen laboratory and the result was sent to our hospital.

In June 2004, an alert describing the antibiotic resistance pattern of the IR-Kp was posted on the website of the French institute of public health surveillance (Institut de Veille Sanitaire), and sent to the Early Warning Response System of the European health authorities.

The extended measures were maintained until the end of October 2004 when the last IR-Kp patient was discharged from the abdominal surgery care centre. This patient was transferred to the infectious disease unit located in a separated building of the hospital, where the same control measures were taken (i.e. establishing a special unit with dedicated nursing staff) until the patient died on 15 December. Screening of contact patients for IR-Kp was maintained at each re-admission of in- or outpatients to our hospital until the end of 2005. This measure was stopped because the number of contact patients coming back to the abdominal surgery care centre had decreased and all had had at least three (on average 10) successive negative screenings.

Microbiological diagnostic

Rectal swabs were plated on Drigalski agar containing 0.5 mg/L of cefotaxime, MacConkey agar containing 2 mg/L of ceftazidime. In Period 2 we added Drigalski agar plates containing 4 mg/L of imipenem until the end of 2005. Antibiotic susceptibility testing, including for imipenem, was performed by agar disk diffusion test according to the recommendations from the French Society for Microbiology [19] A disk synergy test between imipenem and EDTA was used to detect MBL production. The presence of the ESBL in MBL-producing strains was detected by a synergy test between ceftazidime or cefepime and clavulanic acid by adding 4 µl of 0.5 M EDTA pH 8 on the disk of clavulanic acid [17]. Polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE) were used to type IR-Kp strains [17].

All IR-Kp strains shared closely related resistance patterns: a high level of resistance to beta-lactams including imipenem (minimum inhibitory concentration (MIC) ≥32 mg/L), aminoglycosides (except gentamicin), fluoroquinolones and co-trimoxazole; a low level of resistance to gentamicin (MIC=8 mg/L) and susceptibility to colistin [17]. All strains carried the bla_{VIM} and $bla_{SHV\text{-}5}$ genes. The isolates from our eight patients had the same restriction pattern, identical to the strain K5 reported in teaching hospitals in Greece [8,17]. The outbreak was consequently ascribed to the spread of a single strain. This strain has not been isolated in our hospital again since the last case of this outbreak was discharged.

Epidemiological analysis

Rates of IR-Kp infection or colonisation were calculated for all patients admitted between December 2003 (month of the first case) and June 2004 (month of the last case). Rates were calculated separately for those cases who had a liver transplantation and those who stayed in the intensive care unit. Fischer's exact test was used to compare categorical variables and $p<0.05$ was considered significant.

Period 1

During Period 1, 325 patients stayed in the intensive care unit (237 of them stayed also in the acute care facility) and 375 further patients were admitted only to the acute care facility (total=700 patients). Five secondary IR-Kp cases (Cases 2 to 6) were detected: all had IR-Kp infections (two bacteraemias, one bronchopulmonary infection, two urinary tract infections) (Table 1). Analysis of the synoptic curve of the patients' charts suggested that three of them (Cases 4, 5 and 6) acquired the infection in the intensive care unit, and the two others (Cases 2 and 3) in the acute care facility on the fourth floor before surgery (Figure 1).

Based on the analysis of the stays of the index case and the above secondary cases, 324 of the 700 patients admitted during this period were retrospectively defined as contact patients and had been exposed to IR-Kp. Of those, 171 were screened during their stay in abdominal surgery care centre. The remaining 153 contact patients, discharged during Period 1, were not screened in the abdominal surgery care centre, predominantly due to the short duration of their stay, and were the target of the screening campaign organised in Period 2 (see below).

Period 2

During the screening campaign (26 July to 6 August 2004) 75 of the 153 contact patients who had not been screened in Period 1 called the helpline for information and 67 subsequently accepted to be screened (54 in our hospital and 13 in another facility). A further 37 patients did not call the helpline during this campaign but were screened after the helpline had been closed when visiting our hospital as outpatients. Overall, 104 of the 153 patients who were not screened during Period 1 were screened in Period 2. None of them were found to carry IR-Kp.

In addition, two secondary IR-Kp cases (Cases 7 and 8) were identified in June and August 2004 after the extended infection control measures. Case 7 was a contact patient who had stayed in the intensive care unit from 8 March to 3 June, at the same time as Cases 2, 3 and 4. After discharge, this patient visited the abdominal surgery care centre as an outpatient on 17 June. According to the measures in force at this time, this patient was screened and identified as a IR-Kp carrier. Case 8, who had stayed in the acute care facility for four days in January 2004, at the same time as the index case, was re-admitted on 28 July on the fourth floor in the 'contact acute care patients section' where all patients were IR-Kp-negative (Figure 1). This patient was screened and transferred to the 'IR-Kp patients section' after IR-Kp was detected in the rectal swab. Based on the synoptic curve (Figure 1), Cases 7 and 8

are most likely to have acquired IR-Kp during Period 1. They did not develop IR-Kp infection.

Attack rate

A total of 277 of the 324 contact patients (85%) were screened (Figure 2). The global attack rate of secondary IR-Kp cases (n=7) among the screened contact patients (n=277) was 2.5%.

The attack rate of secondary cases was significantly higher among patients with liver transplant (six of 45 patients; 13%) as compared to screened patients without liver transplant (one of 230 patients; 0.4%) (p=0.0001). Although all secondary cases stayed at least some days in the intensive care unit, the likely place of IR-Kp acquisition for three of them was the acute care facility. Which hospital unit the patient stayed in was not a risk factor for acquisition of IR-Kp, as the attack rate was 1.2% in patients staying in the intensive care unit (four of 325 patients) and 0.6% in other patients (three of 375 patients) (p=0.4)

Cost of the screening campaign

The estimated cost of the screening campaign organised in period two (personnel costs for two seniors physicians and one resident physician maintained on duty, telephone bills, mailing costs, sampling and cultures) was EUR 18,830, i.e. a mean of EUR 190 per screened patient. The annual cost of the routine weekly screening for ESBL- or carbapenemase-producing *Enterobacteriaceae* that has been maintained up to now for the entire abdominal surgery care centre (81 beds) is approximately EUR 60,000.

Discussion

The emergence and spread of carbapenem-resistant *Enterobacteriaceae* due to MBL is an increasing international health problem. In Europe, the proportion of *K. pneumoniae* strains resistant to imipenem (IR-Kp) that are isolated from blood cultures is far below 5% in most countries but had in the year 2007 already reached a rate of 22% in Israel and 42% in Greece. In France, the incidence is still very low (<0.1%) and the very few identified cases are in relation with patients transferred from foreign countries [20].

We describe here the epidemiological characteristics and control of the first outbreak of MBL-producing *K. pneumoniae* that occurred in France, after the admission of a patient transferred from a hospital in Greece, where these bacteria are common [8]. To our knowledge, it is the first report on the control of an IR-Kp outbreak in a country with a low rate of these bacteria.

The national measures for controlling cross-transmission of multidrug-resistant bacteria in hospital [18], designed mainly for meticillin-resistant *Staphylococcus aureus*, were implemented during the first six months of the outbreak (Period 1) but were insufficient to achieve control, as five secondary cases occurred during that period. After implementation of the extended infection control measures (Period 2), the only two secondary cases identified were in fact, the consequence of acquisition during Period 1. The extended measures were mainly based on (i) separating IR-Kp patients, contact patients and new patients in distinct sections of the hospital, each with dedicated staff, in order to minimise cross-transmission, (ii) stopping transfer of IR-Kp patients to other units or hospitals to block the extension of the outbreak and (iii) careful identification, screening and follow up of the contact patients. The outbreak was most likely due to cross-transmission by the staff since all the patients in abdominal surgery care centre facility are placed in single bedrooms, particularly in intensive care unit. Successive attempts failed to detect the epidemic strain in environmental samples (data not shown).

The present study was not a randomised controlled trial assessing causality between intervention and outcome. Indeed, the rapid spread of IR-Kp in our hospital triggered rapid and strong action to control the outbreak, making a randomised intervention impossible. However, the fact that the strength and nature of the implemented measures differed markedly during Periods 1 and 2 as well as the length of the follow-up (six years) suggest a causal association between reinforced measures and the control of the outbreak. Causes other than the intervention (e.g. regression to the mean or maturation effect) could have been involved in the decrease in IR-Kp cases [21]. However, spontaneous cessation of IR-Kp outbreaks was not observed in Greece, Israel or the New York City area, where IR-Kp are endemic [8,22-24]. In one setting the incidence of endemic IR-Kp was partially reduced by implementing strict infection control measures comparable to our control programme, whereas contact isolation alone was unsuccessful [25]. Similar measures have also been implemented successfully to control the emergence of vancomycin-resistant enterococci (VRE) [26-28] and are now recommended for VRE outbreaks in France [29] and in the Netherlands [25]. These measures, proposed by the central infection control team of AP-HP, are more stringent than those proposed by the US Centers for Disease Control and Prevention [30].

We faced some difficulties in the implementation of reinforced infection control measures. Defining distinct sections in the hospital to separate IR-Kp carriers, contact patients and IR-Kp-free patients required a strong involvement of hospital managers and the support of the central infection control team of the AP–HP institution. Dedicating teams of nurses to each of these sections was a difficult challenge. Assigning the re-admitted contact patients to the adequate sections required careful examination of medical records. Reinforcing screening procedures increased the workload markedly and required a reorganisation of the laboratory. Maintaining the activity of liver transplantation was made possible by referring part of the patients who did not require transplantation to other care centres. Careful and clear information of patients and staff while avoiding stress and panic required psychological tact.

Although extended measures such as those applied in our setting are difficult to implement and maintain for a long period of time, we believe that they are adequate to control outbreaks of emerging multiresistant organisms, particularly in countries where the incidence is very low [25,31,32]. Recent recommendations advocate implementing similar measures for the sporadic occurrence of IR-Kp [29]. The speed and strength of the intervention is likely to be crucial in limiting the size and duration of IR-Kp outbreaks.

The present study also suggests that pre-emptive isolation of patients at risk of carrying multidrug-resistant strains (i.e. resistant to VIM, KPC, NDM-1), particularly patients transferred from countries where these organisms are endemic, could help to prevent outbreaks. This measure should be combined with a preparedness plan to facilitate urgent and rapid action whenever a first case is detected [31].

Acknowledgements

The authors are grateful to Professor Vatopoulos (University of Athens) for providing IR-Kp strains, all staff at the Centre Hépato-Biliaire, the Infectious Disease Department, and the bacteriology laboratory, and Bruno Coignard at InVS. We also thank the hospital administration for their support.

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Appropriateness of antimicrobial therapy: a multicentre prevalence survey in the Netherlands, 2008–2009

I Willemsen[1], T van der Kooij[2], B van Benthem[2], J Wille[3], J Kluytmans (jankluytmans@gmail.com)[1,4]
1. Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, the Netherlands
2. Netherlands Centre for Infectious Disease Control, National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), Bilthoven, the Netherlands
3. Dutch Institute for Healthcare Improvement (CBO), Utrecht, the Netherlands
4. Department of Microbiology and Infection Control, VU University Medical Center (VU medisch centrum, VUmc), Amsterdam, the Netherlands

Citation style for this article:
Willemsen I, van der Kooij T, van Benthem B, Wille J, Kluytmans J. Appropriateness of antimicrobial therapy: a multicentre prevalence survey in the Netherlands, 2008–2009. Euro Surveill. 2010;15(46):pii=19715. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19715

Article published on 18 November 2010

A survey was carried out to determine the prevalence and appropriateness of antimicrobial therapy (AMT) in the Netherlands and to identify determinants for inappropriate AMT. Prevalence surveys of patients hospitalised in the Netherlands were performed three times in 2008 and 2009. Patients' demographic, infection-related and AMT-related data were collected from hospital wards. A total of 19 hospitals participated, consisting of a mix of university, teaching and general hospitals, which were distributed evenly across the country. The appropriateness of AMT was assessed using a standardised algorithm based on local AMT prescription guidelines. A total of 7,853 patients were included, of which 2,327 (29.6%) patients were on AMT (range: 20.8–39.5%). In 372 patients (16% of patients on AMT), treatment was considered inappropriate. In 265 (11.4%) patients on AMT, appropriateness of treatment was not judged because of insufficient information. The percentage of patients without a judgment varied considerably between the participating hospitals (range: 1.3–36.2%). Appropriate AMT use was significantly associated with a patient being in an intensive care unit, having a central venous catheter and being given beta-lactamase-sensitive penicillins. The use of fluoroquinolones was significantly associated with more frequent inappropriate use. There was considerable and significant variation between the participating hospitals in the amount of antimicrobials prescribed and the appropriateness of their use. To improve the completeness and reliability of such surveys, there is a need for intensive training of observers and medical staff in recording information.

Introduction

Point prevalence surveys are useful ways of investigating healthcare-related events, including antimicrobial use. The first report on antimicrobial use measured in prevalence surveys was published in 1983 [1]. More recently a European project – the European Surveillance of Antimicrobial Consumption (ESAC) – has standardised a method to determine the prevalence of antimicrobial therapy (AMT) in hospitals [2]. In a previous study, performed in a teaching hospital in the Netherlands, we showed that besides the prevalence of AMT, the appropriateness of AMT for individual patients could also be determined, basing the judgement on local antibiotic prescription guidelines [3]. This enables researchers to quantify the number of patients who are treated even when treatment is not indicated or who are treated with a drug that is not the preferred choice. In addition, it was possible to identify determinants of inappropriate use of AMT [3]. The objective of the current study was to determine whether prevalence studies could be used in other hospitals as well and whether they could also be used as a tool for benchmarking.

The study was coordinated by the PREZIES (Prevention of Nosocomial Infections by Surveillance) network – a collaboration between participating hospitals, the Dutch Institute for Healthcare Improvement (CBO) and the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM).

Methods

Prevalence surveys

Hospitals were recruited from PREZIES. Those that had already participated in prevalence surveys for nosocomial infections were invited to add AMT use to this survey. Participation was on a voluntary basis.

Three prevalence surveys of hospitalised patients were performed in spring 2008, autumn 2008 and spring 2009. All patients that were present in the hospital at 00:01 on the day of the survey were included. Patients in day care (including haemodialysis patients) and psychiatric wards were excluded. Data from the hospital wards were collected by infection control practitioners. A standardised case record form, to document patients' demographic, infection-related and AMT-related data, was used. The infection control practitioners received

training during workshops on how to collect the data and how to judge the appropriateness of AMT. The following demographic variables were recorded: age, sex, medical speciality, type of ward and presence of infection on admission. Nosocomial infections were recorded using the definitions from the United States Centers for Disease Control and Prevention (CDC) and patients had to be symptomatic or still being treated on the day of the survey [4,5]. Furthermore, the use of antimicrobial agents and dosage were noted. If more than one antimicrobial was prescribed for one patient, all antibiomicrobials, up to a maximum of three, were registered. Antifungal and antiviral therapy as well as medication for tuberculosis were excluded from the study. The main reasons were that not all local guidelines had specific recommendations for these agents and susceptibility of pathogens to these agents was not always determined by the local microbiology laboratories.

Appropriateness of antimicrobial therapy

The appropriateness of AMT was determined using a standardised method developed by Gyssens *et al.* [6], using the following classifications: correct decision, incorrect decision, incorrect choice or insufficient data. This classification system obviously only takes into account patients that are on AMT. However, using our approach it is possible to examine the appropriateness of not prescribing AMT also, as described in our previous study [3]. A correct decision was deemed appropriate; incorrect decision and incorrect choice were considered inappropriate (the evaluation criteria are summarised in Table 1).

The appropriateness of AMT was judged according to the local AMT prescription guidelines present in all participating hospitals. These local guidelines are based on the national policy developed by the Dutch Working Party on Antibiotic Policy (Stichting Werkgroep Antibiotica Beleid, SWAB) [7,8]. The infection control practitioners assessed the appropriateness of AMT initially: if they could not decide, a consultant microbiologist or infectious disease physician made the final judgment. The consultant microbiologist or infectious disease physician also judged all patients in intensive care units, all patients who received AMT without having an active infection (according to the survey), all patients who did not receive AMT and did have an active infection and all patients who received AMT that was not according to the local AMT guidelines.

If all the antimicrobial agents that a patient received were considered correct, the treatment was considered appropriate. If one or more of the antimicrobial agents was considered incorrect, the treatment was considered inappropriate. If it was not possible to decide whether use of a particular antimicrobial agent was correct due to incomplete information, treatment was recorded as insufficient information. We did not assess the reproducibility of the judgments.

Table 1

Evaluation criteria for appropriateness of antimicrobial therapy, the Netherlands, 2008–2009

Categories and criteria
1. Correct decision (appropriate use)
1.1 No AMT and no infection and no AMT needed
1.2 No AMT and infection and no AMT needed
1.3 AMT and infection and appropriate choice and appropriate use
2. Incorrect decision (inappropriate use)
2.1 No AMT and infection and AMT needed
2.2 AMT and no infection and no prophylaxis and no AMT needed
2.3 AMT and no infection and prophylaxis and no AMT needed
3. Incorrect choice (inappropriate use)
3.1 Divergence from guidelines
4. Missing data (insufficient information)
4.1 No AMT and not enough diagnostic information about infection
4.2 Infection and not enough diagnostic information if AMT is needed
4.3 AMT and not enough diagnostic information about infection
4.4 Infection and not enough information about AMT

AMT: antimicrobial therapy.

Data analysis, quality control and statistical analysis

Privacy of patients is ensured by decoding all data, as required by the privacy regulations in the Netherlands. Data were entered in the PREZIES database or a hospital-owned database and subsequently coded and transferred to PREZIES.

Data were analysed using SPSS version 17.0. Treatment for which there was insufficient information was recorded as a missing value. Categorical variables were analysed by Fisher's exact test or chi-square test where appropriate: continuous variables were analysed using a t-test or Mann–Whitney U test where appropriate. Binary logistic regression analysis was performed: all variables with a p value less than 0.1 in univariate analyses were entered into the multivariate model. Statistical significance was accepted when the chance for coincidence was less than 5%. Finally, a sensitivity analysis was performed. In this, the univariate and multivariate analyses were repeated: once categorising AMT use as appropriate for all patients for whom AMT use could not be judged and once categorising it as inappropriate.

Results

A total of 7,853 patients were included, from 19 hospitals. They were a mix of university, teaching and general hospitals, which were distributed evenly across the country. Of these, 13 participated in one of the three surveys, five participated in two surveys and one participated in all three. A mean of 302 patients were included per hospital per prevalence survey (range: 103–552; standard deviation: 149).

Patient characteristics and nosocomial infections

Overall 3,784 (48.2%) patients were male, and the mean age was 62 years (median: 67 years). On the day of the survey 426 patients (5.4%) had at least one active nosocomial infection.

Antimicrobial therapy

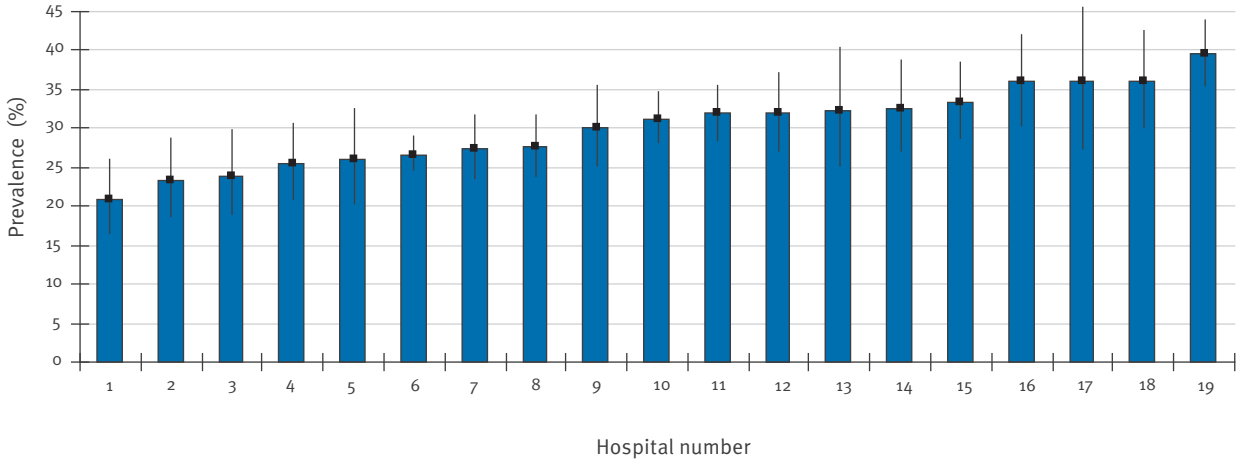
A total of 2,327 patients (29.6%) were on AMT (range: 20.8–39.5%). The mean prevalence of AMT per participating hospital is shown in Figure 1.

Of the 2,327 patients on AMT, 433 (18.6%) were treated with two antimicrobials, and 58 (2.5%) were treated with three or more. In total 2,876 courses of antimicrobial agents were administered, of which 1,709 (59.4%) were given intravenously (range: 42.2–75.9%).

The first antimicrobial agent was considered appropriate in 1,690 (72.6%) patients. In 149 (6.4%) patients the first antimicrobial agent was considered not justified

Figure 1

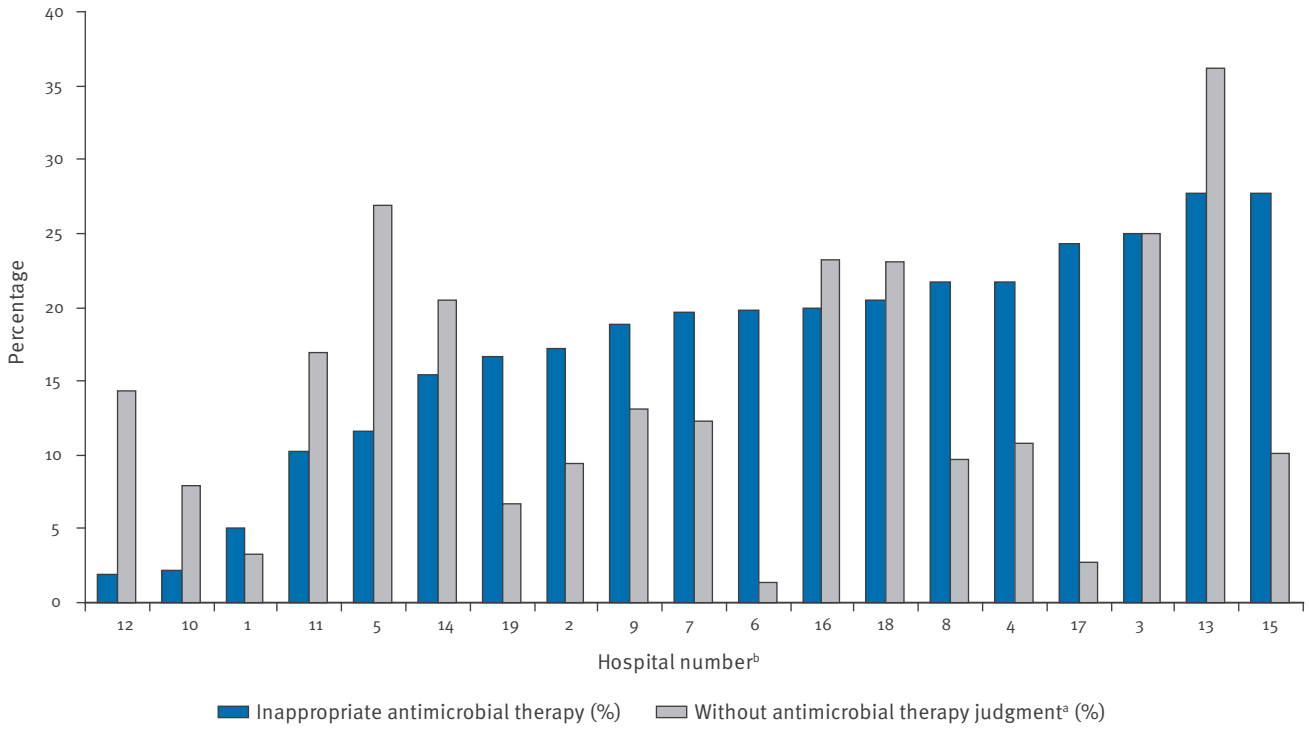
Mean prevalence of antimicrobial therapy per participating hospital, the Netherlands, 2008–2009



The hospitals are shown in increasing order of prevalence. Vertical bars represent the 95% confidence intervals.

Figure 2

Inappropriate antimicrobial therapy and proportion of patients without antimicrobial therapy judgment[a], by participating hospital, the Netherlands, 2008–2009



The hospitals are shown in order of increasing proportion of inappropriate use.

[a] Due to missing information.

[b] The numbering of hospitals is identical to the hospital numbers in Figure 1.

and therefore inappropriate. In 223 (9.6%) patients AMT was justified, but the choice of the agent was not according to the guidelines. In 265 (11.4%) patients no decision was made due to insufficient information. The second antimicrobial agent was considered appropriate in 384 patients (78.2% of the 491 patients treated with more than one antimicrobial agent), not justified in 26 (5.3%) and justified but an incorrect choice in 39 (7.9%) patients. In 42 (8.6%) patients no choice was made due to insufficient information. The third antimicrobial was considered appropriate in 50 of the 58 patients treated with at least three antimicrobial

Table 2

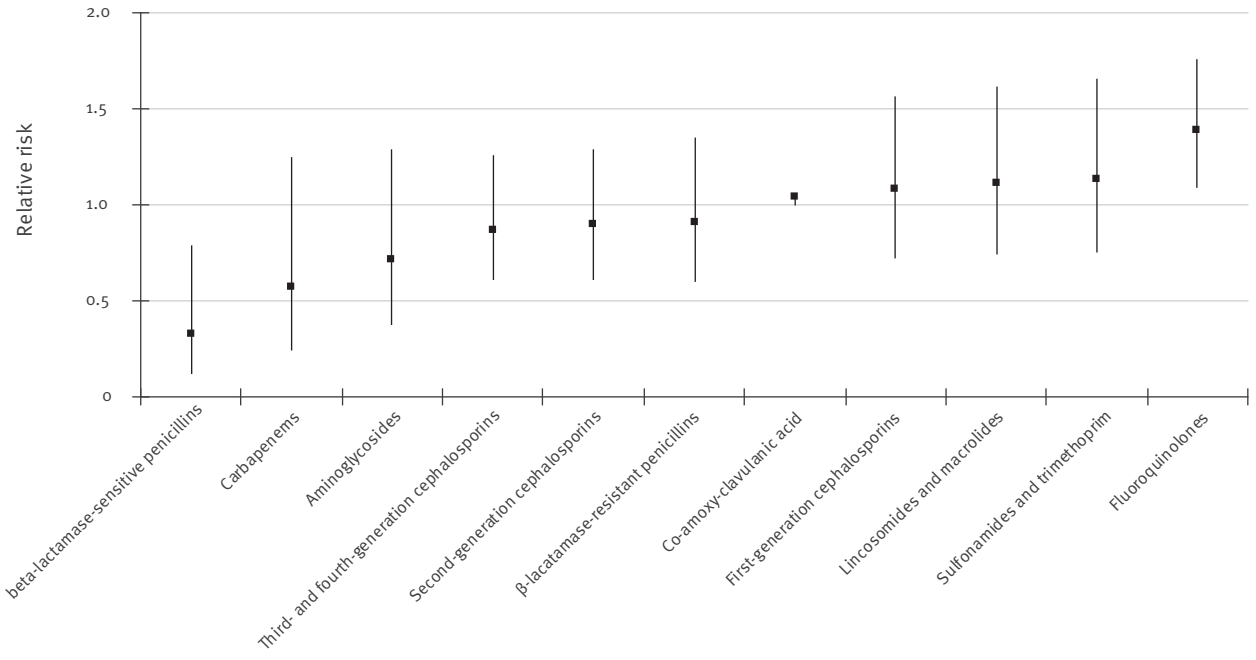
Use of antimicrobial agents in participating hospitals, the Netherlands, 2008–2009

Antimicrobial agent	First antibiotic		Second antibiotic		Third antibiotic		Total	
	n	%	n	%	n	%	n	%
beta-lactamase-sensitive penicillins	58	2.5	12	2.4	1	1.7	**71**	**2.5**
beta-lacatamase-resistant penicillins	150	6.4	17	3.5	1	1.7	**168**	**5.8**
Carbapenems	44	1.9	5	1.0	2	3.4	**51**	**1.8**
Co-amoxicillin-clavulanic acid	715	30.7	42	8.6			**757**	**26.3**
First-generation cephalosporins	124	5.3	2	0.4	1	1.7	**127**	**4.4**
Second-generation cephalosporins	147	6.3	35	7.1	2	3.4	**184**	**6.4**
Third- and fourth-generation cephalosporins	177	7.6	30	6.1	4	6.9	**211**	**7.3**
Co-piperacillin-tazobactam	63	2.7	7	1.4	3	5.2	**73**	**2.5**
Sulfonamides and trimethoprim	107	4.6	15	3.1	3	5.2	**125**	**4.3**
Fluoroquinolones	303	13.0	93	18.8	8	13.8	**404**	**14.0**
Glycopeptides	37	1,6	16	3,3	2	3,4	**55**	**1,9**
Imidazole derivates	55	2,4	68	13,9	4	6,9	**127**	**4,4**
Lincosamides and macrolides	84	3,6	39	8,0	10	17,2	**133**	**4,6**
Broad-spectrum penicillin	128	5,5	34	6,9	2	3,4	**164**	**5,7**
Aminoglycosides	30	1,3	40	8,2	8	13,8	**78**	**2,7**
Tetracyclines	42	1,8	6	1,2	1	1,7	**49**	**1,7**
Other antimicrobials	63	2,7	30	6,1	6	10,3	**99**	**3,4**
Total	**2,327**	**81**	**491**	**17**	**58**	**2**	**2,876**[a]	**100**

[a] Total number of courses of antimicrobial agents administered that were recorded on the survey days.

Figure 3

Relative risk for inappropriate use of antimicrobial therapy by group of antimicrobial agent[a], the Netherlands, 2008–2009



Vertical bars represent the 95% confidence intervals.

[a] Co-amoxycillin-clavulanic acid as reference.

agents, not justified in two and justified but an incorrect choice in two patients. Four were not judged due to insufficient information.

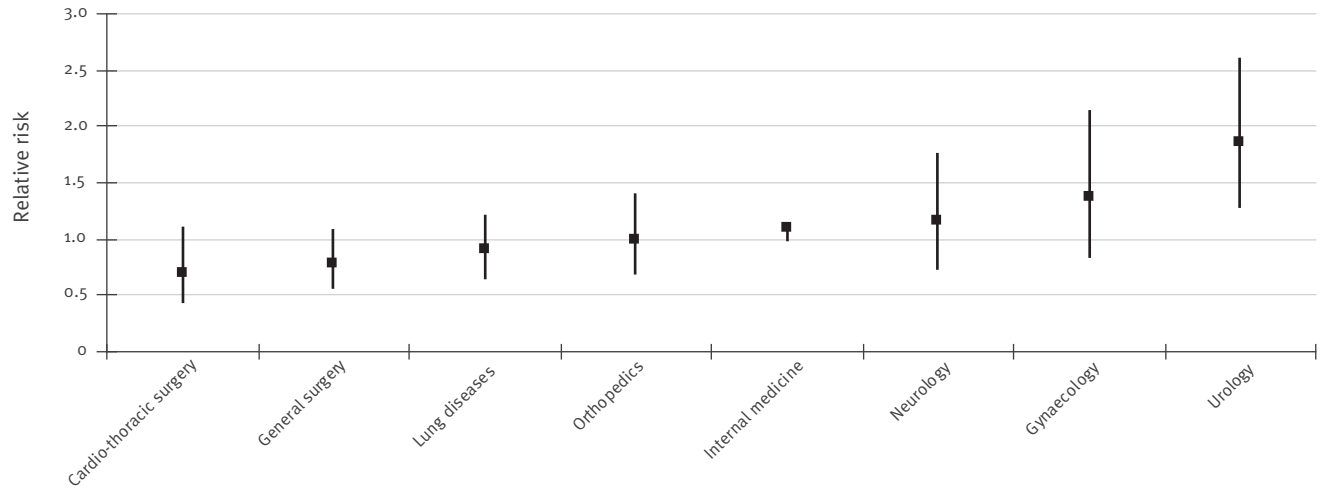
Judgment of the appropriateness of AMT per patient showed that 372 patients (16% of the patients on AMT; 4.7% of the total population) were treated inappropriately. Figure 2 shows the variations in the proportion of AMT considered inappropriate in the different hospitals (range: 5.0–32.4%).

For 265 patients (11.4%) on AMT it was not possible to judge appropriateness because of insufficient information. Figure 2 shows the variations in the proportion of patients on AMT who could not be judged in the participating hospitals (range: 1.3–36.2%).

Of the patients who did not receive AMT (n=5,526), 945 were not judged for the appropriateness of the decision not to treat. This was mainly due to four hospitals that did not judge patients who were not receiving AMT. Of the 4,581 patients not receiving AMT who were judged, the decision not to use AMT was considered appropriate for 4,497 (98.2%) patients. For 22 patients (0.5% of those not on AMT who were judged), patients did not receive AMT, although this was indicated. For 62 (1.4%) patients not on AMT it was not possible to assess the appropriateness because of insufficient information.

Figure 4

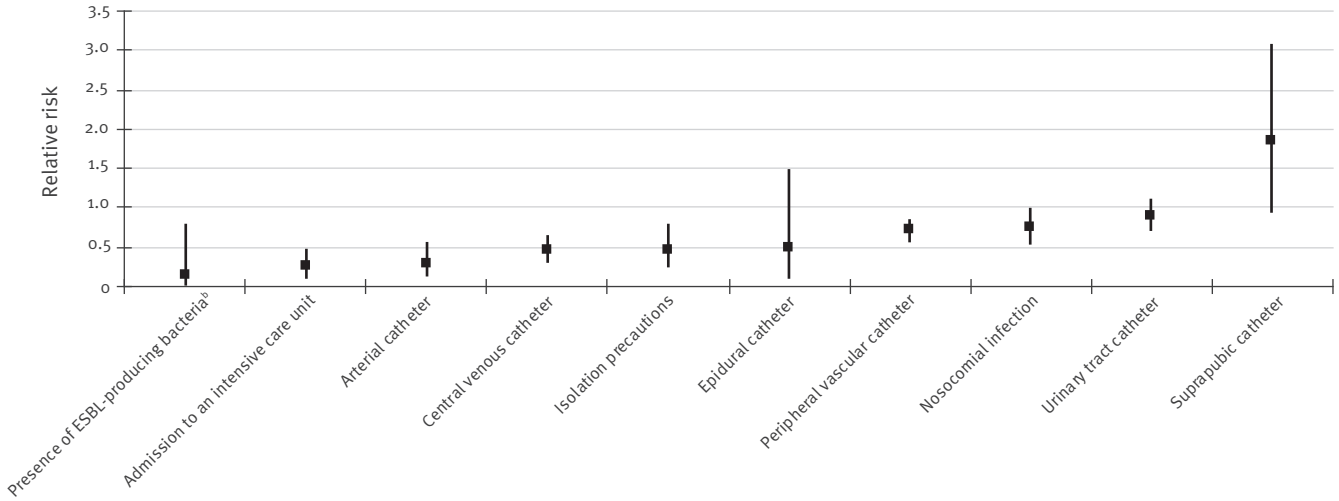
Relative risk for inappropriate use of antimicrobial therapy by medical specialty[a], the Netherlands, 2008–2009



Vertical bars represent the 95% confidence intervals.
[a] Internal medicine as reference.

Figure 5

Relative risk for inappropriate use of antimicrobial therapy[a], the Netherlands, 2008–2009



Vertical bars represent the 95% confidence intervals.
[a] All determinants are dichotomous variables, which are compared to their counterpart.
[b] Extended-spectrum beta-lactamase-producing Gram-negative rods.

Table 2 shows the distribution of the use of various antimicrobials. Co-amoxicillin-clavulanic acid was most commonly used, second were the fluoroquinolones and the third were the third- and fourth-generation cephalosporins.

Determinants of inappropriate use of antimicrobial therapy

In the univariate analysis, use of fluoroquinolones was significantly associated with more frequent inappropriate use of AMT (relative risk: 1.4). The use of beta-lactamase-sensitive penicillins was significantly associated with more frequent appropriate use of AMT (relative risk: 0.3) (Figure 3).

Considering the use of AMT in the different medical specialties, urology (p=0.002) proved to be significantly associated with more frequent inappropriate use (Figure 4). None of the specialties was significantly associated with more frequent appropriate use.

Figure 5 shows that the presence of a suprapubic catheter was significantly associated with more frequent inappropriate use (relative risk: 1.9). The following factors were associated with more frequent appropriate use of AMT: having a central venous catheter, a peripheral vascular catheter or an arterial catheter, the presence of ESBL (extended-spectrum beta-lactamase-producing bacteria, being admitted to an intensive care unit, being in isolation precautions and having a nosocomial infection.

In multivariate analyses, taking the effects of all the above-mentioned variables into account, we found that the hospitals themselves were important determinants associated with appropriate or inappropriate use of AMT (Table 3). Furthermore, increasing age (p=0.024), being in an intensive care unit (p=0.002), having a central venous catheter (p=0.12), peripheral vascular catheter (p=0.005) and nosocomial infection (p=0.049) and use of beta-lactamase-sensitive penicillins (p=0.017) were significantly associated with appropriate use in multivariate analyses. The presence of a suprapubical catheter (p=0.017) or the use of fluoroquinolones (p<0.001) were significantly associated with inappropriate use of AMT. No colinearity was found between the variables in the multivariate model.

Discussion and conclusions

The mean prevalence of AMT in this study was 29.6% (range: 20.8–29.5%). The most recent study that can be used for comparison is from the European Surveillance of Antimicrobial Consumption (ESAC) which found a similar prevalence of 30% (range: 19 –59%) in 20 European hospitals in 2006 [2]. Other prevalence studies in hospitals in the United Kingdom and Turkey also showed similar rates of antimicrobial use [9-11]. Although the overall prevalence in our study is comparable to that of other large surveys, there were large variations between the participating hospitals. This range of appropriate AMT use can be explained by differences in the patient populations and by differences in prescription policies between hospitals and between individual prescribers.

Of all patients on AMT in this study, the use was considered inappropriate in 372 (16%; range: 1.9–27.7%). The patients concerned comprised 4.7% of the total number of patients, which may seem relatively unimportant. However, this means that annually approximately 10,000 days of unjustified AMT are given in a hospital with 200,000 patient days a year. Treating patients with AMT when such treatment is not indicated is known to be associated with higher costs, more side effects and more antimicrobial resistance [12,13].

Our study showed that the proportion of patients for whom AMT was judged to be inappropriate varied between hospitals. AMT use could not be judged for 265 patients due to insufficient information. Deciding on the appropriateness of AMT use is often not easy. However, the difference between the hospitals is remarkable. The hospitals with the lowest proportion of cases that could not be judged were hospitals with previous experience with this kind of survey. Possibly these kinds of judgments require more extensive training. During a session that was organised with the infection control practitioners and consultant microbiologists to discuss the findings, it was thought that more training and discussion of difficult cases in the study group would probably result in a reduction of the number of cases that could not be judged.

Our study showed that the participating hospital is a determinant itself and had a great influence in the analyses of determinants associated with inappropriate or appropriate AMT use. We were unable to identify specific characteristics of the hospitals that were responsible for more frequent inappropriate use. Nevertheless, use of fluoroquinolones proved to be a significant risk factor for inappropriate use of AMT. Fluorouinolones were the second most frequently used antimicrobials. The ESAC reported that use of fluoroquinolones increased most rapidly of all groups of antimicrobial agents, with a rise of 15% or more from 2000 to 2005 in almost half of all participating countries [14]. At the same time antimicrobial resistance against the fluoroquinolones increased from 5% (in 2001) to 14% (in 2008) in *Escherichia coli* and from 4% (in 2005) to 8% (in 2008) in *Klebsiella pneumoniae* [15]. This highlights the importance of undertaking targeted interventions to reduce inappropriate use of fluoroquinolones. Data from prevalence surveys such as those described here provide support for such action.

Other determinants associated with a more appropriate use of AMT were variables associated with the clinical complexity of the patients (e.g. being admitted to an intensive care unit and having a central venous catheter). In the Netherlands, a microbiologist or infectious disease physician is almost always consulted in the assessment of these complicated cases. However,

the largest group of patients being treated with antibiotics comprises relatively uncomplicated cases: most of these are not monitored by the microbiologist or infectious disease physician. A prevalence survey does include this group of patients and delivers information on the appropriateness of use. In our experience, it is this group in which a substantial improvement of the quality of antibiotic prescription can be achieved.

The fact that the treatment or lack of treatment of some patients could not be judged may have affected the outcome of our study. However, a sensitivity analysis showed that this did not affect the conclusions about the appropriateness of AMT use in the participating hospitals. We did not collect information on what factors in the hospitals with higher inappropriate use may have contributed to this. Further studies are warranted, since they may offer clues for further improvement.

In this study we identified those patients who inadvertently did not receive AMT (22 patients, 0.3% of total study population). There was no further analysis of the 22 patients. In an earlier single-centre study, we found a similar fraction of such patients (25 patients, 0.6% of total study population); further investigation showed that those patients were not adversely affected at discharge [3].

The extent of intravenous administration of antimicrobials (59.4%) suggests that there is room for improvement. Intervention studies performed in the Netherlands showed that intravenous administration can be reduced relatively easily by targeted interventions [16,17]. A switch to oral therapy often results in a shorter hospital admission. In our study, the appropriateness of the route of administration was not assessed

In conclusion, we have demonstrated that it is possible to collect prevalence data on use of AMT at a national level. Individual hospital data can be very helpful in initiating targeted interventions to improve AMT use [17]. However, in order to produce more reliable results of such surveys, the number of patients for whom the appropriateness of AMT use could not be judged has to be reduced. Therefore training of infection control practitioners and consultant microbiologists has to be intensified and medical staff need to be trained in how to record information, in order to get an unambiguous assessment of use of AMT.

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Notes

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