ECDC TECHNICAL REPORT

Risk assessment on the spread of carbapenemase-producing Enterobacteriaceae (CPE)

through patient transfer between healthcare facilities, with special emphasis on cross-border transfer

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Summary and conclusions of ECDC risk assessment and guidance for prevention and control

In May 2010, due to concern about the increasing number of outbreaks and the spread of carbapenemase-producing Enterobacteriaceae (CPE) in healthcare facilities across Europe, the EU Member States submitted a proposal to the European Centre for Disease Prevention and Control's Advisory Forum for a risk assessment. The purpose of the *Risk assessment on the spread of carbapenemase-producing Enterobacteriaceae through patient transfer between healthcare facilities, with special emphasis on cross-border transfer* was to evaluate the risk to the citizens of Europe of CPE spread through patient mobility and to assess the effectiveness of infection control methods to stop the spread of CPE within healthcare institutions.

Carbapenemases, a group of clinically important β-lactamases that efficiently hydrolyse most β-lactams, including the carbapenems, have emerged and spread among the Enterobacteriaceae family of bacteria worldwide. Although the exact prevalence of CPE in healthcare facilities and within the community in Europe is unknown, publications from Member States and surveillance systems indicate that CPE is endemic in certain countries. Similar risk factors to those associated with other multidrug-resistant organisms (MDROs) have been identified for CPE and include severity of illness, a history of hospitalisation or a stay in an intensive care unit, prior antimicrobial use and immunosuppression. Patient mobility has also recently been highlighted as a risk factor for the acquisition of CPE in many reports by Member States discussing the introduction and spread of carbapenemases into healthcare settings as a result of patient transfer, mostly from endemic areas, across borders.

Transfer of patients across borders has been shown to be a documented risk factor for the introduction of carbapenemase-producing Enterobacteriaceae into healthcare settings and systems. CPE are easily introduced because they are highly transmissible, resulting in colonisation or infection of patients. Dissemination of mobile genetic elements coding for resistance and of epidemic, multidrug-resistant strains has been the cause of many reported outbreaks. Infections with CPE are a threat to patient safety due to their resistance to multiple antimicrobials, meaning that there are very few therapeutic options with which to treat infected patients. Furthermore, human infections with CPE are associated with poorer patient outcomes, increased morbidity, mortality and higher hospital costs. The risk for humans becomes greater since therapeutic options are limited because there are very few novel antimicrobial agents in the development pipeline.

This risk assessment is based on two systematic reviews. The first review looked at the risk factors for patient colonisation or infection with CPE and the second examined the effectiveness of using screening and/or targeted infection control measures to decrease the incidence of colonisation or infection in acute healthcare settings. In addition, a group of ten experts in infectious diseases, infection control, public health and microbiology attended a meeting in Stockholm, Sweden on 24 November 2010 to give feedback on the systematic reviews, and to provide their expert opinion and recommendations, which are all included in the risk assessment. The following document presents the conclusions from this risk assessment.

Cross-border transfer of patients

There is strong evidence from the descriptive studies included in the systematic literature review that when patients infected or colonised with carbapenemase-producing Enterobacteriaceae are transferred across borders this increases the risk of CPE being introduced and spread into healthcare facilities in the country of destination. Cross-border transfer of patients poses a clear risk for the transmission of CPE, especially when patients are transferred from areas with high rates of CPE to healthcare facilities in another country or have received medical care abroad in areas with high rates of CPE.

The group of experts concurred with these conclusions and stated that, despite the potential publication bias and/or ascertainment bias of the descriptive studies included, the evidence was still compelling and the risk was still inherent. Publication and ascertainment bias, however, can obscure the exact prevalence of CPE in Europe.

Good data is therefore required on the epidemiology of CPE in Europe. Countries are encouraged to actively report cases of CPE by making all clinical cases notifiable to public health authorities. There is a need to have a European network counting cases/outbreaks of infection with CPE, to implement early warning electronic platforms like the Epidemic Intelligence System (EPIS) or the Early Warning and Response System (EWRS), and to perform regular surveys to collect data on the prevalence of CPE in Europe.

Prudent use of antimicrobials

The experts agreed with the results from the systematic review that, prior use of all antimicrobial agents (more specifically the carbapenems, 3rd and 4th generation cephalosporins and fluoroquinolones) increased the risk of infection or colonisation with CPE. Additionally, they stressed the importance of recognising that antimicrobial pressure is associated with the emergence and spread of resistance determinants in general. High rates of MDROs, e.g. extended-spectrum β-lactamase (ESBL)-producing bacteria, represent an indirect risk for the spread of carbapenem resistance mechanisms because they are associated with an increased prescription of carbapenem antibiotics to treat patients infected with these MDROs. It is therefore imperative not only to control the misuse of antimicrobial agents, but also the high rates of MDROs (e.g. ESBL-producing Enterobacteriaceae), since their presence necessitates the use of antimicrobials, especially the carbapenems. The result is an interminable cycle of antimicrobial use, antimicrobial pressure and high rates of antimicrobial resistance. Decisive action is therefore needed to promote prudent use of antimicrobial agents.

Infection control measures

The results of the systematic review demonstrate that there is limited evidence available on the effectiveness of infection control measures to prevent and control nosocomial transmission of CPE in acute healthcare facilities, and no evidence for other healthcare settings. The effectiveness of containment strategies, to combat secondary transmission following cross-border CPE transmission due to patient transfer, is also unclear because reporting on infection control management in the studies is incomplete.

The group of experts emphasised that for infection control purposes CPEs will behave similarly to other MDROs. Therefore, infection control measures able to effectively halt the spread of other MDROs, e.g. ESBL-producing Enterobacteriaceae, in acute healthcare settings should also be used and recommended for CPEs.

Evidence from outbreaks of other MDROs, such as ESBL-producing Enterobacteriaceae in acute care hospitals, consistently supports the effectiveness of a) early implementation of active surveillance by rectal screening for CPE carriage, b) additional precautions for the care of CPE-positive patients, including the wearing of disposable gloves and gown and c) cohort nursing by a separate, dedicated team.

Existing guidance documents from the USA and Europe and the group of experts recommend the implementation of comprehensive, multifaceted infection control programmes, with well-defined structures and processes, and continuous evaluation of the implemented measures to prevent the spread of CPE in acute care facilities.

Use of Standard Precautions, and especially adherence to hand hygiene policies, is the cornerstone for preventing transmission of MDROs, including CPE, in healthcare settings. Colonisation and/or infection of patients with MDROs may be unknown and it is therefore of paramount importance that healthcare workers adhere strictly to basic hand hygiene policies during patient care to prevent cross-infection.

Additional recommended infection control measures include: active screening cultures on admission or transfer of all high-risk patients; routine use of clinical laboratory screening tests for accurate detection of CPE; pre-emptive isolation of high-risk patients pending the results of the active surveillance and, if positive, continuous active surveillance; contact precautions and isolation or cohorting care for all CPE-colonised patients; dedicated staff and cohort nursing for all isolated patients who are carriers of CPE; prudent use of antimicrobial agents and a system for monitoring compliance with all the aforementioned measures.

Active screening

Active surveillance by rectal screening of any patient transferred across borders into a healthcare facility in another country is strongly recommended by the group of experts. However, drawing up lists of high-risk countries from which transferred patients should be screened for CPE is discouraged. Due to patient mobility and the unknown reservoir of CPE in Europe and globally, any patient transferred from any country is at risk of carrying CPE. The group of experts emphatically recommended that any patient transferred across borders between healthcare systems should be screened upon admission and that all countries should develop a guidance document that includes this recommendation. This is already standard practice in countries such as France, Norway, Sweden and Israel and is consistently reported as an integral part of the success of national task forces to control and prevent CPE.

Detection

Detection, diagnosis and confirmation of the presence of carbapenemases is important for surveillance, infection control and treatment purposes. Necessary elements include a local microbiological laboratory performing highly sensitive tests for rapid detection of carbapenemases and CPE, especially for active screening purposes, and a fast diagnostic turnaround time and timely communication of laboratory results to physicians, nurses and the

infection control team. Confirmatory tests for the presence of carbapenemases are widely available and easy to implement as long as the necessary resources are available and laboratory staff have received the appropriate training. If this is not possible locally, isolates should be sent to reference laboratories, which may cause delays, hampering implementation of infection control measures. Both the experts and guidance documents strongly recommended that all confirmed clinical cases of carbapenemase and CPE should be notifiable to the public health authorities.

Public health

As the spread of CPE continues and new carbapenemases are reported, it is clear that public health preparedness for the surveillance and containment of CPE in Europe needs to be intensified. Guidelines and reports from countries with recent and/or ongoing epidemics have underscored the need for better public health infrastructure, including the creation of public health laboratory networks and national task forces focusing on infection control.

Other proposed measures for improving public health infrastructure include the standardisation of laboratory testing methodologies; use of similar interpretive criteria to ensure adequate EU-wide laboratory capacity for timely and accurate detection of carbapenemases and CPE; making public health reporting of CPE mandatory; strengthening collaboration between reference laboratories and the European Antimicrobial Resistance Surveillance Network (EARS-Net) and ensuring that countries actively participate in electronic early warning platforms such as the Epidemic Intelligence System (EPIS) or the Early Warning and Response System (EWRS).

Research needs

To better assess the effectiveness of infection control and other measures for the prevention and control of CPE transmission in acute and other healthcare facilities in endemic and non-endemic regions, there is a need for better designed and reported studies of the benefit and harm of infection control measures.

In order to obtain the best data from case-control studies and, where possible, eliminate bias these studies should be designed to clearly identify and report whether bacterial isolates represent cases of infection or colonisation. A guidance document specifying quality indicators for the design and reporting of these studies would be a helpful tool to ensure that all necessary elements are included.

Lastly, in order to avoid publication and ascertainment bias, it is necessary to encourage countries to more actively report cases of cross-border transfer of CPE from countries in Europe and globally. Issues of awareness, lack of resources and laboratory capacity and unwillingness to report data may limit the implementation of such a system.

1 Background on carbapenemase-producing Enterobacteriaceae

Introduction

Over the past decade carbapenemases, a group of clinically important β-lactamases have emerged and spread among *Enterobacteriaceae* (1-4). One of the milestones in the emergence of carbapenemases in Enterobacteriaceae was the detection of a novel carbapenemase, *Klebsiella pneumoniae* carbapenemase (KPC), in a *Klebsiella pneumoniae* isolate in North Carolina, USA, which later successfully spread throughout the world [1]. Since then, most acquired carbapenemases have been found and reported in carbapenemase-producing Enterobacteriaceae (CPE) globally [2, 3].

Carbapenemases are enzymes that can efficiently hydrolyse most β-lactams, including carbapenems [4, 5]. In addition, many CPE strains frequently carry additional resistance determinants to other non-β-lactam antibiotics, making these organisms resistant to most antibiotics. CPE commonly remain susceptible to only a few classes of antimicrobials, commonly the polymyxins, tigecycline, fosfomycin, and nitrofurantoin. There is no proven clinical efficacy against these strains and in fact there are reports of clinical failures [6] and emerging resistance to these antimicrobials [7-10].

The emergence and spread of CPE has also been identified as a public health threat, especially since recent studies on CPE [11, 12] and carbapenem-non-susceptible Enterobacteriaceae (CNSE) [13, 14] have shown that infection or colonisation has been associated with higher in-hospital mortality. Similarly, prior studies of outcomes, involving patients infected with multidrug-resistant organisms (MDROs), show that an inadequate choice or the delayed administration of antimicrobial therapy is associated with poorer patient outcomes, increased morbidity, mortality, increased length of hospital stay and increased costs [15-20]. The risk to patients infected with these MDROs becomes even greater, given the very limited number of new antimicrobial agents that are in the developmental pipeline [21, 22].

1.1 Classification, epidemiology and worldwide spread

The most commonly used classification for carbapenemases is that defined by Ambler, although the one by Bush-Jacoby is also used. The Ambler classification separates β-lactamases into four classes A-D, based on their molecular structure [5, 23]. Ambler classes A, B and D will be used throughout this document when referring to carbapenemase classification. An additional classification for carbapenemases has recently been proposed by other experts, whereby extended-spectrum β-lactamases (ESBLs) with hydrolytic activity against carbapenems above a quantitatively defined threshold are designated as $ESBL_{CARBA}$ [24].

Class A carbapenemases are serine β-lactamases and contain serine at their active site [3, 23]. KPC is the most frequently encountered Class A carbapenemase and, along with its variants KPC-2 to KPC-13, which differ solely by amino-acid mutations, it has spread throughout the USA and globally [2]. The bla_{KPC} gene is plasmid-mediated and is transported in a Tn3-based transposon, *Tn4401*, which makes it readily transferable between bacterial isolates [25].

Following the first report of a *K. pneumoniae* isolate harbouring bla_{KPC} from USA [1], $bla_{KPC,}$ spread efficiently with patient mobility and disseminated across borders internationally [26-28]. KPCs are predominantly found in Enterobacteriaceae, most commonly in *K. pneumoniae* isolates, but have recently also been reported in non-fermentative bacteria such as *Pseudomonas* spp. [29, 30] and *Acinetobacter* spp. [31]. KPC has now become endemic in many areas of the world, including north-eastern USA [32, 33], Greece [34, 35], Israel [28, 36, 37], Colombia [38] and Puerto Rico [39-40].

Class B carbapenemases, also known as metallo-β-lactamases (MBLs), are zinc-dependent at their active site. Originally, MBLs were described in non-fermentative bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp. [41, 42], and more recently have also been described in Enterobacteriaceae [43, 44]. The most commonly found Class B carbapenemases are of the VIM-type, [45] which have been identified on all continents [46], but are found mostly in southern Europe [40, 45, 47]. Evidence for the emergence of newer carbapenemases is the description of a novel type of MBL carbapenemase, the New Delhi metallo-β-lactamase-1 (NDM-1) [48-50], mostly associated with travel to the Indian subcontinent, where it appears to be endemic [49, 51]. NDM-1 has also been reported from other countries including China, Australia [52], the USA [53], Canada [54, 55] and many countries in Europe [49, 50, 56-58], most recently the Balkan region [50, 59-61]. These isolates are reported either as cases of returned travellers from the Indian subcontinent, autochthonous cases in countries with no travel association or contact with infected individuals, or as cases of in-country secondary transmission. A recent report of NDM-2 from the north of Africa is worrying testimony that new variants of NDM have begun to emerge [62].

Class D carbapenemases are oxacillinases [46] and include the OXA-type carbapenemases, predominantly in *Acinetobacter* spp. (mainly OXA-23, -24, -58, and -143) [46, 63, 64] but also in *P. aeruginosa* (mainly OXA-40) [65]. The first report of OXA-48 in Enterobacteriaceae was from Turkey [66, 67] and it has since been reported from other countries in the Mediterranean basin, including Israel [68], Tunisia [69], Morocco [70] and Spain [71]. Cross-border transfer of OXA-48-producing Enterobacteriaceae into healthcare facilities in the destination countries (e.g. France which has seen a sharp increase in cases recently) is being reported more frequently in the literature, suggesting that the mode of introduction into healthcare facilities is patient mobility [72-74].

1.2 Worldwide spread

In the last decade, there has been a rapid increase in carbapenem-non-susceptible Enterobacteriaceae (CNSE) [2, 40] worldwide, with certain areas reporting higher rates of all classes of carbapenemases: KPC type from USA [32, 33], Israel [28, 36, 37], Greece [34, 35], Puerto Rico [39], and Colombia [38]; VIM type from the southern Mediterranean region, e.g. Greece [41, 45, 75] and NDM-1 from the Indian subcontinent [48-50].

All types of these carbapenemases have been described in members of the Enterobacteriaceae family, including *E. coli*, *Serratia marcescens*, *Citrobacter* spp., *Enterobacter* spp. and interspecies spread has also been reported, perhaps demonstrating the facility with which the genetic elements can disseminate [45, 76-79].

In Europe, antimicrobial susceptibility testing data and trends for *K. pneumoniae* resistance to carbapenem antimicrobials have been reported annually since 2005 through the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS[1]). EARS-Net data from 2009 showed that, while in most European countries rates of carbapenem resistance in invasive *K. pneumoniae* isolates from blood and cerebrospinal fluid were below 1%, Greece, Cyprus and Italy reported resistance rates of 43.5%, 17.0% and 1.3% respectively, representing a rise since 2005. Similar rates have also been shown in reports from these countries at national level [45, 47].

The dissemination of these mobile genetic elements (e.g. transposon and plasmids) and of epidemic strains occurs through human population mobility and, more specifically, through patient transfer between healthcare facilities, not only within the same country but also across borders [28, 37, 80, 81]. In fact, the first epidemiological evidence of intercontinental spread of KPC was described in reports from France [82] and Israel [37] after KPC was detected following introduction from the USA. Since then, many other reports have documented cross-border importation between healthcare facilities resulting from patient transfer, not only within Europe but also from outside European borders. Secondary transmission of these organisms has been reported, leading to outbreaks, epidemics, and in some countries endemicity [2, 27, 40, 83-87].

1.3 Issues in laboratory detection

Detecting carbapenemases can be particularly challenging for a number of reasons, ranging from clinical and infection control to laboratory issues. Clinical or infection control issues can include lack of hospital or national infection control protocols that suggest active screening; incomplete evaluation of which patients should be actively screened or cultured, and resource-poor settings where implementation of infection control measures is difficult once detection of carbapenemases is suspected or confirmed.

In order to implement infection control in a timely manner, but also for therapeutic purposes, it is important that local microbiology laboratories should be able to detect carbapenem resistance in a timely manner and with high sensitivity at the point of care. Similarly, it is important for local and/or reference laboratories to be able to quickly confirm the presence of carbapenemases in Enterobacteriaceae [40, 44, 88, 89].

As previously stated, carbapenemases are enzymes that can efficiently hydrolyse most β-lactams, including carbapenems [4, 5]. One of the main reasons that timely detection can be challenging in the laboratory is that not all carbapenemases will confer clinical carbapenem resistance and this is particularly true for the Enterobacteriaceae. The definition of a carbapenemase therefore relies not on the ability to confer clinical resistance to carbapenems but on the hydrolytic capacity of carbapenems measured by quantitative spectrophotometry. Based on this definition, detection of the genes encoding the enzymes regarded as carbapenemases is usually an appropriate confirmation of carbapenemase production.

However, detection of either carbapenem-resistance or the presence of carbapenemases can be compromised by various diagnostic difficulties. CPEs can demonstrate significant variation in their carbapenem minimum inhibitory concentrations (MICs), even falling within the susceptibility range defined by either the Clinical Laboratory Standards Institute (CLSI) [90] or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [91]. In order to circumvent this problem, the CLSI recently proposed revised MIC clinical breakpoint interpretive criteria, which are lower than previously published, to better detect carbapenem resistance [92].

[1] Available from: http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx

The diagnostic accuracy for the detection of carbapenem resistance and the presence of a carbapenemase can be affected by a number of factors, including the bacterial species being tested; the class of carbapenemase produced by the organism [44, 93]; the geographical origin of the bacterial species; heteroresistance [94] and the presence of other resistance mechanisms, such as ESBLs, porin mutations and/or presence of efflux pumps [95-98].

Furthermore, certain testing methods, such as automatic testing, have been shown to not always distinguish between Enterobacteriaceae that produce carbapenemases and those carrying other mechanisms of resistance (e.g. ESBLs and/or porin loss) [99-101]. Difficulties also exist when using automated diagnostic testing systems to detect specific carbapenemases, e.g. OXA-48, because these isolates can remain susceptible to extended-spectrum cephalosporins and monobactams, but resistant to carbapenems [40, 101]. They may also have lower MICs to carbapenem antimicrobials, and therefore go undetected.

Recently, phenotypic tests have become available that correlate well with the presence of clinically important carbapenemases. However, false positive and false negative results have been reported, especially when using the Modified Hodge Test (MHT) and therefore caution should be exercised in interpreting results [102, 103]. False detection (false positive MHT) of a carbapenemase can occur because of the presence of other resistance mechanisms, e.g. ESBLs and/or porin loss [102]. False negative results of the MHT have also been recently reported when testing NDM-1-producing bacterial strains [103].

In order to identify isolates with specific types of carbapenemase production more accurately, other screening methods have been proposed and validated. Examples of these are disk diffusion synergy tests, using carbapenemase-inhibiting compounds such as boronic acid for KPC and dipicolinic acid for MBL [44, 97, 104-106]. The use of selective chromogenic agar media has also been proposed for rapid screening purposes [40, 107, 108]. Finally, molecular confirmation tests, such as the single or multiplex PCR, which are usually limited to use in reference laboratories or under epidemic conditions, have also been evaluated and have shown good results [109, 110].

1.4 Issues in infection control

Carbapenemase-producing Enterobacteriaceae can colonise or infect not only those patients who are debilitated, immune-compromised or critically ill, but also those who were previously healthy and became colonised or infected in healthcare settings practicing poor infection control. This poses an obvious threat to patient safety since infection with these organisms is associated with worse outcomes, prolonged hospitalisation and higher mortality rates [12, 13, 111].

It is necessary to curb the spread of CPE in healthcare facilities after their importation, or even when they have already become endemic in a healthcare system. Knowing which infection control measures are effective and should be implemented is of paramount importance. Because of the difficulty in assessing the effectiveness of these measures, the ORION statement [112] was developed as a standard for the transparent reporting of infection control interventions during outbreaks.

In response to the growing threat of CPE spread in healthcare systems, two guidance documents were recently published providing recommendations for the implementation of multimodal infection control interventions to prevent the spread of CPE in acute healthcare facilities. These guidelines, one by the US Centers for Disease Control and Prevention (CDC) [89] and one from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [81], are mainly based on prior guidelines for prevention and control of MDROs [113] and on expert opinion.

Many countries in Europe have addressed the spread of CPE by creating new or modified guidelines or strategies for other MDROs, or by creating national task forces [88] and developing local or national strategies to tackle this public health threat (Table 1).

Table 1. Updated national guidance documents on Enterobacteriaceae producing New Delhi metallo-β-lactamase-1 (NDM-1) or carbapenemases in general, as reported in 16 European countries (June, 2011)

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; National guidelines in preparation	http://www.referenzzentrum.at
Belgium	•	•	•	•		http://www.nsih.be/surv_carba/carbapenemase_fr.asp
Czech Republic	•	•	•			http://www.szu.cz/uploads/documents/CeM/Zpravy_EM/18_2009/3_brezen/100_betal.pdf
Estonia	•					http://www.elmy.ee/public/files/Enterobacteriaceae%20algoritmide%20selgitused%20ver1.0.doc
Finland	•	•	•	•		http://www.ktl.fi/portal/17160
France	•	•	•	•		http://www.hcsp.fr/explore.cgi/hcspr20101116_bmrimport.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/index.php?option=com_content&view=article&id=190%3A2010-12-01-05-45-22&catid=64%3A2010-08-04-08-56-37&Itemid=1
Ireland	•		•	•	Interim guidance available; national guidelines in development	http://ndsc.newsweaver.ie/epiinsight/x3k8tfcgbkctx2boyfzyr4
Netherlands	•	•	•	•		Please see references [114, 115]
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	•	•	•	•		http://www.dgs.pt/?f=3&id=16683 http://www.dgs.pt/upload/membro.id/ficheiros/i013491.pdf
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

Adapted and updated with permission from "New Delhi metallo-β-lactamase-1-producing Enterobacteriaceae: emergence and response in Europe". Struelens MJ, et al. Euro Surveill 2010;15(46):pii=19716 [50].

1.5 Origin of ECDC risk assessment

The need for this risk assessment was prompted by an increasing number of publications and outbreak reports discussing the spread of carbapenemase-producing Enterobacteriaceae throughout healthcare facilities and across borders in Europe. France expressed its concern regarding this problem at the ECDC Advisory Forum on 6 May 2010, asking whether there was a need for a European strategy for a European risk assessment (ECDC), including routine epidemic intelligence activities[2] and proposing that a risk assessment be performed.

Subsequent data reported by the ECDC National Antimicrobial Resistance (AMR) Focal Points regarding uncontrolled epidemics caused by a diversity of strains in different countries then led to this risk assessment being expanded to include all carbapenemases and all Enterobacteriaceae.

[2] (J. C. Desenclos, Advisory Forum, ECDC, 6 May 2010).

2 Objectives

The aim of this risk assessment was to evaluate the human risk associated with the spread of carbapenemase-producing Enterobacteriaceae (CPE) between healthcare facilities, with a particular focus on cross-border transfer. The assessment aimed to answer the following questions:

- What are the risk factors for patient colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the effectiveness of using screening and/or targeted or other infection control interventions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the clinical diagnostic accuracy of tests for screening, detection and confirmation of carbapenemase-producing Enterobacteriaceae?

3 Methods

3.1 Defining the eligibility criteria for the inclusion of studies in the systematic reviews

3.1.1 Evolution of questions, initial search for studies and re-organisation of questions

During the first internal meeting at ECDC on 10 June 2010, the core ECDC and internal ECDC ad hoc working groups met and drafted the following questions to be addressed in the risk assessment:

- What are the risk factors for patient colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the effectiveness of using standard precautions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the effectiveness of using targeted infection control interventions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the effectiveness of using screening, in addition to targeted infection control interventions, to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the clinical diagnostic accuracy of tests for screening, detection and confirmation of carbapenemase-producing Enterobacteriaceae?

After describing the population, intervention, comparator and outcome (PICO) and performing preliminary literature searches, it became evident that there was insufficient evidence available to answer all five questions and these were merged by two of the authors (AM and MS) and the medical librarian into the following three:

- What are the risk factors for patient colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the effectiveness of using screening and/or targeted or other infection control interventions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?
- What is the clinical diagnostic accuracy of tests for screening, detection and confirmation of carbapenemase-producing Enterobacteriaceae?

The lists of synonyms for each concept and a detailed description of the PICO and the complete search strategy for each question appear below.

3.1.2 Defining the population, intervention, comparator and outcome (PICO) for each question

Systematic review #1

What are the risk factors for patient colonisation or infection with carbapenemase-producing Enterobacteriaceae?

Types of study considered for inclusion:
All case-control studies or cohort studies were considered for inclusion in this review.

Population:
Defined as: 'all patients admitted or transferred to healthcare facilities who were at risk of becoming colonised or infected with carbapenemase-producing Enterobacteriaceae'. Healthcare facilities were defined as 'secondary and tertiary healthcare facilities, acute care facilities, hospitals, ICUs, long-term care facilities, nursing homes, rehabilitation centres and step-down units'.

Intervention(s) included:
There were no interventions.

Outcome measure(s):
Colonisation or infection with carbapenemase-producing Enterobacteriaceae.

Systematic review #2

What is the effectiveness of using screening and/or targeted or other infection control interventions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?"

Types of study considered for inclusion:
All prospective randomised controlled trials (RCTs), or non-randomised clinical trials (CCTs) (e.g. interrupted time-series analyses, controlled before-and-after studies (CBAs), uncontrolled before-after-studies, outbreak reports and other observational studies) were considered for inclusion in this review.

Population:
Defined as: 'all patients admitted or transferred to healthcare facilities who were at risk of becoming colonised or infected with carbapenemase-producing Enterobacteriaceae'. This included patients who were exposed to CPE introduced by cross-border transfer or by introduction of CPE into non-endemic or endemic healthcare facilities and countries and during an outbreak. Healthcare facilities were defined as 'secondary and tertiary healthcare facilities, acute care facilities, hospitals, ICUs, long-term care facilities, nursing homes, rehabilitation centres and step-down units'.

Intervention(s) included:
The systematic review aimed to assess the effectiveness of implementing targeted or non-targeted infection control measures compared to standard precautions or active patient screening alone.

Definitions and synonyms:

Screening:

- Performing active surveillance cultures, active screening tests or contact screening of at-risk patients to detect colonisation with carbapenemase-producing Enterobacteriaceae.
- Sites of screening included the rectum, active wounds and other relevant superficial body sites.
- Timing of screening was left open to include 'on admission', 'on discharge', in the ICU, daily or weekly or in serial point-prevalence surveys.

Additional targeted infection control precautions (in addition to standard precautions) included:

- Precautions restricted to the care of patients colonised or infected with carbapenemase-producing Enterobacteriaceae, patient cohorting (i.e. physical separation and/or nursing team separation for colonised and non-colonised patients), barrier precautions, barrier nursing, contact isolation, contact precautions, use of gloves, gowns and face masks.

Other infection control measures:

- Pre-emptive patient isolation and contact precautions for patients at high-risk of colonisation with carbapenemase-producing Enterobacteriaceae, contact precautions for all patient care, ward closure, environmental cleaning and disinfection, antibiotic restrictions or antibiotic class shift.

Outcome measure(s):
Transmission or spread of carbapenemase-producing Enterobacteriaceae within a healthcare facility, measured by the frequency/incidence of colonisation and/or infection with these organisms.

Caveats for outcome reporting in this systematic review included the following:

- All outcome types, case definitions, incidence metrics and statistical analysis methods were reported where available.
- Risk reduction effect was summarised as a risk ratio (RR) associated with infection control intervention(s) versus pre-intervention baseline (and 95% confidence interval of the RR estimate).
- Where only raw data were reported, e.g. number of cases over time periods, these were reported as such.
- Studies not reporting data on acquisition outcomes were excluded.

Systematic review #3

What is the clinical diagnostic accuracy of tests for screening and confirmation of carbapenemase-producing Enterobacteriaceae?

Types of studies considered for inclusion:
The types of studies included were not specified; the search included studies retrieved by the population, intervention, reference test and testing (PIRT) results.

Population:
Defined as 'all patients admitted or transferred to healthcare facilities who were at risk of becoming colonised or infected with carbapenemase-producing Enterobacteriaceae'. Healthcare facilities were defined as 'secondary and tertiary healthcare facilities, acute care facilities, hospitals, ICUs, long-term care facilities, nursing homes, rehabilitation centres and step-down units'.

Intervention(s) included:
The use of screening and confirmatory testing for carbapenemase production (e.g. automated susceptibility testing, Modified Hodge Test (MHT), double disk synergy testing, E-test and PCR).

Reference test(s):

The accepted reference standard or a designated comparator test for screening, detection and confirmation of carbapenemases in Enterobacteriaceae.

Testing result(s):

Accurate screening, detection and confirmation of carbapenemases in Enterobacteriaceae accompanied by reporting sensitivity and specificity of the test.

3.1.3 Search and identification of studies

Information sources

The data sources used for the systematic search of English and non-English publications were Ovid Medline (from 2000 to the third week of August 2010), the Cochrane Library (1960-2010), EMBASE (2000-2010), PubMed (2000 to the third week of August 2010), EMBASE (2000 to the third week of August 2010) and the Health Technology Assessment (HTA) website (1988 to August 2010).

Developing the search strategy

- A search strategy was developed by a medical librarian with input from two of the authors (AM and MS). The original five questions were analysed by population, intervention, control and outcome (PICO), the thesaurus search terms (i.e. KPC) synonyms and equivalent text words. Broader topics were mapped out using prior references in the existing literature and other equivalent medical terms.
- Medical Subject Headings (MESH terms) were included in the search terms and sought in the OVID Medline and PubMed databases. Emtree terms were sought in the EMBASE database.
- A Cochrane Library Database review did not retrieve any relevant RCTs or systematic reviews in this field. Date limits were set as 2000–present, language limits were set at the end of the systematic search to English, French, Spanish, Italian and Greek and limits were also set to humans, but only for questions 1 and 2.
- In order to capture the largest number of relevant articles but limit the number of retrieved citations, containing terms that were not relevant to the questions, the following modifications were made to the search strategy:
  - Enterobacteriaceae and its synonyms were defined in two ways when creating the search strategy for OVID Medline and PubMed: a) by using the MeSH term 'Enterobacteriaceae' and b) by using Enterobacteriaceae as a text word. *Salmonella*, *Shigella* and *Yersinia* were then excluded: 'Enterobacteriaceae' ('NOT *Salmonella, Shigella, Yersinia*').
  - Since there is a large body of literature on the subject of carbapenemases in *Acinetobacter spp*. and *Pseudomonas spp*. and these bacteria are not addressed in the review, they were also excluded from the entire search ('NOT *Acinetobacter*, NOT *Pseudomonas*').
  - Many articles in OVID Medline and PubMed are indexed using β-lactamases as a MeSH term. Even though carbapenemases are a type of β-lactamase, no such subheading exists under 'β-lactamases'. In order to retrieve all citations indexing β-lactamases as a MeSH term, we included 'β-lactamases' in our search strategy, retrieving a very large number of articles containing 'extended-spectrum β-lactamases' ('ESBLs'). To avoid this, the authors decided to include the term 'β-lactamases' as a MeSH term, but to remove articles that contained mention of ESBLs ('NOT extended-spectrum β-lactamases').

Searching other sources

The authors decided that for the three systematic reviews citations could only be included if they were from peer-reviewed and published articles. Preliminary search terms were entered into Google before starting the systematic search, and very pertinent results were cross-checked with results from preliminary searches

performed on PubMed, Embase and OVID. An additional advantage of doing so was that articles not yet indexed in PubMed or OVID Medline could be found on Google.

The 'grey literature' was not searched, no hand searching was performed, reference lists of full-text articles were not searched systematically and an automatic e-mail notification from PubMed was set up for newly published articles. For all these searches, the fourth week of 2010 was the cut-off date beyond which no new article could be added to the studies.

3.1.4 Methods of abstract selection and inclusion of full-text documents

3.1.4.1 Excluding studies based on evaluation of the retrieved abstract

Each of the questions presented in this risk assessment represents one systematic review in itself. Initially, search strategies were developed for each of the original five questions. However, the systematic search in OVID Medline, PubMed and EMBASE produced such a large number of overlapping citations that one of the authors (AM) decided to streamline these citations by merging all retrieved studies into one large database and removing all duplicates. The citations retrieved during the systematic search were placed in a central Endnote database and any further work on these was performed by creating separate Endnote databases for the individual questions or tasks performed.

It also became apparent that there were insufficient studies to answer all five questions separately and the questions were therefore merged and reduced to three (see Objectives section).

One of the authors (AM) removed all irrelevant abstracts from the central Endnote database and all duplicates were then deleted. The abstracts were then re-evaluated and more abstracts were removed by one author (AM) based on irrelevance to the PICOs. Any e-mail notifications from 'My NCBI' were added to this number. No further search was undertaken after the fourth week of August 2010.

3.1.4.2 Study pre-selection based on abstract

The abstracts retrieved from the systematic search were then independently reviewed by two of the authors for each question (question # 1: AM and MS, question #2: MS and AM and question #3 :AJ and AM) and excluded. Abstracts that were not relevant to the questions were excluded. Abstracts were only included if their content was in accordance with the PICO for the question. If the reviewers' opinions differed about whether the abstracts met the eligibility criteria, as described by the PICO, this was resolved by discussion and the final number of abstracts agreed upon by the two reviewers.

3.1.4.3 Full-text document inclusion for each systematic review

The full-text documents were then retrieved and pre-defined exclusion and inclusion criteria were applied, by the three authors (AM, MS and AJ) and two members of the ECDC ad hoc working group (FF and DM). The author of each systematic review evaluated his/her full-text articles individually, according to the criteria listed below, and decided which articles should be excluded and included.

3.2 Expert meeting

As part of the risk assessment, a meeting of external experts in infectious diseases, microbiology and infection control was arranged. The purpose of the meeting was for the experts to provide feedback and expert opinions on the three systematic reviews that had been performed. This meeting took place in Stockholm on 24 November 2010.

There was much deliberation as to how the feedback and expert opinion should be incorporated into the risk assessment. The decision was that it should be incorporated in three parts: a) suggestions for the systematic reviews, incorporated as changes in the body of the document; b) any clarifications, as well as proposals for the future, to be included in a section called 'Contributions from the Expert Meeting' and c) all other proposals and suggestions for research and actions for the future to also be included under the heading 'Contributions from the Expert Meeting'.

4 Results

4.1 Results of the search strategy

For the full search strategy, please see Annexes I-IV.

ESBL

By including 'β-lactamases' in the search strategy, even after removing the term 'ESBL', an excessive number of articles were retrieved, even after repeated modifications of the search terms, referring only to 'ESBL' and 'Enterobacteriaceae'. This most likely occurred because the MeSH term 'β-lactamases' was part of the search strategy. It was therefore decided to manually remove all articles that reported only ESBL.

4.2 Process of retrieving and retaining all relevant abstracts

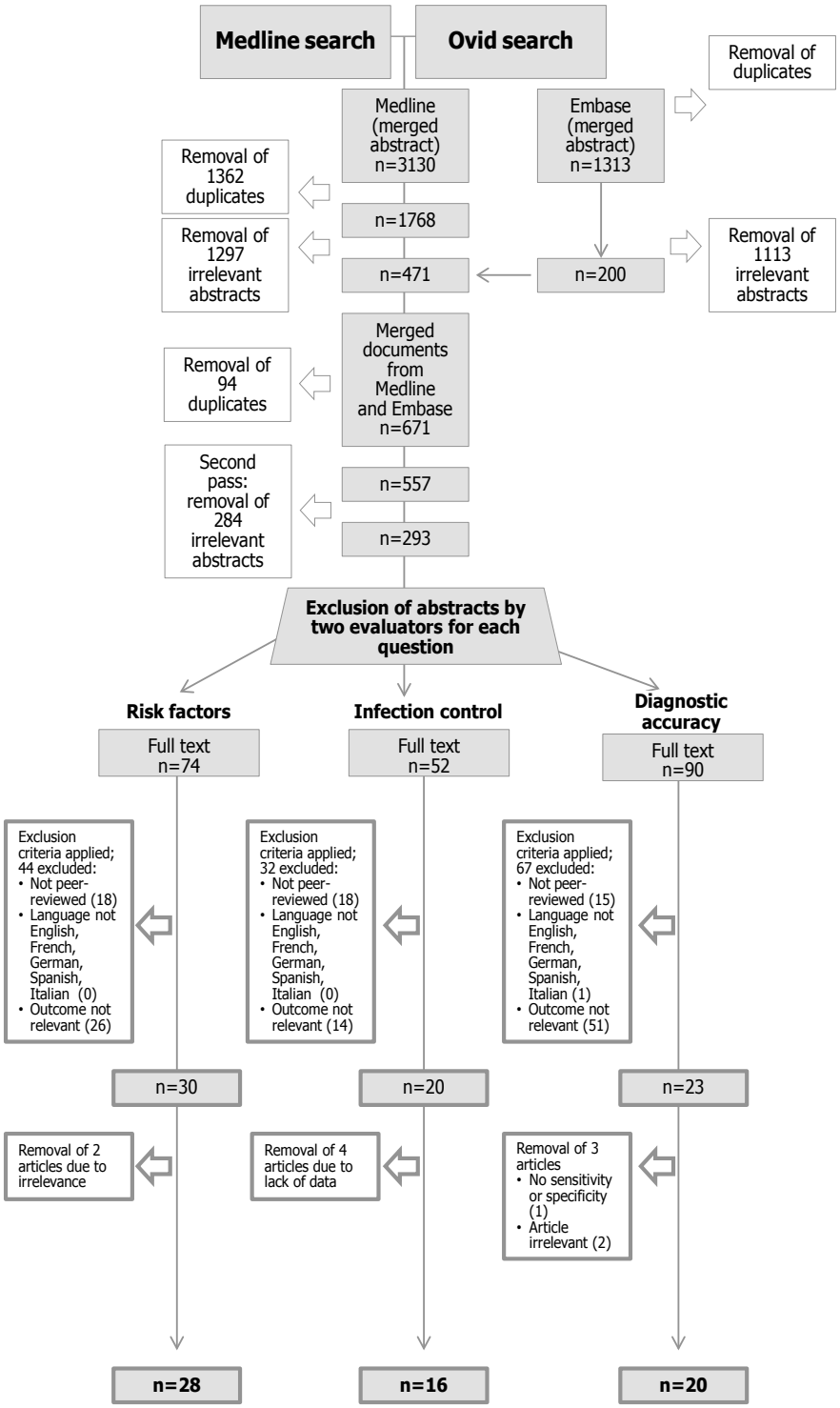
All citations retrieved in OVID Medline and PubMed were merged (n=3 130) and duplicates (n=1 362) were removed leaving 1 768 citations. Of these, 1 297 abstracts were not relevant to the PICO for any of the three questions and were removed by one of the authors (AM), leaving 471 OVID Medline and PubMed abstracts.

Similarly, all citations from EMBASE were merged and duplicates removed, leaving 1 313 citations. From these 1 113 were not relevant to the PICO for any of the three questions and were removed, leaving 200 studies from EMBASE.

The remaining citations from OVID Medline and EMBASE were combined (n=671) and 94 duplicates removed, leaving 577 citations. A second review was performed by one of the authors (AM), resulting in the removal of 284 irrelevant abstracts, leaving 293 abstracts in total. One additional study was added to the total number, from a 'My NCBI' e-mail alert, bringing the total number of studies to 294.

This process is summarised in the flowchart presented in Figure 1.

Figure 1. Flowchart illustrating how studies were selected for systematic review



4.3 Results of full-text inclusion for each question

(For questions see 'Methods' section).

Inclusion of the full-text documents for each question required the establishment of exclusion criteria. The first set of minimal exclusion criteria were common. An article was excluded immediately if it was not peer-reviewed, was not written in one of the six languages specified or did not have a relevant outcome. Inclusion criteria were then developed by the authors of the systematic reviews (AM, AJ and MS) and two members of the internal ECDC ad hoc working group.

The author of each systematic review then applied the exclusion and the inclusion criteria to his/her abstracts and decided which full-text articles should be included. Appendix V, Table V.1 lists the excluded abstracts and the reasons for exclusion. Appendix V, Table V.2 shows the full-text articles included. The flowchart in Figure 1 shows the entire process of study selection and full-text inclusion.

4.3.1 Finding tables and extracting data

The authors of the systematic reviews (AM, AJ and MS) and one member of the internal ECDC ad hoc working group (FF) created a summary of findings table to help select the data to be extracted from the full-text articles. Various guidance documents were consulted [112, 116, 117] to ensure that all the necessary data were extracted, reported and appraised. The study data extraction templates created by the group were subsequently pilot-tested using full-text documents and then further refined as the data were being extracted.

4.3.2. Methodological assessment of bias in included studies

Assessment of bias and reporting of internal validity were discussed at length for each of the three questions by all authors (AM, AJ and MS) and one member of the internal ECDC ad hoc internal working group. Discussions were based on documents regarding the assessment of bias in observational epidemiological studies [116-119].

5 Systematic review #1

What are the risk factors for patient colonisation or infection with carbapenemase-producing Enterobacteriaceae?

5.1 Background

Risk factors for colonisation or infection with MDROs, including CPE, appear to be quite similar and include prior antimicrobial use, severity of illness and ICU stay. Recent reports discussing the apparent spread of CPE across borders and into healthcare facilities via patient transfer have highlighted the risk of transmission to humans and the spread of these MDROs.

Most cross-border transfer of CPE is reported from areas of high endemicity, as evidenced by the intercontinental spread of KPC from the USA to France [82] and Israel [37]. Since then, many other reports have been published detailing cross-border transfer of patients across healthcare facilities, countries and continents [27, 37, 87, 120, 121]. This assessment presents the methodology, findings and discussion of a systematic review evaluating the risk factors for colonisation or infection with CPE, with a special emphasis on cross-border transfer.

5.2 Methods

For this risk assessment the authors decided to include all studies looking not only at CPE, but also CNSE. They did this for two reasons: firstly because the resistance mechanisms were not always described in the studies and secondly, because it was also important to evaluate risk factors for the acquisition of all CNSE, regardless of the resistance mechanism. The latter is significant because these organisms may exhibit similar behaviour.

Thus, the studies included were those that reported:

- Confirmed carbapenemase-producing Enterobacteriaceae isolates;
- Carbapenem-non-susceptible (not further defined) Enterobacteriaceae isolates;
- A mixed population of Enterobacteriaceae isolates that were carbapenem-non-susceptible and had subsequently been confirmed as carbapenemase-producing.

Study selection

Exclusion and inclusion of abstracts for question #1

A total of 294 citations were retrieved from the systematic search and two authors (AM and MS) independently reviewed these abstracts to assess whether they should be included. Abstracts were included only if their content was in accordance with the PICO for the question. If the reviewers' opinions differed as to whether the abstracts met the eligibility criteria described by the PICO, this was resolved by discussion before agreeing upon the final number of abstracts included.

The full-text documents were then retrieved and detailed exclusion and inclusion criteria applied by the author of the systematic review (AM) (see criteria below) to determine which full-text articles would be included in the review.

Exclusion criteria:

Full-text articles were excluded if:

- the article was not peer-reviewed;
- the article was not in English, Spanish, Italian, Greek, French or German;
- the outcome(s) of the study was not relevant to the PICO.

Inclusion criteria:

The article had to be a primary research article and any one of the following:

- An analytic study (i.e. a cohort or case-control study)
- A case report or case series associated with international cross-border transmission and/or outbreaks containing a description of surveillance and/or infection control measures (a cross-border transmission study).

Data extraction and analysis

Description of included studies

Analytic studies and descriptive studies were separated according to the inclusion and exclusion criteria above. The author of this systematic review (AM) extracted data on study design, population and setting, primary objectives, methods and outcomes from the selected studies using a standard form. A table summarising the findings can be found in Appendix VI (Table VI.1).

For analytic studies, 23 items were included in the data extraction form for risk factors. Based on extracted data two summary tables were prepared: Table VI.1 (Appendix VI) on study description (seven items) and Table VI.2 (Appendix VI) on appraisal of methodological quality and risk of bias (five items).

For cross-border transmission studies (n=18), nine items were extracted to summarise findings. No systematic quality appraisal or analysis of the effect of control measures was performed due to variable precision in the reporting of methods and outcomes.

Assessment of methodological quality and risk of bias in included analytic studies

The presence of selection bias, information bias and confounding in the case-control studies under review was evaluated by the author (AM) and reported in Appendix VI, Table VI.2. Relevant literature discussing bias in observational studies was used for this purpose [118, 119, 122, 123].

Overall strength and volume of evidence

The authors anticipated that only a limited number of studies of heterogeneous intervention with low level of quality study design would be available for this review. They therefore planned a narrative summary of the overall strength and volume of evidence.

5.3 Results

Study selection

A total of 74 abstracts were included by the two reviewers (AM and MS). Full-text articles were obtained and the exclusion/inclusion criteria were applied by the author of the systematic review (AM). In all, 44 full-text articles were excluded and 30 included, 12 of which were analytic studies and 18 descriptive studies. When abstracting the data, a further two analytic studies and one descriptive study were excluded because they did not match the PICO of the question. This left a total of 27 studies. Of these, nine were analytic studies with varied study designs (e.g. case-control, matched case-control, case-case control) and 18 were descriptive reports of cross-border transmission.

Description of studies

Analytic studies (Appendix VI, Table VI.2)

The studies, which were all written in English, were conducted in four countries: Greece (n=1), the USA (n=3), Israel (n=3) and South Korea (n=2). The designs were case-control studies (n=4), matched case-control studies (n=4) and case-case control studies (n=1) looking at risk factors for the acquisition of carbapenem-resistant Enterobacteriaceae.

The Enterobacteriaceae included in the nine case-control studies were: *K. pneumoniae* isolates harbouring KPC, seven isolates from Gregory et al. [124] and isolates from four other studies [8, 11, 111, 125]; *K. pneumoniae* isolates that were carbapenem-resistant (no further information was provided regarding the mechanism of resistance), 19 isolates from Gregory et al. [124] and isolates from three other studies [14, 126, 127]. Jeon et al. [128] included *E. coli* isolates that were imipenem-resistant and Marchaim et al. [12] included *Enterobacter spp.* isolates that harboured KPC (Appendix VI, Table VI.1).

Of the nine studies included, resistance to carbapenems alone was reported in four studies, whereas four additional studies reported the exact mechanisms of carbapenemase-production. The remaining study reported a combination of both.

Appendix VI, Table VI.3, provides information on whether the isolates included in each study were from areas of high endemicity. Where available, information on which clinical breakpoints used in the studies to determine susceptibility to carbapenem is also provided.

Descriptive studies (Appendix VI, Table VI.4)

A total of 18 studies reporting cross-border transmission were selected and included. Reporting countries were France (n=6), Belgium (n=1), USA (n=3), UK (n=2), Colombia (n=1), Israel (n=1), Finland (n=1), Sweden (n=1), Norway (n=1) and Germany (n=1). The countries of origin were Greece (n=11), Italy (n=1), USA (n=3),

Israel (n=3) and the Indian subcontinent (n=2). The design was retrospective case series with detailed microbiological investigations.

All reports concerned *K. pneumoniae* isolates producing KPC, VIM and NDM-1, *Enterobacter spp*. isolates producing KPC and NDM-1 and *E. coli* producing NDM-1.

The primary aim of these articles was to describe the cross-border importation of CPE. In nine out of the 18 studies, there was strong evidence of importation by direct transfer of patients from a foreign hospital and concordant molecular typing or genetic context of the resistance gene from the country of origin. Five studies showed moderate evidence of importation by reporting concordant molecular typing or genetic context of the resistance genes from the country of origin, but no clear evidence of hospital transfer. One study showed moderate evidence of importation with a clear history of direct hospital transfer, but without performing concordant molecular typing (although this was the first case reported in the country originating from an endemic area). One study showed strong evidence of importation with direct transfer across healthcare facilities (within the same country) and concordant molecular typing. Meanwhile, one case report showed weak evidence of importation, since there was no clear direct hospital transfer but evidence that the resistance genes had a genetic context within the country of origin.

5.4 Summary of findings for risk factors from the analytic studies included

The studies included by the author (AM) in this review are those that evaluated risk factors by using multivariate analysis; those that included isolates which were carbapenemase-producing and/or described as being carbapenem-resistant (Appendix VI, Table VI.1) and those that met the exclusion and inclusion criteria.

5.4.1 General comments: Interpretive criteria

Within the studies included, results for antimicrobial susceptibility testing were very dependent on the interpretive criteria used. Overall, there was a lot of heterogeneity present amongst the studies.. The variability is due to the fact that different clinical breakpoints were used (where available these have been stated; see Annex VI, Table VI.1) and that recently the Committee for Laboratory Standards Institute (CLSI) [92] lowered their breakpoints for carbapenem resistance in Enterobacteriaceae.

5.4.2 Risk factors

General risk factors

Risk factors found to be associated with colonisation or infection with CPE were: prior antimicrobial use; length of stay (time at risk); severity of illness; mechanical ventilation; admission to the ICU; high procedure score; presence of wounds; positive culture from a blood isolate; transfer between hospital units within the same hospital; prior surgery; prior hospital stay; presence of a biliary catheter and recent transplantation.

Prior antimicrobial use

Like other studies on other MDROs [129-132], prior antibiotic exposure was found to be a risk factor for colonisation and infection with CPE. In the nine case-control studies, for which a summary of findings appears in Appendix VI, Table VI.1, prior antibiotic use was identified as a risk factor, both for the acquisition of carbapenem-resistant *K. pneumoniae* [14] and KPC-producing *K. pneumoniae* [125].

Carbapenems

Prior use of a carbapenem was identified as an independent risk factor for the acquisition of KPC-producing *K. pneumoniae* [111, 125], for carbapenem-resistant *K. pneumoniae* and carbapenem-resistant *E. coli* [127, 128].

Cephalosporins

Prior use of an extended-spectrum cephalosporin was identified as a risk factor for the acquisition of KPC-producing *K. pneumoniae* [11, 111]. Their use was also found to be a risk factor for the acquisition of carbapenem-resistant *K. pneumoniae* (mechanism of resistance not specified) [127].

Fluoroquinolones

Prior use of a fluoroquinolone was identified as an independent risk factor for the acquisition of KPC-producing *K. pneumoniae* [11, 125] and as an independent risk factor for the acquisition of carbapenem-resistant *K. pneumoniae* [14, 126]. In one study, fluoroquinolones had a protective effect [127].

Other antimicrobials

Other antimicrobial agents associated with a risk of acquiring carbapenem-resistant Enterobacteriaceae were the anti-pseudomonal penicillins [126] and metronidazole [128].

Other risk factors

High procedure scores and severity of illness were found to be associated with a risk of acquiring *Enterobacter* spp. harbouring *bla*$_{KPC-2}$ [12] and *K. pneumoniae* harbouring *bla*$_{KPC}$ [11], respectively.

The risk of acquiring *K. pneumoniae* harbouring *bla*$_{KPC}$ was also associated with admission to an ICU [125], mechanical ventilation, a longer length of hospital stay and recent solid organ transplantation (SOT) or stem-cell transplantation (SCT) [111]. Acquisition of carbapenem-resistant *E. coli* was associated with the presence of a biliary catheter and a prior hospital stay of less than a year prior to positive culture [128]. Risk factors associated with the acquisition of carbapenem-resistant *K. pneumoniae* (only seven of the 26 isolates had been tested and found positive for *bla*$_{KPC}$) were previous surgery, transfer between hospital units and the presence of wounds [124].

5.5 Summary of findings from descriptive studies

Appendix VI, Table VI.4 presents a summary of findings from descriptive studies reporting cross-border spread of CPE. Eighteen studies reported and illustrated transmission of various species of CPE across healthcare facilities and as a result of cross-border transfer of colonised or infected patients (two *Enterobacter* spp. isolates, one *E. coli* isolate and the remaining isolates were *K. pneumoniae*. These isolates carried *bla*$_{KPC,}$ *bla*$_{VIM-1}$ or *bla*$_{NDM-1}$).

The primary aim of these articles was to describe the cross-border importation of CPE. In nine out of the 18 studies there was strong evidence of importation, as a result of direct transfer of patients from a foreign hospital, and concordant molecular typing or genetic context of the resistance gene from the country of origin. Five studies showing moderate evidence of importation reported concordant molecular typing or genetic context of the resistance genes from the country of origin, but no clear evidence of hospital transfer. One study showed moderate evidence of importation with a clear history of direct hospital transfer, but without concordant molecular typing (although this was the first case reported in the country originating from an endemic area). One study showed strong evidence for importation with direct transfer across healthcare facilities (within the same country) and concordant molecular typing. Finally, one case report showed weak evidence of importation, since there was no direct hospital transfer but a genetic context was established linking the resistance genes to the country of origin.

From the studies included, there is evidence of cross-border transmission by direct hospital-to-hospital transfer of patients through the identification of six *K. pneumoniae* isolates (five *K. pneumoniae* harbouring *bla*$_{KPC}$ isolates [86, 133-135] and one *K. pneumoniae* isolate with *bla*$_{VIM-1}$ [85]), transferred from Greece to France and the three *K. pneumoniae* isolates carrying *bla*$_{KPC}$ transferred from Greece to Belgium [83]. These were obtained by active screening or clinical culture in the country of origin.

Israel also reported cross-border transfer of *K. pneumoniae* with *bla*$_{KPC-3}$ in three studies. One case involved a patient who was transferred to Colombia [120], the second to the UK [27] and the third to Sweden [87]. Evidence of cross-border transmission from USA to Israel, despite the lack of an index case, was supported by the spread of a hyperepidemic clone of *K. pneumoniae* carrying *bla*$_{KPC-3}$ within Israel, with an indistinguishable pulsed-field gel electrophoresis (PFGE) pattern from *K. pneumoniae* isolates which at that time only existed in the USA [37].

Cross-border transmission was also reported from the USA. One isolate was a *K. pneumoniae* harbouring *bla*$_{VIM-1}$ from a patient transferred directly from a hospital in Greece [136]. Three more isolates, a *K. pneumoniae*, an *E. coli* isolate, and an *Enterobacter cloacae* isolate, all carrying *bla*$_{NDM-1,}$ were found in patients who had received recent medical care on the Indian subcontinent [53]. Cross-border transmission of *K. pneumoniae* isolates with *bla*$_{NDM-1}$ from patients who had recently travelled to India was also reported in UK. Although, there was no direct hospital transfer of the cases from UK, 14 of the 17 reported cases had previously been hospitalised on the Indian subcontinent [49]. This indicates that since NDM-1 appears to be endemic in the Indian subcontinent the importation of these strains into UK was due to cross-border transmission, either by patients or by secondary transmission from cases returning from that region.

Germany [137] reported an outbreak involving nine patients caused by *K. pneumoniae* isolate harbouring *bla*$_{KPC-2}$ with an epidemiological link to a previously hospitalised patient from Greece and a bacterial isolate with an indistinguishable PFGE pattern. There was also strong evidence for two *K. pneumoniae* isolates harbouring *bla*$_{KPC-2}$ being imported into Finland. These isolates, identical to isolates from their countries of origin, were imported as a result of direct hospital-to-hospital patient transfer from Italy and Greece respectively [138]. The first isolate of *K. pneumoniae* with *bla*$_{KPC-2}$ reported in Sweden was found in a patient directly transferred from Greece, although no molecular typing was performed. Four *K. pneumoniae* isolates carrying *bla*$_{KPC-2}$ were reported from Norway, two of which were obtained from patients who had undergone direct hospital-to-hospital transfer from Greece, and the other two were from patients who had a remote (<3 months prior) hospitalisation in Greece. All isolates belonged to a clonally related strain from Greece [87].

The only report of CPE importation between healthcare facilities but not across borders was a report identifying *K. pneumoniae* isolates carrying bla_{KPC} in the USA [121].

5.6 Discussion

General

Despite limitations such as heterogeneous study design, differences in the definition of controls and internal bias, the risk factors identified from the nine case-control studies were similar to those found in previous studies on other MDROs [130, 131, 139-142]. These include prior exposure to antimicrobial agents; the presence of indwelling devices; severity of illness; admission to an ICU; transfer between hospital units; previous surgery; prior hospital stay (<1 year prior); recent SOT (solid organ transplant) or SCT (stem cell transplant); presence of wounds; presence of biliary catheter and mechanical ventilation.

Significant risk factors relating to antimicrobial use were prior exposure to antimicrobials: unspecified [14, 125], carbapenems [111, 125, 127, 128], fluoroquinolones [11, 14, 125-127] cephalosporins [11, 111, 127], anti-pseudomonal penicillins [126] and metronidazole [128] (Appendix VI, Table VI.3).

The evidence from the descriptive studies included in this systematic review (see Annex VI, Table VI.4) clearly gives the overall message that transmission of CPE across healthcare facilities and borders is an active process strongly associated with patient transfer. More specifically, strong evidence exists that cross-border transfer of patients is associated with a risk of transmission of CPE when: a) patients are transferred from areas with high rates of CPE to healthcare facilities in another country and b) patients had received medical care abroad in areas with high rates of CPE.

Most of the cases of CPE in this review were transferred across borders from areas where CPE is endemic, such as Greece [45, 143], Israel [28, 37], the north east of USA [144] and the Indian subcontinent [5, 49] into countries which, until that time, had detected very few similar CPE isolates or none at all [27, 53, 136, 138]. Molecular typing by PFGE, further sequencing and the identification of similar genetic contexts of the resistance genes provided proof of clonally related isolates in specific geographical areas [25, 145].

It is important to note that the detection of CPE and CNSE isolates in these studies was only possible as a result of clinical cultures and active screening of these patients. It is therefore evident that, without these crucial actions to identify CPE-positive patients it would have been impossible to halt the introduction of CPE into healthcare settings through infection control measures to prevent clonal spread [85, 86, 120, 121, 135, 137].

Bias and limitations of the analytic studies included

Appendix VI, Table VI.2 presents a summary of internal bias found in the case-control studies included in this review.

Due to the retrospective nature of the case-control studies included (n=9) and differences in defining the control groups, which is a common difficulty in case-control studies for antimicrobial resistance, these studies all contain inherent bias. Wherever the source of bias could not be identified, because of incomplete reporting of adjustments for bias and confounders, the author (AM) assigned a bias value of + on a scale of 0 to ++ (Appendix VI, Table VI.2).

Selection bias

Selection bias was also present in these studies since bias is introduced if random selection is not performed [123]. This has previously been reported in studies for other MDROs [118, 123, 146].

This type of bias can also arise when heterogeneous bacterial species are included in the studies, since their properties can be divergent. This could be the case in the current review since the isolates were *K. pneumoniae* (n=8), *Enterobacter* spp. (n=1) and *E. coli* (n=1).

Information bias

Information bias can arise from the misclassification of infected and colonised patients, rendering cases and controls more divergent and creating greater odds ratios [122, 123, 130]. It can also arise from incomplete data collection due to the retrospective nature of the studies, varying results for Sensitive (S), Intermediate (I), Resistant (R), depending on which clinical breakpoints are applied and the use of different methods for screening and confirmation when identifying isolates as carbapenemase producers. In the studies included (Table VI.2, Appendix VI), the breakpoints used for carbapenem resistance were frequently different and the resistance mechanism was not reported systematically.

5.7 Conclusions

- A limited number of analytic studies with a great deal of heterogeneity have addressed risk factors for colonisation or infection with CPE. Further case-control studies are needed. These studies should be of similar design in order to limit the internal bias so that the identification of risk factors and the estimation of risk can be as accurate as possible.
- Nevertheless the risk factors identified were similar in most studies: prior use of antimicrobials, in particular carbapenems, fluoroquinolones, cephalosporins, anti-pseudomonal penicillins and metronidazole.
- There is strong evidence that cross-border transfer of patients is associated with a risk of CPE transmission when: a) patients are transferred from areas with high rates of CPE to healthcare facilities in another country and b) patients have received medical care abroad in areas with high rates of CPE.
- There are limited data available from the studies on inter-healthcare transmission of CPE within countries, although many imported CPE isolates were responsible for secondary transmission within the healthcare facilities of the destination country.
- In order to avoid publication and ascertainment bias, it is necessary to encourage more active reporting of CPE cases associated with cross-border transfer from all countries in Europe and globally in order to have a complete epidemiological picture of the true risk of CPE spread.

6 Systematic review #2

What is the effectiveness of using screening and/or targeted or other infection control interventions to decrease the incidence of healthcare facility and intensive care unit (ICU)-acquired colonisation or infection with carbapenemase-producing Enterobacteriaceae?

6.1 Background

The generic principles for controlling multidrug-resistant bacteria in healthcare facilities are based on over 100 observational and outbreak studies in hospitals and the general population from several countries. These principles encompass the following elements [113]:

- Healthcare facility organisation and preparedness – including institutional commitment to antibiotic resistance control as a patient safety goal, allocation of structural and personnel resources and epidemic management plans;
- Education of healthcare workers and promotion of best practices for standard precautions;
- Prudent use of antimicrobial agents;
- Surveillance of resistance – including active surveillance of colonisation in high-risk patients with epidemiologically significant, transmissible MDRO;
- Implementation of additional precautions including:
  - Reinforced active surveillance of MDROs
  - Physical cohorting and care cohorting of patients colonised with MDRO
  - Restricted admission or unit closure
  - Environmental decontamination
  - Patient decolonisation.

In both the USA and Europe, guidelines for the control of CPE [81, 89] have recommended the following measures for acute care facilities:

- Routine use of clinical laboratory screening tests for accurate detection of CPE;
- Screening surveys on admission for patients at high risk of CPE colonisation; if positive, continuous active surveillance;
- Contact precautions and, if necessary, cohorting care for CPE-colonised patients.

6.2 Methods

Exclusion and inclusion of abstracts

Two hundred and ninety-four citations were retrieved from the systematic search and two authors (AM and MS) independently reviewed these abstracts to assess whether they should be included. Abstracts were only included if their content was in accordance with the PICO for the question. If the reviewers' opinions differed regarding whether the abstracts met the eligibility criteria, as described in the PICO, this was resolved by discussion.

The full-text documents were then retrieved and detailed exclusion and inclusion criteria were applied by the author of the systematic review (AM) (see criteria below) and the appropriate full-text articles were included in the review.

Excluded studies and the reasons for their exclusion are listed in Appendix VI, Table VI.1.

Exclusion criteria

Full-text articles were excluded if:

- the article was not peer-reviewed;
- the article was not in English, Spanish, Italian, Greek, French or German;
- the outcome(s) of the study was not relevant to the PICO.

Inclusion criteria:

The article was a primary research study on one of the following topics:

- A study of a planned infection control intervention or an outbreak report that described outbreak control measures and results (an intervention study);

- A case report or case series associated with international cross-border transmission and/or outbreaks with description of surveillance and/or infection control measures (a cross-border transmission study).

Data extraction and analysis

Description of included studies

The description and analysis of studies was stratified according to the inclusion criteria above. One author (MS) extracted data on study design, population and setting; primary and secondary objectives and endpoints; intervention, methods and outcomes from the selected studies using a standard form. No attempt was made to contact authors of studies to request additional information.

For intervention studies, 38 items were included in the infection control data extraction form. Three summary tables were prepared on the basis of extracted data and these can be found in Appendix VII. Table VII.1 on study description (eight items), Table VII.2 on quality and risk of bias (nine items) and Table VII.3 on effect of infection control measures (five items).

For cross-border transmission studies, nine items were extracted in order to summarise their findings. No systematic quality appraisal or analysis of the effect of control measures was performed for these studies due to the very limited reporting of methods and outcomes.

Assessment of methodological quality and risk of bias in included studies

For intervention studies, a novel quality appraisal tool consisting of 18 quality items was developed, based on current guidance for reporting and appraisal of observational studies and quasi- experimental studies, with special reference to nosocomial and antimicrobial control intervention studies and outbreak reports [112, 116, 119, 147].

These items included the following elements: study design, source of funding and authors' interests; description of primary and secondary objectives; population; epidemiological setting; baseline infection control and intervention; monitoring of compliance with baseline and intervention measures; study endpoints; case definition and incidence metrics; case detection methods; statistical analysis methods; sample size and duration of follow-up; appropriate time series data description and analysis; estimated size of effect and likely presence of selection bias, detection bias and confounding bias. Potential confounders checked for assessment or control were: compliance with standard infection control precautions, including hand hygiene; healthcare worker to patient staffing ratio; antibiotic use; patient case-mix and length of hospital stay; colonisation pressure and regression to the mean.

From these elements, the findings of eight items of key relevance to the quality of selected studies were summarised in Appendix VII, Table VII.2 and a summary internal validity quality score was given to each study, with three levels indicating the decreasing likelihood of bias explaining the observed effect (0,+,++).

Summary measures of effect in individual studies

For intervention studies, the following measures of effect were extracted and summarised in Appendix VII, Table VII.3: outcome metric, comparison of incidence rates at baseline and during intervention and duration of intervention follow-up. We had intended to analyse the risk ratio estimate (RR and 95% confidence interval) of preventive effects associated with intervention(s), but the data necessary for calculating this estimate were available in only a minority of studies.

Overall strength and volume of evidence

We anticipated that only a limited number of studies containing heterogeneous interventions would be available and that the study design would be low level of quality design, thereby precluding any meaningful meta-analysis of effect. We therefore planned a narrative summary of the overall strength and volume of evidence supporting the potential effectiveness of interventions.

6.3 Results

Study selection

Fifty-two abstracts were included by the two reviewers. Full-text articles were obtained and the exclusion/inclusion criteria applied. Thirty-two full-text articles were excluded and 20 were tentatively included for data extraction. While data was being extracted for these studies, a further four outbreak studies were excluded because of the lack of relevant outcome data. Thus, the final number of studies included in the systematic review was 16, eight of which were intervention or outbreak control studies [124, 137, 148-153] and eight cross-border transmission reports [83, 85-87, 120, 135, 138, 154]. Detailed reasons for exclusion can be found in Appendix VI, Table VI.1.

Description of studies

The 16 studies included are presented in Appendix VII, Table VII.1 (eight intervention studies) and Appendix VII, Table VII.4 (eight cross-border transmission studies).

Intervention studies

The studies were written in English and came from four countries: Australia (n=1), Germany (n=1), Israel (n=1) and USA (n=5) (Appendix VII, Table VII.1). One study was a retrospective quasi-experimental planned intervention study and seven were retrospective outbreak reports, with uncontrolled before-after study of control outcome. There was no controlled trial, controlled before-after study or prospective planned intervention study with interrupted-time series.

Etiologic agents included *K. pneumoniae* (n=7 studies) and multiple species of Gram-negative bacteria (n=1). Carbapenemases included KPC (n=5 studies), IMP (n=1) and undefined carbapenem resistance mechanism (n=1). These were single-centre studies conducted in an ICU (n=3), a tertiary care teaching hospital (n=3) and a long-term acute care hospital (n=2). The epidemiological setting was an endemic unit from an endemic region (n=1), a hospital outbreak in endemic region (n=6) and a hospital outbreak in non-endemic region (n=2).

The primary aim of the study was to describe an outbreak (n=5 studies) or to assess the effect of control measures (n=3). Interventions were based on a combination of two to seven infection control measures which were implemented simultaneously (n=7 studies) or sequentially (n=1). These included: active surveillance culture (n=7 studies); daily internal reporting and external notification of new CPE patients (n=2); contact precautions for CPE patients (n=4); universal contact precautions (n=1); pre-emptive isolation with contact precautions of high-risk patients (n=1); single room isolation of CPE patients (n=2); physical cohorting of CPE patients (n=1); cohorted nursing care of CPE patients (n=5); computer alert of CPE patient re-admission (n=1); antibiotic restriction (n=1); ICU closure (n=1); promotion of hand hygiene (n=1); promotion of environmental surface decontamination (n=3); patient decolonisation with antiseptic bathing (n=2) and healthcare staff education (n=3).

Methodological quality and risk of bias in infection control studies.

Appendix VII, Table VII.2 summarises the quality of design, conduct and reporting for the studies included, as well providing an assessment of the risk of bias. Overall, these studies were retrospective, uncontrolled observational studies and outbreak reports which are, by design, subject to a high risk of bias and difficult to adjust for natural fluctuations of incidence over time. No study conformed completely with current methodological and reporting guidelines for this type of observational study [112, 147]. Two of them [149, 152] provided an adequate level of completeness and clarity in reporting interventions and one [152] used an appropriate time series analysis of effect adjusted for colonisation pressure. Other studies either did not use any statistical analysis (n=5), which may be appropriate for small outbreaks and short follow-up periods, or used inappropriate methods (n=2) that failed to account for time and person dependence of communicable disease risk.

In addition, no study monitored adherence to measures, which is recognised as a major bottleneck for all clinical quality improvement studies. The risk of selection bias was generally low in five studies that included all consecutive admissions to a given facility over limited time periods, ranging from three months to four years. In contrast, potential detection bias was present in six studies that used non-comparable intensity and accuracy of case detection in before-after intervention periods (Appendix VII, Table VII.2). Similarly, only two studies addressed potential confounders (colonisation pressure, antibiotic use and length of stay) in the data analysis, while no study controlled or adjusted for other relevant confounders (compliance with standard infection control precautions, including hand hygiene, healthcare worker to patient staffing ratio and patient case-mix).

Effects of infection control measures

Appendix VII, Table VII.3 presents a summary of outcome measures and size of effect reported in the infection control studies. The outcome metric used was a case count per period (n=5), the monthly or quarterly incidence of nosocomial acquisition per hospital patient-days (n=2) and point prevalence in a cross-sectional survey (n=1). The follow-up period ranged from six weeks to 20 months. All measures were reported as successful in reducing the occurrence or incidence of epidemic or endemic CPE cases, with three studies indicating a statistically significant effect, one of which was based on appropriate methods. The size of effect, where evaluable, ranged from 100% relative risk reduction, or complete outbreak eradication (n=2) to 62% risk reduction in reducing endemic transmission (n=1).

It is impossible to compare the size of effect by intervention type due to the limited number of studies, the complex nature of interventions and the heterogeneity of endpoints and methods. Nevertheless, it is worth noting that most interventions (n=6) combined at least active surveillance cultures and control measures targeted at CPE colonised patients, such as contact precautions and/or cohort nursing.

6.4 Cross-border transmission reports with information on surveillance and/or control measures

These reports were written in English. A total of 18 cases were included relating to international importation of CPE-colonised or -infected patients after hospitalisation in another country with ongoing epidemics of CPE (Appendix VII, Table VII.4). Reports were from seven countries: Belgium (n=1), Colombia (n=1), Denmark (n=1), Finland (n=1), France (n=2), Norway and Sweden (n=1). Countries of origin included Greece (n=7), Israel (n=2) and Italy (n=1). The design was a retrospective case series with detailed microbiological investigations but scant description of surveillance and control measures.

All reports concerned *K. pneumoniae* producing KPC (n=7 studies) or VIM (n=1). These were single-centre studies (n=5) or multicentre studies (n=3) in ICUs, organ transplant and surgery units and tertiary care hospitals.

The primary aim of the studies was to describe cases of cross-border importation of CPE. In six of eight studies there was strong evidence of importation by direct transfer of patient from a foreign hospital, and concordant molecular typing with epidemic strain or genetic context of the resistance gene from the country of origin. In 15 of 18 cases, CPE was detected within a few days of admission, either from clinical cultures of active infection present on admission or by admission surveillance (rectal culture) for multi-drug resistant Gram-negative bacilli (n=7 studies). Infection control precautions were unclear but appeared to include pre-emptive contact precautions or contact precautions for detected CPE patients in at least two to four studies. Four studies reported contact tracing by means of surveillance cultures for contact patients sharing the same healthcare personnel or invasive device. Five studies reported secondary nosocomial transmission to a further two to 84 patients associated with cross-infection and endoscope contamination. These secondary outbreaks affected the receiving hospital unit (n=2 studies), the hospital (n=1) or several regional hospitals (n=1).

Due to insufficient data provided on surveillance and infection control measures, it is impossible to compare the risk of nosocomial transmission following an index case of importation into a non-endemic hospital according to type of surveillance or control measures.

6.5 Summary of findings

We did not identify any trials or controlled intervention studies, only a limited number of observational studies of CPE outbreaks (n=8) and endemic transmission control measures (n=1) in acute healthcare facilities. The results offered suggestive and consistent evidence of the effectiveness of combined interventions, including active surveillance culture for early detection of CPE-colonised patients, contact precautions and cohort nursing care for CPE-colonised patients. The evidence is more difficult to interpret for other measures such as antibiotic restriction; promotion of hand hygiene and environmental surface decontamination; patient decolonisation with antiseptic bathing and healthcare staff education. These measures have not been investigated to the same extent and only in combination with targeted precautions.

However, the substantial risk of bias in the studies available limits the strength of evidence supporting these interventions. There was considerable variation in the magnitude of effect, with eradication of transmission observed only in a minority of small-size outbreaks, and more limited reduction of transmission in relation to large outbreaks in endemic regions. This supports the empirical concept that early initiation of surveillance and control measures is more effective in eradicating CPE transmission. However, the majority of these studies were conducted in tertiary care centres in endemic regions, particularly in the USA. It is uncertain whether the study findings can be generalised for other healthcare facilities in non-endemic countries

The studies of cases associated with patient cross-border transfer from hospitals in endemic countries to hospitals in non-endemic countries illustrate the risk of secondary transmission within the receiving facility and in regional hospitals by shared healthcare workers or invasive procedures. This risk is present even in facilities where active surveillance screening of transfer from foreign hospital is performed. Insufficient data on surveillance and control procedures or the heterogeneity of contact surveillance intensity across studies makes it impossible to analyse the determinants of effective prevention of secondary transmission in this context.

6.6 Conclusions

- To date there is only limited evidence available on the effectiveness of infection control measures for the prevention and control of nosocomial CPE transmission in acute healthcare facilities and no evidence as regards to other healthcare settings.
- The low-grade evidence available, derived from outbreaks in acute care hospitals, consistently supports the effectiveness of early, active surveillance for CPE carriage by rectal screening and additional precautions for the care of CPE-positive patients, including wearing disposable gloves and gown and cohort nursing by a separate, dedicated team.
- Other non-targeted infection control measures may be beneficial but the evidence supporting their effectiveness is less clear due to even less data being available.

- The evidence of effective containment of secondary transmission following cross-border CPE transmission through patient transfer between acute care facilities is unclear due to incomplete reporting of infection control management and outcome in the case series available.
- There is a need to improve the design of studies on the benefit and harm of infection control measures to prevent and control nosocomial CPE transmission. This applies to acute care and other healthcare facilities in endemic and non-endemic regions, including in the context of cross-border care.

7 Systematic review #3:
What is the clinical diagnostic accuracy of tests for screening and confirmation of carbapenemase-producing Enterobacteriaceae?

7.1 General

The third systematic review was performed in parallel with the other two reviews included in this risk assessment. After much deliberation ECDC decided that, although it had been presented to the expert group for feedback, this third systematic review would not be included in the risk assessment and would possibly be addressed at a later date.

The PICO for this question was used in the overall literature search strategy, as presented in Figure 1 and Appendices I-IV. Information on the methodology and the results of abstract and full-text selection are set out in this section.

7.2 Methods

Exclusion and inclusion of abstracts

Two hundred and ninety-four citations were retrieved from the systematic search and two authors (AM and MS) independently reviewed these abstracts to assess whether they should be included. Abstracts were included only if their content was in accordance with the PICO for this question. If the reviewers' opinions differed regarding whether the abstracts met the eligibility criteria, this was resolved through discussion. The full-text documents were then retrieved, detailed inclusion and exclusion criteria were applied by the author of the review (AJ) (see criteria below) and the appropriate full-text articles included.

Exclusion criteria:

Full-text articles were excluded if:

- the article was not peer-reviewed;
- the article was not in English, Spanish, Italian, Greek, French or German;
- the outcome(s) of the study was not relevant to the PICO.

Inclusion criteria:

- All studies had to report sensitivity and specificity (otherwise they were not included);
- Studies that evaluated in vitro detection of carbapenem resistance or carbapenemase activity in CPE isolates, with mechanistic and antimicrobial susceptibility testing, including:
  - Identification of CPE using phenotypic tests;
  - Identification of genes encoding for production of carbapenemase in Enterobacteriaceae;
- Studies that evaluated 'rapid screening' (active surveillance) for detection of CPE-colonised patients;
  - Identification of CPE using phenotypic tests.

7.3 Results

Study selection

Ninety abstracts were included by the two reviewers. Full-text articles were obtained and the author of this systematic review (AJ) applied the exclusion and inclusion criteria to the full text articles (see Figure 1 for flowchart). Sixty-seven full-text articles were excluded and 23 included. When data were abstracted from these studies, an additional four were excluded by the author (AJ); three because they were incompatible with the PICO for the question and one because it did not report on sensitivity and specificity (one of the minimal inclusion criteria). In total, twenty full-text articles were included in the systematic review. Details of the reasons for exclusion can be found in Appendix V, Table V.1

8 Contributions from the expert meeting

Background

On 24 November 2010, a group of ten experts on infectious diseases, infection control, public health and microbiology were invited to a meeting in Stockholm, Sweden. Prior to this meeting, the three systematic reviews had been sent to the experts via e-mail for review. The purpose of this one-day meeting was to obtain feedback from the experts on the systematic reviews performed by ECDC.

The experts were reminded that the results of the systematic reviews were based on a complete literature review, performed after following a complete search strategy and that strict criteria had been applied for exclusion and inclusion of studies. Only peer-reviewed and original studies had been included. Conference proceedings and abstracts were therefore, by default, ineligible and were not considered in the systematic reviews. The studies included were considered to be the 'best available' evidence to date.

8.1 Specific comments and expert feedback on the systematic reviews

Cross-border transmission of CPE and bias

The experts agreed with the conclusions of the systematic reviews that there was strong evidence from the descriptive studies indicating that transfer of patients across borders between healthcare facilities is a risk factor for the transmission and spread of CPE.

The experts, however, suggested that caution should be exercised when creating a final epidemiological picture of Europe using only the included studies, because of the presence of publication bias. The true extent of the reservoir of CPE in all EU Member States is unknown and our current knowledge suffers from publication bias, reporting bias and ascertainment bias. In addition, although emergence of CPE has been reported in many European countries, it has not always been associated with evidence of cross-border transfer. Cross-border transfer of patients could have been the original source of CPE in these countries. However, the lack of epidemiological information from regions neighbouring the European Union (e.g. some countries in the Balkan region, Turkey and North Africa) should be taken into account, as these regions could be a significant source of CPE.

Were relevant descriptive studies missed?

The experts were asked whether there were any studies that had been missed during the systematic search. Obviously, studies published after the cut-off date of the third week of August 2010 were not included in the systematic reviews. A good example of publication bias is the lack of published cases referring to cross-border transfer of OXA-48, an important carbapenemase. Many countries already had data on cross-border transfer of OXA-48, but these had not yet been published by the cut-off date and were therefore not retrieved by our systematic search. Ascertainment bias may also have accounted for the lack of studies on cross-border transfer of OXA-48, since it can be difficult to detect in the microbiology laboratory.

Suggested additional studies

Two such studies meeting the criteria for the inclusion of descriptive studies but published after the cut-off date, are listed below, although they could not be formally added to the systematic review:

Goren MG, Chmelnitsky I, Carmeli Y, Navon-Venezia S. Plasmid-encoded OXA-48 carbapenemase in *Escherichia coli* from Israel. J Antimicrob Chemother 2011;66(3):672-3 [68];

Samuelsen Ø, Thilesen CM, Heggelund L, Vada AN, Kümmel A, Sundsfjord A. Identification of NDM-1-producing *Enterobacteriaceae* in Norway. J AntimicrobChemother 2011;66(3):670-2 [155].

8.2 Recommendations from experts to ensure better data reporting in Europe

- To assess the true prevalence of CPE in Europe, sound epidemiological surveillance data must be available from each European country.
- Countries should be encouraged to actively report cases of CPE by making all clinical cases notifiable to public health authorities, keeping in mind that reporting and publication will be subject to delay and that the true epidemiological picture may not be reflected at any given moment. Issues of awareness, lack of resources, laboratory capacity and unwillingness to report data may limit the implementation of such a system.

- It would also be helpful to encourage the ECDC National Contact Points for Antimicrobial Resistance (AMR Surveillance and the National AMR Focal Points to collect data from outbreaks in their countries, since much of this information is not published.
- Implementing structures that would aid in the notification of such cases at an early stage, such as the Epidemic Intelligence System (EPIS) and an Early Warning System (EWRS) already being used successfully in some countries (e.g. France), would be useful tools for the whole of Europe.
- Regular surveys to collect data on the prevalence of CPE in Europe should be considered.
- The classification scheme for assessing the stages of CPE expansion nationwide, as used by Grundmann, et al. in their article [40] may prove useful to evaluate the prevalence of CPE within Europe and for future risk assessments, both at national and European level.

What are the key issues creating bias in case-control studies for risk factors and how should the outcomes be presented?

Infection vs. colonisation

The majority of the case-control studies included did not clearly state whether they were reporting clinical cases or colonisation. This was a problem frequently encountered in the literature and during the meeting and it was noted as an action point for improvement in future studies.

Eliminating bias from the included case-control studies

In order to eliminate internal bias, ensure more accurate outcome measures and perform a meta-analysis, the experts suggested separating the case-control studies a) by case types and control groups included (e.g. patients with resistant vs. sensitive bacterial isolates), b) according to whether checks had been made on time-at-risk and c) whether checks had been made for confounding.

Breakpoints in included studies and misclassification bias

The experts cautioned that the Clinical Laboratory Standards Institute (CLSI) [92] had recently lowered their interpretive criteria for the carbapenems and many of the studies used different interpretive criteria. This resulted in considerable variability in the use of breakpoints which would create misclassification bias. An additional suggestion was to include a statement in the risk assessment that 'not all carbapenemase-producing organisms are carbapenem-resistant'.

Recommendations from experts on how to eliminate bias in case-control studies

- Highlight the need for analytic studies that clearly identify whether the bacterial isolates represent clinical cases or colonisation.
- Design case-control studies looking at any risk factor associated with CPE transmission into European healthcare facilities, including all types of population mobility;
- Specify quality indicators for case-control studies that will eliminate bias to the extent possible and construct a guidance document outlining these indicators, as the ORION statement does for infection control [112].

8.3 Other recommendations from the expert group

Prudent use of antimicrobials and antimicrobial resistance

Exposure to antimicrobials, particularly to the third and fourth generation cephalosporins and the carbapenems, are risk factors for infection and/or colonisation with CPE. It is important that both clinical and public health professionals realise that the recent emergence of CPE, although extremely alarming, should not come as a surprise. The evolution of clinically relevant β-lactamases follows a trajectory dictated by the misuse and overuse of several generations of β-lactam antibiotics over the last 40–50 years. This destructive pattern can only be broken if decisive action is taken in the areas of prudent antibiotic use and infection control. Furthermore, high levels of multidrug-resistant bacteria (e.g. those that produce ESBLs) represent an indirect risk for the spread of carbapenem resistance mechanisms because they are associated with an increase in the prescription of carbapenem antibiotics.

Recommendations from experts on prudent use of antimicrobials

- Recognise the importance of antibiotic selection pressure and the emergence and spread of resistance determinants.
- Control CPEs and all other MDROs (e.g. ESBL-producing organisms) to prevent the overuse of antibiotics, especially the carbapenems.

Curbing the spread of CPE into healthcare facilities due to cross-border patient transfer

Active screening of patients

In Europe and globally, many countries have created and implemented national and/or local policies for the active screening of patients who are admitted or transferred to healthcare facilities (Table 1). These guidance documents are frequently created from previous guidance documents, the best available evidence, the grey literature and expert opinion.

Important questions in relation to the active screening of patients entering a healthcare facility are: Who should be screened? Should there be lists of countries from which, when patients are transferred, active screening should be performed? In fact, such lists had been created by some EU countries, recommending the screening of all patients transferred from areas with a high prevalence of CPE. However, the lists are becoming obsolete, since it is evident that patients from all countries are at risk as the true prevalence and magnitude of the CPE reservoir in Europe and globally is unknown.

Strict active screening of all patients who come from a healthcare system in any foreign country is a strategy already being implemented in countries such as France, Norway, Denmark, Sweden and Israel.

Recommendations from experts on the active screening of patients and infection control to prevent CPE spread with cross-border transfer of patients.

- All countries should develop local guidance documents recommending the active screening of any patient transferred from a healthcare facility in a foreign country.
- ECDC should develop infection control guidance relating to the transmission of CPE into healthcare facilities as a result of cross-border patient transfer.
- The contents of all national guidance documents for control and surveillance of CPE, listed in Table 1, should be compared.
- The experts all agreed that some guidance for infection control should be included in the current ECDC risk assessment. Recommendations were to base it on the limited evidence revealed by the systematic review, to rely heavily on expert opinion and to include content from current guidance documents on the prevention of MDRO transmission in acute healthcare facilities.

Long-term healthcare facilities (LTCFs)

There is lack of data on the prevalence and magnitude of the CPE reservoir in LTCFs since most data on CPE in Europe comes from acute care facilities.

Some countries, such as Israel, have collected data on infection control measures in LTCFs. Implementation of specific infection control measures, such as active screening of new admissions, enforcement of standard precautions (not contact precautions unless the patients were incontinent of stool or were receiving antimicrobial therapy) and cohorting in a long-term healthcare facility, decreased the average colonisation from 12–8.2% within a year.

To further complicate matters, there is no uniform definition for LTCFs within Europe and as a result, it would be difficult to create recommendations for them. If necessary, the required data could be obtained in the first instance from the ECDC European Point Prevalence Survey or the Healthcare-Associated Infections in LTCFs (HALT) study. However, the final decision from the expert group and ECDC was not to include LTCFs in this risk assessment.

9 Final considerations for assessment of risk and provision of guidance on prevention and control

The purpose of this ECDC risk assessment is two-fold. Firstly, to present evidence on the risk of colonisation or infection with CPE, including the risk of CPE importation into healthcare facilities through cross-border patient transfer. The second purpose was to provide guidance on controlling the spread of CPE, by combining the conclusions of the systematic reviews, the expert opinion and recommendations from key guidance infection control documents.

9.1 Points included from expert opinion

9.1.1 Expert opinion on risk factors

The experts concurred with the conclusions that, despite the publication bias of the descriptive studies included, there is strong evidence that cross-border transfer of patients across healthcare facilities is a risk factor for the transmission and spread of CPE into healthcare settings.

The experts stressed the need to obtain reliable data on the epidemiology of CPE in Europe and to encourage countries to actively report cases of CPE. Recommendations by the expert group were to achieve this by making all clinical cases notifiable to public health authorities, or establishing a European network to count cases/outbreaks of CPE infection, implement electronic early warning platforms, such as the Epidemic Intelligence System (EPIS) [156] or the Early Warning and Response System (EWRS), and perform surveys on a regular basis to collect data on CPE prevalence in Europe.

The expert group also agreed with all other risk factors identified by the systematic review, i.e. the prior use of antimicrobial agents, immunosuppression and invasive devices, since most of these have been previously associated with colonisation or infection with other MDROs. In addition, the group stressed the importance of recognising that antimicrobial pressure is associated with the emergence and spread of resistance determinants.

It is imperative to control not only the misuse of antimicrobial agents, but also patient-to-patient spread of MDROs in general (e.g. ESBL-producing Enterobacteriaceae), since their presence encourages the use of antimicrobials, especially the carbapenems, and results in an interminable cycle of antimicrobial use, antimicrobial pressure and high rates of antimicrobial resistance. Decisive action is needed to promote the prudent use of antimicrobial agents.

9.1.2 Expert opinion on how to control CPE spread in healthcare facilities

The experts agreed that CPEs should behave similarly to other MDROs and that generally, the infection control measures effective for other MDROs should also be used to control the spread of CPEs in healthcare settings. This was based on their expert opinion in infection control and on guidelines for other MDROs. Use of standard precautions, and especially adherence to hand-hygiene policies, is essential for preventing transmission of MDROs in healthcare settings. Colonisation and/or infection of patients with these organisms can be known or unknown and it is therefore of paramount importance that healthcare workers strictly adhere to basic hand hygiene policies during patient care [89, 113] to prevent cross-infection.

However, the experts also concurred with the findings from the second systematic review, that there are limited reliable data currently available to support the effectiveness of infection control measures in curbing the spread of CPE in healthcare facilities and that further studies are needed. The experts supported the conclusions of the systematic review, stating that the following infection control are effective: active screening of all high-risk patients, use of additional contact precautions and dedicated staff/cohort nursing for all isolated patients who were confirmed carriers of CPE.

9.1.3 Expert opinion on active screening

Identifying high-risk patients and performing active surveillance by rectal screening of any patient transferred from a healthcare facility in another country is essential in preventing introduction and transmission of CPE.

In assessing which patients are considered high-risk the experts stated that, since any country can be considered a source of CPE, patients from any country should be considered high-risk and should be screened. Furthermore,

all countries should develop guidance documents with recommendations for active screening of all patients transferred from a healthcare facility abroad.

9.1.4 Expert opinion on the importance of timely detection and confirmation

The experts highlighted the fact that detection, diagnosis and confirmation of the presence of carbapenemases is important for surveillance, infection control and treatment purposes. Ideally, detection of carbapenemases for active screening purposes should have a fast turnaround time and be available at the point of care to ensure timely implementation of infection control measures in order to effectively prevent spread. Confirming the presence of carbapenemases is also important, but this is often only possible at reference laboratories. Consequently, locally performed phenotypic tests can be extremely helpful, especially for clinical specimens. These tests can prove even more useful if they are interpreted in conjunction with data on the background prevalence of CPE in a specific region. Fast diagnostic turnaround time and timely communication of laboratory results to physicians, nurses and the infection control team are extremely important for infection control and clinical therapy.

9.2 Recommendations included from published guidance documents

Due to the growing and ongoing threat of MDRO transmission in healthcare facilities, the medical community has issued some key documents containing recommendations and describing processes to prevent spread of MDROs [113] and, more specifically, CPE [81, 88, 89] in acute healthcare facilities. Preventing transmission of these microorganisms in acute healthcare facilities is vital in terms of patient safety since MDRO infections are associated with increased morbidity, mortality, length of hospitalisation and healthcare costs [13, 15, 17, 20, 111]. It is also imperative to prevent horizontal transmission of mobile genetic elements to other microorganisms.

Overall, the recommendations suggest implementing comprehensive, multifaceted infection control programmes, with well-defined structures and processes, and continuous evaluation of implemented measures [81, 113]. Recommendations from these documents are based on strong and weak evidence from the literature, but also rely heavily on expert opinion due to the scarcity of data available on the effectiveness of measures.

9.2.1 Laboratory detection

It is important to have a local microbiological laboratory that can perform highly-sensitive tests with a rapid diagnostic turnaround time for active screening. Prompt notification and timely communication of laboratory results to physicians, nurses and the infection prevention team are important for infection control and clinical therapy. Confirmatory tests (phenotypic tests) for the presence of carbapenemases are widely available and easy to implement for most laboratories, provided that the resources are available and laboratory staff have been trained. If these tests can be performed at local laboratories, it would allow a fast turnaround time for the diagnosis and confirmation of CPE. If this is not possible at the local level, isolates should be sent to reference laboratories, however this will result in significant delays in the confirmation of carbapenemases, which may ultimately hamper the implementation of infection control measures. It is strongly recommended that all confirmed clinical cases be notifiable to the public health authorities [40, 50, 81, 89, 113, 114, 157].

9.2.2 Infection control measures

Infection control measures to prevent the spread of CPE in acute healthcare facilities following importation and during CPE outbreaks include the following:

Screening

Identifying patients who are at high risk of colonisation or infection with MDROs (this includes CPE) and performing active screening by rectal swab on admission to healthcare facilities has been strongly advocated and this practice is now becoming more widespread in healthcare settings [50, 81, 89, 113, 114, 157]. The implementation of more extensive active surveillance during outbreaks has also been recommended (e.g. follow-up surveillance at regular time intervals and/or for all contacts with confirmed cases).

Additional measures for infection control

In guidance documents, the most commonly recommended infection control measures for reducing transmission are: pre-emptive isolation of high-risk patients pending the results of active surveillance cultures; isolation or cohorting of all patients with suspected or confirmed CPE colonisation/infection; use of contact precautions for all isolated patients; having dedicated staff and cohort nursing for all isolated patients who are CPE carriers; focused efforts to promote the prudent use of antimicrobial agents and a system for monitoring compliance with all the aforementioned measures [50, 81, 89, 113, 114, 157].

Annex 1. Original search strategy 2010

Methods used to perform the systematic search

Electronic searches

The following electronic databases were searched for all relevant studies. Limits to language were placed for all three questions at the end of the search, for Questions 1 and 2 limits for 'human' were also placed.

- Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2010)
- Ovid MEDLINE (2000 to the third week of August 2010)
- PubMed (2000 to the third week of August 2010)
- Ovid EMBASE (2000 to the third week of August 2010)
- Health Technology Assessment (HTA) website (1988 to August 2010).

Annex 2. Ovid MEDLINE search strategy

1 carbapenemase.mp. (473)
2 ((carbapenem* or meropenem* or ertapenem* or imipenem*) and resist*).ti,ab. (5411)
3 (ndm or kpc or vim or mbl or oxa 48 or metallo beta lactamase).ti,ab. (3831)
4 1 or 2 or 3 (8745)
5 enterobacteriaceae/ or exp citrobacter/ or exp enterobacter/ or exp escherichia/ or exp hafnia/ or exp klebsiella/ or kluyvera/ or exp morganella/ or exp proteus/ or providencia/ or exp serratia/ (237100)
6 (enterobacter* or klebsiella or citrobact* or escherichia or hafnia or morganell* or proteus or serratia).ti,ab. (213053)
7 5 or 6 (303132)
8 4 and 7 (2430)

Risk factors

9 exp risk/ or exp cross infection/ or disease outbreaks/ or carrier state/ or community-acquired infections/ (726275)
10 (comorbid* or co-morbid* or case-control stud*).ti,ab. (92948)
11 (risk factor* or outbreak* or patient transfer* or patient colonization* or patient colonisation* or cross infect* or hospitalis* or hospitaliz* or carrier*).ti,ab. (491848)
12 case-control studies/ or exp hospitalization/ (249941)
13 9 or 10 or 11 or 12 (1210873)
14 8 and 13 (695)
15 limit 14 to (humans and yr=’2000–Current’) (528)

Infection control

16 exp infection control/ or handwashing/ or exp protective clothing/ (53042)
17 (infection control* or precaution* or surveillan* or patient isolat* or barrier* or cohorting).ti,ab. (205618)
18 (handwash* or cloth* or glove* or mask* or face shield* or protective measure* or pre-emptive isolat* or protective device* or patient cohort* or preemptive isolat*).ti,ab. (63048)
19 16 or 17 or 18 (307495)
20 8 and 19 (437)
21 limit 20 to (humans and yr=’2000–Current’) (319)

Diagnostic accuracy

22 (screening or rectal swab* or surveillanc*).ti,ab. or rectum/mi (313699)
23 8 and 22 (385)
24 limit 23 to (humans and yr=’2000–Current’) (283)

Annex 3. Pubmed search strategy

#1	Search (Enterobacteriaceae[MeSH Terms] OR Enterobacteriaceae Infections[MeSH Terms] OR enterobacter*[tiab] OR Klebsiella[tiab]) AND (carbapenem* OR VIM OR OXA-48 OR ndm OR MBL OR IMP OR imipenem OR meropenem OR ertapenem OR doripenem OR biapenem OR panipenem OR pz-601 OR β-Lactamases[MeSH Terms])	8448
#2	Search #1 NOT acinetobacter NOT pseudomonas NOT shigella NOT salmonella NOT Yersinia	5856
#3	Search ESBL[ti] OR ESBLs[ti] OR ESBe*[ti] OR extended[ti]	16110
#4	Search #2 NOT #3	4521

Risk factors

#5	Search #4 AND (cross infection OR nosocom* OR risk factors OR spread OR outbreak OR transmission OR transfer OR safety OR ventilat* OR catheter* OR carrier* OR carriage OR hospitali* OR colonis* OR coloniz* OR environment* OR emerging OR emergence)	1248

Infection control

#6	Search #4 AND (precaution* OR prevention OR control OR Communicable Disease Control OR cohorting OR surveillance OR intervention OR interventions OR incidence OR hygiene OR handwashing OR protection OR protective OR Protective Clothing OR gloves OR Gloves, Protective OR mask OR masks)	974

Screening

#7	Search #4 AND (screening OR surveillance OR diagnosis OR specimen handling OR rectal OR rectum OR faecal OR faeces OR fecal OR feces)	986

Diagnostic accuracy

#8	Search #4 AND (test OR tests OR testing OR screening OR molecular diagnostic techniques OR microbiological techniques OR 'clover leaf' OR cloverleaf OR hodge OR boronic OR phenylboronic OR PCR OR EDTA OR PBA OR chromogen* OR culture media OR microbial sensitivity tests OR 'double disk' OR breakpoint*) AND (accuracy OR reliability OR reliance OR detection OR detect OR confirmation OR confirm OR sensitivity and specificity OR validity OR validation OR identif* OR evaluat* OR reproducibility of results)	890
#9	Search #5 OR #6 OR #7 OR #8 Limits: Publication Date from 2000	1395
#10	Search #5 Limits: Humans, publication date from 2000	604
#11	Search #6 Limits: Humans, publication date from 2000	509
#12	Search #7 Limits: Humans, publication date from 2000	545
#13	Search #10 OR #11 OR #12 Limits: Humans, publication date from 2000	824
#14	Search #8 NOT #13 Limits: publication date from 2000	296

Annex 4. EMBASE.COM search strategy

Search query

Risk factors

☐	#21 #20 NOT #6 AND [2000-2011]/py	577
☐	#20 #6 NOT ('drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis OR 'warfarin'/exp OR warfarin:ab,ti OR 'hiv'/exp OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [embase]/lim AND [2000-2011]/py	1,971

Diagnostic accuracy

☐	#19 #17 NOT #18 AND [2000-2011]/py	313
☐	#18 #4 AND (test:ab,ti OR tests:ab,ti OR testing:ab,ti OR screening:ab,ti OR 'clover leaf':ab,ti OR cloverleaf:ab,ti OR hodge:ab,ti OR boronic:ab,ti OR pcr:ab,ti OR edta:ab,ti OR pba:ab,ti OR 'double disk':ab,ti OR chromogen*:ab,ti OR (diagnostic AND procedure OR microbiological AND 'examination'/exp OR culture AND medium)) AND (accuracy:ab,ti OR reliability:ab,ti OR reliance:ab,ti OR detection:ab,ti OR detect:ab,ti OR confirmation:ab,ti OR confirm:ab,ti OR sensitivity:ab,ti AND specificity:ab,ti OR validity:ab,ti OR validation:ab,ti OR identif*:ab,ti OR evaluat*:ab,ti OR reproducibility:ab,ti) AND [medline]/lim AND [2000-2011]/py	418
☐	#17 #4 AND (test:ab,ti OR tests:ab,ti OR testing:ab,ti OR screening:ab,ti OR 'clover leaf':ab,ti OR cloverleaf:ab,ti OR hodge:ab,ti OR boronic:ab,ti OR pcr:ab,ti OR edta:ab,ti OR pba:ab,ti OR 'double disk':ab,ti OR chromogen*:ab,ti OR (diagnostic AND procedure OR microbiological AND 'examination'/exp OR culture AND medium)) AND (accuracy:ab,ti OR reliability:ab,ti OR reliance:ab,ti OR detection:ab,ti OR detect:ab,ti OR confirmation:ab,ti OR confirm:ab,ti OR sensitivity:ab,ti AND specificity:ab,ti OR validity:ab,ti OR validation:ab,ti OR identif*:ab,ti OR evaluat*:ab,ti OR reproducibility:ab,ti) AND [2000-2011]/py	731

Screening

☐	#16 #15 NOT (immunodeficien*:ab,ti OR immunocompromi*:ab,ti OR transfusion:ab,ti OR 'drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity:ab,ti OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis:ab,ti OR 'warfarin'/exp OR warfarin:ab,ti OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [2000-2011]/py	212
☐	#15 #13 NOT #14 AND [2000-2011]/py	249
☐	#14 #4 AND (screening:ab,ti OR surveillance:ab,ti OR diagnos*:ab,ti OR swab*:ab,ti OR specimen:ab,ti OR rectal:ab,ti OR rectum:ab,ti OR stool:ab,ti OR faecal:ab,ti OR fecal:ab,ti OR faeces:ab,ti OR feces:ab,ti) AND [humans]/lim AND [medline]/lim AND [2000-2011]/py	564
☐	#13 #4 AND (screening:ab,ti OR surveillance:ab,ti OR diagnos*:ab,ti OR swab*:ab,ti OR specimen:ab,ti OR rectal:ab,ti OR rectum:ab,ti OR stool:ab,ti	813

OR faecal:ab,ti OR fecal:ab,ti OR faeces:ab,ti OR feces:ab,ti) AND [humans]/lim AND [2000-2011]/py

Infection control

☐	#12 #9 NOT #8 AND [2000-2011]/py	212
☐	#11 #7 NOT #10 AND [2000-2011]/py	227
☐	#10 #4 AND (precaution*:ab,ti OR prevention:ab,ti OR control:ab,ti OR cohorting:ab,ti OR surveillance:ab,ti OR intervention:ab,ti OR interventions:ab,ti OR incidence:ab,ti OR hygiene:ab,ti OR handwashing:ab,ti OR protection:ab,ti OR protective:ab,ti OR gloves:ab,ti OR mask:ab,ti OR masks:ab,ti) AND [humans]/lim AND [embase]/lim AND [medline]/lim AND [2000-2011]/py	610
☐	#9 #7 NOT ('drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis OR 'warfarin'/exp OR warfarin:ab,ti OR 'hiv'/exp OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [humans]/lim AND [embase]/lim AND [2000-2011]/py	770
☐	#9 #7 NOT ('drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis OR 'warfarin'/exp OR warfarin:ab,ti OR 'hiv'/exp OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [humans]/lim AND [embase]/lim AND [2000-2011]/py	770
☐	#8 #7 NOT ('drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis OR 'warfarin'/exp OR warfarin:ab,ti OR 'hiv'/exp OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [medline]/lim AND [humans]/lim AND [embase]/lim AND [2000-2011]/py	558
☐	#7 #4 AND (precaution*:ab,ti OR prevention:ab,ti OR control:ab,ti OR cohorting:ab,ti OR surveillance:ab,ti OR intervention:ab,ti OR interventions:ab,ti OR incidence:ab,ti OR hygiene:ab,ti OR handwashing:ab,ti OR protection:ab,ti OR protective:ab,ti OR gloves:ab,ti OR mask:ab,ti OR masks:ab,ti) AND [humans]/lim AND [embase]/lim AND [2000-2011]/py	837
☐	#6 #5 NOT ('drug hypersensitivity'/exp OR 'hypersensitivity' OR hypersensitivity OR 'hemolysis'/exp OR 'hemolysis' OR hemolysis OR 'warfarin'/exp OR warfarin:ab,ti OR 'hiv'/exp OR hiv:ab,ti OR tuberculosis:ab,ti OR diabetes:ab,ti OR malignan*:ab,ti) AND [embase]/lim AND [medline]/lim AND [2000-2011]/py	1,394
☐	#5 #4 AND ('cross infection'/exp OR 'cross infection' OR nosocom* OR 'risk' OR 'risk'/exp OR risk OR 'risk factor'/exp OR 'risk factor' OR spread OR outbreak OR transmission OR transfer OR 'safety' OR 'safety'/exp OR safety OR ventilat* OR catheter* OR carrier* OR carriage OR hospitali* OR colonis* OR coloniz* OR environment*) AND [humans]/lim AND [embase]/lim AND [2000-2011]/py	2,202
☐	#4 #3 AND ('enterobacteriaceae'/exp/mj OR 'enterobacteriaceae' OR 'enterobacteriaceae infection'/exp/mj OR 'enterobacteriaceae infection') AND [embase]/lim AND [2000-2011]/py	5,509

☐	#3 #2 NOT (esbl:ti OR esbls:ti OR esbe*:ti OR extended*:ti) AND [embase]/lim AND [2000-2011]/py	28,377
☐	#2 #1 NOT (acinetobacter:ti OR pseudomonas:ti OR shigella:ti OR salmonella:ti OR yersinia:ti) AND [embase]/lim AND [2000-2011]/py	29,607
☐	#1 'carbapenem' OR 'carbapenem'/exp OR carbapenem OR carbapenemas* OR vim OR 'oxa 48' OR ndm OR mbl OR 'imp' OR 'imp'/exp OR imp OR 'imipenem' OR 'imipenem'/exp OR imipenem OR 'meropenem' OR 'meropenem'/exp OR meropenem OR 'ertapenem' OR 'ertapenem'/exp OR ertapenem OR 'doripenem' OR 'doripenem'/exp OR doripenem OR 'biapenem' OR 'biapenem'/exp OR biapenem OR 'panipenem' OR 'panipenem'/exp OR panipenem OR 'pz 601' OR 'beta lactamases'/exp OR 'beta lactamases' AND [embase]/lim AND [2000-2011]/py	32,473

Annex 5. Tables for exclusion and inclusion of full-text articles

Table 5.1 Summary of number and classification of excluded full-text articles

Exclusion criteria	Question on risk factors	Question on infection control	Question on diagnostic accuracy
Original number of studies	74	52	87
Not peer-reviewed	18	18	15
Not in English, French, Spanish, Greek, German, Italian	0	0	1
Outcome irrelevant	27	14	48

Table 5.2 Summary of number and classification of included full-text articles

Inclusion criteria	Question on risk factors	Question on infection control	Question on diagnostic accuracy
Included studies after applying inclusion criteria	29	20	23
Added studies	0	0	0
Additional removal of studies	2	4	3
Final number of included studies	28 (10 analytic and 18 descriptive)	16 (8 analytic and 8 descriptive)	20

Annex 6. Tables for systematic review #1

Table 6.1 Summary of findings: Included studies investigating risk factors for acquisition of carbapenem-resistant Enterobacteriaceae

Falagas, 2007 [126]

Study design	Matched case-control study (1:1 matching): 53 cases and 53 controls, matched for site of infection
Study objectives	To identify risk factors associated with the development of carbapenem-resistant *K. pneumoniae* infections
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae*, and were reported as carbapenem-resistant; no resistance mechanism was reported.
Breakpoints	MIC ≥16 mg/L to imipenem or meropenem
Cases	Any patient infected with carbapenem-resistant *K. pneumoniae* during the time period
Controls	Patients without carbapenem-resistant *K. pneumoniae* from the same eligible population and during the same time period as the cases
Risk factors independently associated with CPE	1. Prior use of fluoroquinolones OR=4.54 (95%CI: 1.78-11.54, P=0.001 2. Prior use of anti-pseudomonal penicillins (OR=2.60, 95%CI: 1.00-6.71, P= 0.04

Gasink, 2009 [11]

Study design	Case-control study (56 cases, 863 controls)
Study objectives	To identify the risk factors associated with infection or colonisation of patients with KPC-producing *K. pneumoniae.* To study the association of infection with KPC-producing *K. pneumoniae* on in-hospital mortality
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae* and all harboured bla_{KPC}
Breakpoints	Not reported
Cases	Any patient aged >18 years, who had an in-patient clinical culture with KPC-producing *K. pneumoniae* during the study period
Controls	All patients who had a positive culture for carbapenem-susceptible *K. pneumoniae* during the study period
Risk factors independently associated with CPE	1. Severity of illness OR=4.31 (95% CI: 2.25-8.25), P<0.001 2. Prior fluoroquinolone use OR=3.39 (95%CI: 1.50-7.66), P=0.003 3. Prior extended-spectrum cephalosporin use OR=2.55 (95% CI: 1.18-5.52), P=0.02 4. Blood isolate (negative association) OR=0.33 (95%CI: 0.12-0.86), P=0.02

Gregory, 2010 [124]

Study design	**Two case-control studies during an outbreak (see below for cases and controls)**
Study objectives	First case-control study: To identify risk factors for acquiring carbapenem- resistant *K. pneumoniae* (CKRP) Second case-control study: To distinguish between risk factors for acquiring CRKP and general risk factors for acquiring *K. pneumoniae.*
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae*. Only seven isolates were submitted for bla_{KPC} detection and sequencing: five expressed a new KPC variant, KPC-8, and the remaining two expressed KPC-2.
Breakpoints	Clinical Laboratory Standards Institute (CLSI), 2009 [90]
Cases	26 cases (19 clinical infections and seven colonised) defined as patients with CRKP from January 2006 to September 2008.
Controls	Different control groups for the two studies: First case-control study: 26 controls hospitalised for at least seven days, 21 years of age at least, no prior history of CRKP infection and admitted within seven days of a case patient. Second case-control study: 26 controls selected among patients aged 21 years and over, with carbapenem-susceptible *K. pneumoniae* (CSKP) isolated from any site.
Risk factors independently associated with CPE	First case-control study: Multivariate analysis adjusted for length of stay: • Presence of wounds Hazard ratio=19.0 (95% CI: 2.5-142) Second case-control study: Multivariate analysis: • Transfer between hospital units Adjusted OR=7.5 (95% CI: 1.8-31.1), P=0.08 • Previous surgery Adjusted OR=4.0 (95% CI: 1.0-15.7), P=0.05 • Presence of wounds Adjusted OR=4.9 (95% CI: 1.1-21.8), P=0.04

Hussein, 2009 [125]

Study design	Case-control study (125 cases and 373 controls)
Study objectives	To identify risk factors for patient infection with carbapenem-resistant *K. pneumoniae*
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae* and all harboured bla_{KPC}
Breakpoints	MIC to imipenem MIC ≥8µg/ml
Cases	All in-patients during the study period who had a clinical culture positive for a K. *pneumoniae* isolate that was intermediately susceptible or resistant to imipenem (MIC ≥8µg/ml).
Controls	All in-patients during the study period who had a clinical culture positive for a K. *pneumoniae* isolate that was sensitive to imipenem (MIC <8µg/ml).
Risk factors independently associated with CPE	1. Prior fluoroquinolone antibiotic use OR=1.87 (95% CI: 1.07-3.26), P=0.026 2. Prior carbapenem antibiotic use OR=1.83 (95% CI: 1.02-3.27), P=0.042 3. Admission to the ICU OR=4.27 (95% CI: 2.49-7.31), P<0.001 4. Exposure to at least 1 antibiotic drug prior to isolation of *K. pneumoniae* OR=3.93 (95% CI: 1.15-13.47), P=0.029

Jeon, 2008 [128]

Study design	Matched retrospective case-control study (1:3 matching): 46 cases and 138 controls
Study objectives	To identify risk factors for patient acquisition of carbapenem-resistant *E. coli* (CREC)
Bacterial species and mechanism of resistance	All isolates were *E. coli*; no resistance mechanism was reported, only that they were resistant to imipenem.
Breakpoints	CLSI, 2004; MIC to imipenem or meropenem ≥8 µg/mL
Cases	Hospitalised patients from whom nosocomially-acquired CREC were isolated, 48 hours after admission to the hospital.
Controls	Controls were randomly selected from the same medical or surgical service as the cases on the day each case patient had a positive culture for CREC. Controls to cases ratio was 3:1, matching as closely as possible for age, sex and admission date. When more than three subjects met these conditions, an independent person chose three subjects at random.
Risk factors independently associated with CPE	Multivariate analysis: 1. Prior carbapenem use OR=6.50 (95%CI: 2.33-18.16), P<0.001 2. Prior use of metronidazole in the 14 days before positive culture OR=4.25 (95%CI: 1.56-11.59), P=0.05 3. Presence of biliary catheter OR=4.59 (95% CI: 1.18-17.78), P=0.028 4. Prior hospital stay (<1 year) OR=1.02, (95% CI: 1.00-1.03), P=0.01

Kwak, 2005 [127]

Study design	Case-control study; (1:4) (30 cases and 120 controls)
Study objectives	To define the risk factors for patient acquisition of carbapenem-resistant *K. pneumoniae* (CRKP).
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae,* no resistance mechanism was reported, just that the isolates were resistant to imipenem.
Breakpoints	National Committee for Clinical Laboratory Standards (NCCLS), 1994; MIC to meropenem ≥8 g/ml.
Cases	Hospitalised patients from whom nosocomially acquired CRKP was isolated 48 hours after hospital admission.
Controls	Controls were randomly selected from the same medical or surgical service as the cases on the day that the CRKP was isolated from each case patient, with a ratio of 4:1.
Risk factors independently associated with CPE	1. Prior carbapenem use OR=28.68 (95%CI: 9.08-90.55), P<0.001 2. Prior cephalosporin use OR=4.10 (95% CI: 1.35–12.43), P=0.013 3. Fluoroquinolones with a negative effect OR=0.26 (95% CI: 0.07-0.97), P=0.045

Marchaim, 2008 [12]

Study design	Matched case-control study; 33 cases and 33 controls
Study objectives	What are the risk factors for isolation of imipenem-resistant *Enterobacter* spp. (IRE) compared to imipenem-susceptible *Enterobacter* spp. (ISE) isolates? Is there an association with mortality when infected with an imipenem-resistant *Enterobacter* spp.?
Bacterial species and mechanism of resistance	All isolates were *Enterobacter* spp. and all isolates harboured bla_{KPC-2}
Breakpoints	CLSI, 2006
Cases	All patients who had had a clinical culture with imipenem-resistant *Enterobacter* spp. between 1 April and 31 December 2006.
Controls	Controls were 1:1 to cases; matched by age group and source of clinical culture
Risk factors independently associated with CPE	High procedure score OR=4.93 (95% CI: 1.3-18.6), P=0.02
Mortality	After controlling for confounding, the presence of an imipenem-resistant *Enterobacter* spp. was independently associated with increased in-hospital mortality OR=8.3 +/- 8.6 (95% CI: 1.07-64), P=0.043

Patel, 2008 [111]

Study design	Matched case-control study (99 cases and 99 controls)
Study objectives	To identify the risk factors associated patients having invasive carbapenem-resistant *K. pneumoniae* infection. To identify the risk factors associated with in-hospital mortality among patients with carbapenem-resistant *K. pneumonia.*
Bacterial species and mechanism of resistance	All isolates were *K. pneumoniae* and all harboured bla_{KPC}
Breakpoints	CLSI, 2006
Cases	Medical records of all patients who received a diagnosis of any type of invasive *K. pneumoniae* infection that was carbapenem-resistant, during the period 1 July 2004–30 June 2006.
Controls	Control group selected from patients with carbapenem-susceptible *K. pneumoniae* infections. Controls were matched 1:1 with cases according to anatomic site of infection; if several control patients for each case, patient closest in age and isolation date of *K. pneumoniae* selected.
Risk factors independently associated with CPE	1. Recent solid organ or hematopoietic stem cell transplantation OR=3.71 (95% CI: 1.41-9.73), P=<0.008 2. Receipt of mechanical ventilation OR=2.44 (95% CI: 1.06-5.61), P=0.04 3. Longer length of stay before infection OR=1.05 (95% CI: 1.01-1.08); P=0.01 4. Cephalosporin exposure OR=2.65 (95% CI: 1.45-6.12), P=0.02 5. Carbapenem exposure OR=14.97 (95% CI: 5.29-42.35), P<0.001
Mortality	Predictors of mortality among cases with invasive *K. pneumoniae:* Independent risk factors for mortality with invasive *K. pneumoniae* infection: • heart disease OR 3.40 (95% CI: 1.31-8.84), P=0.24; • liver disease OR=2.90 (95% CI: 1.15-7.35), P=0.24; • ICU stay OR=5.16 (95% CI: 1.43-18.64), P=0.012; • infection with carbapenem-resistant K. pneumoniae OR=4.69 (95% CI: 1.9-11.58), P=0.001 Independent risk factors for mortality only within the cohort of case patients were: • heart disease OR=3.14 (95% CI: 1.16-8.53), P=0.02; • renal insufficiency OR=2.75 (95% CI: 1.16-6.50), P=0.02; • ICU stay OR=3.29 (95% CI: 1.32-8.19), P=0.009

Schwaber, 2008 [14]

<table>
<tr><th>Study design</th><td colspan="2">Two studies:
Retrospective case-case-control study for risk factors
Retrospective cohort study to determine the in-hospital mortality associated with the isolation of carbapenem-resistant K. pneumoniae (CRKP)</td></tr>
<tr><td>Study objectives</td><td colspan="2">To identify the risk factors for carbapenem-resistant CKRP isolation from patients.
To establish whether in-hospital mortality is associated with isolation of CRKP</td></tr>
<tr><td>Bacterial species and mechanism of resistance</td><td colspan="2">K. pneumoniae: K. pneumoniae isolates were carbapenem-resistant (from area with high endemicity for CPE). No mechanism of resistance was reported.</td></tr>
<tr><td>Breakpoints</td><td colspan="2">CLSI, 2005</td></tr>
<tr><td>Cases</td><td colspan="2">Two case groups:
Case group #1: 48 patients from whom a CRKP isolate was cultured during hospitalisation between 2003–2006; 35 of whom had isolated positive culture in 2006.
Case group #2: 56 patients with carbapenem-susceptible Klebsiella spp. (CSKS) by choosing randomly from lists of patients meeting the criteria for these second cases (54 with K. pneumoniae and 2 with K. oxytoca).</td></tr>
<tr><td>Controls</td><td colspan="2">Randomly selected from lists of patients with no positive cultures for Klebsiella spp.</td></tr>
<tr><td>Risk factors independently associated with CPE</td><td>Case Control Study #1</td><td>Case-Control Study #2</td></tr>
<tr><td></td><td>Poor functional status
OR=15.4 (95% CI: 4.0-58.6), P< 0.001</td><td>Poor functional status
OR=6.3 (95% CI: 2.3-17.2), P< 0.001</td></tr>
<tr><td></td><td>ICU stay
OR=17.4 (95% CI: 1.5-201.9), P=0.02</td><td>ICU stay
OR=12.5 (95% CI: 1.3-125.4), P=0.03</td></tr>
<tr><td></td><td>Use of antibiotics
OR=4.4 (95% CI: 1.0-19.2), P=0.05</td><td>Non-invasive procedure
OR=9.4 (95% CI: 1.0-92.6), P=0.05</td></tr>
<tr><td></td><td>Use of fluoroquinolones
OR=7.2 (95% CI: 1.1-49.4), P=0.04</td><td>Malignancy
OR=3.2 (95% CI: 1.2-9.0), P=0.02</td></tr>
<tr><td></td><td colspan="2">Comparison of risk factors for CRKP and CSKS:
Prior receipt of antibiotics, especially fluoroquinolones</td></tr>
</table>

Table 6.2 Appraisal of the methodological quality of included studies for risk factors

Study	Study design	Presence of potential bias			Overall quality
		Selection	Information/ Misclassification	Confounding	
Falagas, 2007 [126]	Matched case-control	No	Yes	Yes	+
Gasink, 2009 [11]	Case-control	No	Yes	Yes	+
Gregory, 2010 [124]	Case-control	Yes	Yes	Yes	+
Hussein, 2009 [125]	Case-control	Yes	Yes	Yes	+
Jeon, 2008 [128]	Matched case-control	Yes	Yes	Yes	+
Kwak, 2005 [127]	Case-control	Yes	Yes	Yes	+
Marchaim, 2008 [12]	Matched case-control	Yes	Yes	Yes	++
Patel, 2008 [111]	2 matched case-control	Yes	Yes	Yes	+
Schwaber, 2008 [14]	Case-case control	Yes	Yes	Yes	++

Table 6.3 Risk factors associated with acquisition of carbapenem-resistant Enterobacteriaceae

Risk factors	Bibliographic citation and measurement of risk	Resistance mechanisms
Antibiotic use (general)	Hussein, 2009 [125] OR=3.93 (95% CI: 1.15-13.47), P=0.029	Carbapenem-resistant *K. pneumoniae* (from areas where KPC-producing *K. pneumoniae* are endemic)
	Schwaber, 2008 [14] OR=4.4 (95% CI: 1.0-19.2), P=0.05	Carbapenem-resistant *K. pneumoniae* (from areas where KPC-producing *K. pneumoniae* are endemic)
Fluoroquinolones	Gasink, 2009 [11] OR=4.31 (95% CI: 2.25–8.25), P<0.001	*K. pneumoniae* with bla_{KPC}
	Falagas, 2007 [126] OR=4.54 (95%CI: 1.78-11.54), P=0.001	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing and VIM-producing *K. pneumoniae* are endemic)
	Hussein, 2009 [125] OR=1.87 (95% CI: 1.07-3.26), P=0.026	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
	Schwaber, 2008 [14] OR=7.2 (95% CI: 1.1-49.4), P=0.04	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
	Kwak, 2005 [127] *OR=0.26 (95% CI: 0.07-0.97) (negative effect)*	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are not endemic)
Carbapenems	Hussein, 2009 [125] OR=1.83 (95% CI: 1.02-3.27), P=0.042	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
	Jeon, 2008 [128] OR=6.50 (95%CI: 2.33-18.16), P<0.001	Carbapenem-resistant *E. coli*
	Kwak, 2005 [127] OR=28.68 (95%CI: 9.08-90.55), P<0.001	Carbapenem-resistant *K. pneumoniae* (not from area where KPC-producing *K. pneumoniae* are endemic)
	Patel, 2008 [111] OR=14.97 (95% CI: 5.29-42.35), P<0.001	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
Cephalosporins	Gasink, 2009 [11] OR=2.55 (95% CI: 1.18-5.52), P=0 .02	*K. pneumoniae* with bla_{KPC}
	Kwak, 2005 [127] OR=4.10 (95% CI: 1.35-12.43), P=0.013	Carbapenem-resistant *K. pneumoniae* (not from area where KPC-producing *K. pneumoniae* are endemic)
	Patel, 2008 [111] OR=2.65 (95% CI: 1.45-6.12), P=0.02	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
Anti-pseudomonal penicillins	Falagas, 2007 [126] OR=2.60 (95% CI: 1.00-6.71), P= 0.04	Carbapenem resistant *K. pneumoniae* (from area where KPC-producing and VIM-producing *K. pneumoniae* are endemic)
Metronidazole	Jeon, 2008 [128] OR=4.25 (95% CI: 1.56-11.59), P=0.05	Carbapenem-resistant *E. coli*
Severity of illness	Gasink, 2009 [11] OR=4.31 (95% CI: 2.25–8.25), P<0.001	KPC-producing *K. pneumonia*
ICU admission	Hussein, 2009 [125] OR=4.27 (95% CI: 2.49-7.31), P<0.001	Carbapenem resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)

Risk factors	Bibliographic citation and measurement of risk	Resistance mechanisms
High procedure score (multiple invasive devices)	Marchaim, 2008 [12] OR=4.93 (95% CI : 1.3-18.6) P=0.02	*Enterobacter* spp. with bla_{KPC}
Presence of wounds	Gregory, 2010 [124] Hazard Ratio=19.0 (95% CI: 2.5-142), (when CRKP compared to uninfected controls)	Carbapenem-resistant *K. pneumoniae* (few isolates with confirmed bla_{KPC}, from area where KPC-producing *K. pneumoniae* are endemic)
	Gregory, 2010 [124] Adjusted OR=4.9 (95% CI: 1.1-21.8), P=0.04) (when CRKP compared to controls with CSKP)	
Longer length of stay	Patel, 2008 [111] OR, 1.05 (1.01-1.08); P=0.01	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
Blood isolate (negative association)	Gasink, 2009 [11] OR=0.33 (95%CI: 0.12–0.86), P=0.02	*K. pneumoniae* with bla_{KPC}
Transfer between hospital units	Gregory, 2010 [124] Adjusted OR=7.5 (95% CI: 1.8-31.1), P=0.08	Carbapenem-resistant *K. pneumoniae* (few isolates with confirmed bla_{KPC}, from area where KPC-producing *K. pneumoniae* are endemic)
Previous surgery	Gregory, 2010 [124] Adjusted OR=4.0 (95% CI: 1.0-15.7), P=0.05	
Prior hospital stay < 1 year prior	Jeon, 2008 [128] OR=1.02 (95% CI: 1.00-1.03), P=0.01	Carbapenem-resistant *E. coli*
Presence of a biliary catheter	Jeon, 2008 [128] OR=4.59 (95% CI: 1.18-17.78), P=0.028	
Recent solid organ transplantation (SOT) or stem-cell transplantation (SCT)	Patel, 2008 [111] OR=3.71 (95% CI: 1.41-9.73), P=<0.008	Carbapenem-resistant *K. pneumoniae* (from area where KPC-producing *K. pneumoniae* are endemic)
Mechanical ventilation	Patel, 2008 [111] OR=2.44 (95% CI: 1.06-5.61), P=0.04	

Table 6.4 Description of cross-border transmission studies

Study (ref. no.)	Bacterial species, enzyme (no. of cases)	Reporting country	Country of origin	Evidence of importation	Method of CPE detection	Evidence of direct hospital-to-hospital transfer	Evidence of intra-hospital transmission or outbreak	Evidence of cross-border transfer
Barbier, 2009 [133]	*K. pneumoniae,* KPC-2 & VIM-1	France	Greece	Strong	Rectal screening	Yes	Not reported	Yes
Bogaerts, 2010 [83]	*K. pneumoniae,* KPC-2	Belgium	Greece	Strong	Clinical culture and rectal screening	Yes	None reported	Yes
Cuzon, 2008 [134]	*K. pneumoniae,* KPC-2	France	Greece	Strong	Rectal and other site screening	Yes	None reported	Yes
Dortet, 2007 [26]	*Enterobacter* spp., KPC-3	France	USA	Moderate	Clinical culture	Yes	None reported	Yes
Halstead, 2009 [121]	*K. pneumoniae,* KPC-2 & KPC-3	USA	USA	Strong	Clinical culture	Yes	Yes	No; but across healthcare facilities
Kassis-Chikhani, 2006 [85]	*K. pneumoniae,* VIM-1	France	Greece	Strong	Rectal screening	Yes	Yes	Yes
Kassis-Chikhani, 2010 [86]	*K. pneumoniae,* KPC-2	France	Greece	Weak	Rectal screening	No	Yes	Yes
Kumarasamy, 2010 [49]	*K. pneumoniae,* NDM-1	UK	Indian subcontinent	Strong		Not reported	Not reported	Yes
Lopez, 2010 [120]	*K. pneumoniae,* KPC-3	Colombia	Israel	Strong	Clinical culture	No	Clonal spread over months	Yes
MMWR, June 2010 [53]	*E. coli* , *K. pneumoniae* & *E. cloacae* , NDM-1	USA	India	Strong	Not reported	Yes	Not reported	Yes
MMWR, Sept 2010 [136]	*K. pneumoniae,* VIM-1	USA	Greece	Strong	Clinical culture	Yes	No	Yes
Naas, 2010 [135]	*K. pneumoniae,* KPC-2	France	Greece	Weak	Clinical culture and rectal screening	No	Yes and to other hospitals	Unclear
Navon Venezia, 2009 [37]	*K. pneumoniae,* KPC-3	Israel	Probably USA	Moderate	Clinical culture	Unclear	Nationwide spread	Yes
Österblad, 2009 [138]	*K. pneumoniae* KPC-2	Finland	Greece, Italy	Strong	Clinical culture andrectal screening	Yes	None reported	Yes
Samuelsen, 2009 [87]	*K. pneumoniae,* KPC	Norway, Sweden	Greece, Israel	Strong	Clinical culture & perineal screening	Direct for three patients, with time lag for two patients	None reported	Yes
Tegmark Wisell, 2007 [158]	*K. pneumoniae, KPC-2*	Sweden	Greece	Moderate	Clinical culture	Yes	None reported	Unclear
Wendt, 2010 [137]	*K. pneumoniae, KPC-2*	Germany	Greece	Moderate	Clinical culture &rectal screening	Unclear	Yes	Yes
Woodford, 2008 [27]	*K. pneumoniae, KPC-3*	UK	Israel	Moderate	Clinical culture	Unclear	Not reported	Probable

Annex 7. Tables for systematic review #2

Table 7.1 Description of infection control intervention studies and outbreak reports

Study	Study design	Study objectives	Bacterial species, enzyme	Country, year	Care setting	Epidemio-logical setting	Infection control measures	
							Baseline	Intervention(s)
Ben-David, 2010 [152]	Retrospective cohort study with pre-intervention (outbreak) and post intervention phases	To assess the effectiveness of a combined intervention on the incidence of nosocomial infections by KPC-producing *K. pneumoniae*	*K. pneumoniae*, KPC-3	Israel, 2006–07	1600-bed tertiary care teaching hospital	Outbreak in endemic region	Contact precautions for care of KPC cases detected by clinical cultures	• Active surveillance by rectal culture in all patients admitted to ICU & step-down units (admission & weekly during stay) and in patients with epidemiological link to epidemic cases in other units. Daily reporting of cases to unit, hospital manager and national health authority Case flagging for detection of re-admission.
Endimiani 1 2009 [144]	Retrospective outbreak report	To describe microbiological characteristics of outbreak-related isolates	*K. pneumoniae*, KPC	USA, 2008	Long-term acute care hospital	Outbreak in endemic region	Not reported	• Active surveillance by rectal cultures (point prevalence survey) • Contact isolation precautions • Cohort nursing of KPC cases
Gregory, 2010 [124]	Retrospective outbreak report	To describe an outbreak and the successful control measures	*K. pneumoniae*, KPC-8	Puerto-Rico, 2006–08	328-bed tertiary teaching hospital	Outbreak in endemic region	• Contact precautions for care of KPC cases detected by clinical cultures • ICU temporary closure • Restriction of broad-spectrum antibiotics	• Active surveillance by peri-rectal cultures • Cohort nursing of KPC cases
Herbert, 2007 [148]	Retrospective outbreak report	To describe an outbreak and infection control measures	8 species, IMP-4	Australia, 2003–05	320-bed tertiary care teaching hospital	Outbreak in non-endemic region	• Standard precautions, with promotion of alcohol hand hygiene in ICU • Environmental cleaning in ICU	• Carbapenem restriction (all wards) • Universal wearing of gloves and gown (ICU) • Single room isolation of CPE-patients (all wards)
Kochar, 2009 [149]	Retrospective, observational cohort study with planned pre/post interventional phases	To assess the effect of multiple infection control measures on limiting the spread of carbapenem-resistant *K. pneumoniae*	*K. pneumoniae*, Enzyme not reported (carbapenem-resistant)	USA, 2004–07	10-bed medical-surgical ICU in tertiary care hospital	Endemic hospital in endemic region	• Contact isolation precautions, including disposable gloves and gown for care of patients with carbapenem-resistant Gram-negative bacilli, VRE and MRSA • Alcohol hand disinfection. • Daily cleaning & decontamination of environmental surfaces	• Rectal surveillance culture on ICU admission and weekly + notification in medical record • ICU closure and environmental surfaces cleaning and disinfection • CPE patients grouped in one ICU side with dedicated cohort nursing • Promotion of alcohol hand hygiene • Promotion of environmental disinfection

Study	Study design	Study objectives	Bacterial species, enzyme	Country, year	Care setting	Epidemio-logical setting	Infection control measures	
							Baseline	Intervention(s)
Munoz-Price, 2010a [151]	Retrospective outbreak report	To describe the investigation and control of an outbreak	*K. pneumoniae*, KPC	USA, 2009	20-bed surgical ICU in teaching hospital	Outbreak in endemic region	Not reported	• Active surveillance by rectal cultures (point prevalence survey) • Contact isolation precautions • Cohort nursing • Chlorhexidine baths for all SICU patients • Increased environmental surface cleaning and disinfection • Healthcare staff education
Munoz-Price, 2010b [150]	Retrospective outbreak report	To determine the effect of bundle of outbreak control measures on transmission of KPC-producing *K. pneumoniae*	*K. pneumoniae*, KPC	USA, 2008	70-bed long-term acute-care hospital	Outbreak in endemic region	Admission active surveillance cultures	• Active surveillance by rectal cultures (serial point prevalence surveys) • Contact isolation precautions of KPC cases & pre-emptive isolation of high risk patients • Cohort nursing of KPC cases • Chlorhexidine baths for all patients • Increased environmental surface cleaning and disinfection • Healthcare staff education

Table 7.2 Appraisal of the methodological quality of included studies for infection control

Study	Design	Clarity of reporting (ORION)	Statistical analysis		Presence of potential bias			Confounders addressed	Over all quality
			Method	Quality	Selection	Detection	Confounding		
Ben-David, 2010 (152)	Outbreak report	Adequate	Interrupted time series using Poisson regression model of monthly incidence, adjusted for previous month's outcome	Appropriate	No	No	Yes	Colonisation pressure. Regression to the mean.	+
Endimiani 2009 (144)	Outbreak report	Inadequate	None	-	Yes	Yes	Yes	None	0
Gregory, 2010 (124)	Outbreak report	Inadequate	None	-	Yes	Yes	Yes	None	0
Herbert, 2007 (148)	Outbreak report	Inadequate	None	-	Yes	Yes	Yes	None	0
Kochar, 2009 (149)	Planned intervention	Adequate	Student *t* test to compare the mean incidence per period	Inappropriate	No	No	Yes	Antibiotic use. Length of stay.	+
Munoz-Price, 2010a (151)	Outbreak report	Inadequate	None	-	No	Yes	Yes	None	0
Munoz-Price, 2010b (150)	Outbreak report	Adequate	Cochrane-Armitage Chi-square test for linear trend in proportion	Inappropriate	No	Yes	Yes	None	0
Wendt, 2010 (137)	Outbreak report	Inadequate	None	-	No	Yes	Yes	None	0

Table 7.3 Effect of infection control measures

Study	Category	Outcome metric	Effect of measures				Duration of follow-up
			Incidence before measures	Incidence after measures	Risk ratio (95 % CI)	P-value	
Ben-David, 2010 [152]	Outbreak report	Number of nosocomial infections by carbapenem-resistant *K. pneumoniae*/ 10 000 hospital patient-days, per month. Slope of temporal linear trend per intervention phase.	• Increasing incidence trend (slope 0.12) • Last pre-intervention incidence: 6.93/10 000 patient-days	• Decreasing incidence trend (slope -0.07) • Last post-intervention incidence: 1.80/10 000 patient-days	0.26	<0.001	20 months
Endimiani 2009 [144]	Outbreak report	Number of cases with positive culture for KPC-producing *K. pneumoniae*, per period	9 cases/4 weeks	1 case/6 weeks	-	-	6 weeks
Gregory, 2010 [124]	Outbreak report	Number of cases of nosocomial infection by KPC-producing *K. pneumoniae*, per period	26 cases/26 months	1 case	-	-	Not reported
Herbert, 2007 [148]	Outbreak report	Number of cases of colonisation or infection with IMP-4 positive bacteria, per month	2 to 3 cases/month	• Interventions 1+2: increase to seven cases/month • Intervention 3: decrease to 1–2 cases/month	-	-	13 months
Kochar, 2009 [149]	Planned intervention	Number of patients with new positive (clinical) culture for carbapenem-resistant *K. pneumoniae* (acquired in the ICU)/1 000 ICU patient-days, per quarter.	9.7+/- 2.2 (mean +/- SD)	3.7+/- 1.6 (mean +/- SD)	0.38	<0.001	7 months
Munoz-Price, 2010a [151]	Outbreak report	Number of cases with positive culture for KPC-producing *K. pneumoniae*, per period	7 cases/1 survey in 7 months	2 cases/1 survey in 5 months	-	-	5 months
Munoz-Price 2010b [150]	Outbreak report	Point prevalence of rectal colonisation by KPC-producing *K. pneumoniae*, per serial survey	21% prevalence	decrease from 12% to 0% prevalence	0.00	<0.001	3 months
Wendt, 2010 [137]	Outbreak report	Number of cases with positive culture for KPC-producing *K. pneumoniae*, per month	3 cases/1 month	5 cases/1 month then 0 case/6 months	0.00	-	7 months

Table 7.4 Description of cross-border transmission studies

Study	Species, enzyme (no. of cases)	Reporting country	Country of origin	Evidence of import	Method of CPE detection	Infection control	Contact screening	Number of secondary nosocomial cases	Level of spread
Bogaerts 2010 [83]	*K. pneumoniae,* KPC (3)	Belgium	Greece	Strong	Clinical culture & rectal screening	Strict measures	Unclear	0	None
Hammerum, 2010 [154]	*K. pneumoniae,* KPC (2)	Denmark	Greece	Strong	Clinical culture & rectal screening	Pre-emptive isolation	Unclear	0	None
Kassis-Chikhani, 2006 [85]	*K. pneumoniae,* VIM (1)	France	Greece	Strong	Rectal screening	Pre-emptive isolation	Contact in unit	7	Hospital unit
Kassis-Chikhani, 2010 [86]	*K. pneumoniae,* KPC (1)	France	Greece	Weak	Rectal screening	Unclear	Contact in unit	3	Hospital unit
Österblad 2009 [138]	*K. pneumoniae,* KPC (2)	Finland	Greece, Italy	Strong	Clinical culture & rectal screening	Stayed alert	Unclear	Unclear	Unclear
Lopez, 2010 [120]	*K. pneumoniae,* KPC (2)	Colombia	Israel	Strong	Clinical culture	Barrier precautions after outbreak detection	ICU after outbreak detected	84	Hospital
Naas, 2010 [135]	*K. pneumoniae,* KPC (2)	France	Greece	Weak	Rectal screening	Cohorting care after outbreak detection	Surgical unit & endoscope contacts	3 in unit; 6 from endoscope	Regional hospitals
Samuelsen, 2009 [87]	*K. pneumoniae,* KPC (5)	Norway, Sweden	Greece, Israel	Strong	Clinical culture & perineal screening	Unclear	Unclear	2	Unclear

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