Royal College of Physicians of Ireland

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Tús Áite do Shábháilteacht Othar
Patient Safety First

Quality and Patient Safety Directorate

Guidelines for the Prevention and Control of **Multi-drug resistant organisms (MDRO)** excluding MRSA in the healthcare setting

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Executive Summary

Key Recommendations

- Successful implementation of this guidance depends on implementation of a national strategy for Ireland with strong political support, adequate funding and cooperation in both public and private sectors of senior health management, healthcare workers, patients and the public.
- **Key components of a national strategy for the prevention and control of multi-drug resistant organisms (MDRO) in healthcare settings include:**
    - Development of infection control guidelines for MDRO, taking local epidemiology into consideration in all Irish healthcare facilities.
    - Surveillance of invasive infection rates and new acquisition rates for MDRO in Irish hospitals to obtain national surveillance data and to facilitate introduction of key performance indicators.
    - Monitoring of adherence to local guidelines and protocols.
    - Establishment of antimicrobial stewardship programmes in all Irish healthcare facilities.
    - National typing studies to establish the epidemiology of MDRO.
    - Establishment of a national reference laboratory service for MDRO discussed in this document.
    - Continuous education programmes in infection prevention and control for all clinical staff in hospital and long-term care facilities, healthcare managers, patients and the general public.
    - Good communication structures between Irish healthcare facilities.
- **Key infection control recommendations for the acute hospital setting applicable to all MDRO discussed in this document include:**
    - Ideally, every patient who is colonised or infected with MDRO should be isolated in a single room with *en-suite* facilities. Contact Precautions should be applied. If limited isolation facilities are available, a local risk assessment should be undertaken in conjunction with the infection prevention and control team (IPCT) (e.g., Lewisham index, see Appendix 6).
    - Patients should be informed of their status for colonisation or infection with MDRO upon laboratory confirmation. The patient should be provided with an information leaflet (Appendix 9-11).
    - The responsibility for informing patients of their MDRO status and documenting this in the healthcare record lies with the clinical team caring for the patient.
    - The patient's healthcare records should be flagged to highlight the positive MDRO status.
    - Screening of healthcare workers for carriage of MDRO is generally not appropriate.
- **Key infection control recommendations for settings outside the hospital applicable to all MDRO discussed in this document include:**
    - MDRO colonised patients should not be declined admission to a long-term care facility (LTCF), day care facilities or rehabilitation services or have their admission delayed on the basis of positive MDRO colonisation status.
    - Isolation of a resident with MDRO is generally not required in LTCF. Standard Precautions are required for the care of all patients, including patients colonised with MDRO in LTCF. The need to place a MDRO colonised patient in a single room or to use Contact Precautions should be determined based upon local risk assessment on a case-by-case basis (see Chapter 1.4: Patient placement and priority for isolation).
    - Routine screening for MDRO is not recommended for LTCF.
- It is the Committee's view that similar efforts and resources to those committed by the Irish health authorities for the control of meticillin resistant *Staphylococcus aureus* (MRSA), should be committed to the control of other MDRO.

Lay Summary

Infections caused by bacterial organisms resistant to most available antibiotics, called multi-drug resistant organisms (MDRO), have been increasing during the last decade in Ireland. The following document aims to provide information and guidance on how to control the spread of these bacteria inside and outside the hospital both on a local and on a national level. Bacteria discussed in detail in this document are those most frequently found in hospitalised patients: vancomycin-resistant enterococci (VRE) and resistant *Enterobacteriaceae* (for example *Escherichia coli*) and multi-drug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumanii*.

The basis for all control measures is the accurate and timely laboratory identification of bacteria with multi-drug resistance. This will deliver important information for the implementation of infection control measures in hospitals and for regional and national containment strategies. The development of microbiology laboratory reference services specialised in the identification and more detailed characterisation of these organisms in Ireland is therefore an urgent requirement. Hospitals and long-term care facilities need to develop and implement infection control policies for MDRO. Healthcare workers, administrators and patients who are responsible for the successful implementation of infection control policies require information and education on the control of MDRO and adherence to policies should also be monitored. Control can only be achieved if a national strategy is developed and adhered to by all healthcare facilities. More detailed information on infection control measures can be found in chapter 1. Chapters 2 to 4 provide more detailed information about the bacteria listed above.

The Committee developing this guideline document believes that similar efforts to those that have been implemented for the control of MRSA will need to be introduced for the control of other types of MDRO.

Introduction

Public attention and preventive efforts over the last decades have focused primarily on the control of MRSA. Data from the Health Protection Surveillance Centre (HPSC) reports a decrease in the occurrence of MRSA bloodstream infections (BSI) in Ireland from 2005 to 2011. In contrast the numbers of BSIs caused by organisms, such as *Escherichia coli* and *Enterococcus faecium*, a significant proportion of which is vancomycin-resistant *Enterococcus faecium* (VRE) are continuously increasing and have now reached a level that exceeds MRSA. In 2011, 264 MRSA BSI were reported versus 455 BSI due to other multi-drug resistant organisms (MDRO) (Table 1). Estimating the amount of MDRO-associated disease from analysis of BSI alone significantly underestimates the burden of disease. For example, urinary tract infections (UTI) which are predominantly caused by Gram-negative bacteria are one of the most frequently encountered healthcare associated infections (HCAI). Furthermore, multi-drug resistance among Gram-negative organisms causing UTIs is increasing both in the hospital and in the community setting.

Table 1: Irish EARS-Net bloodstream infection (BSI) reports submitted to HPSC in 2011

EARS-Net[1] BSI[2] reports in 2011	Number of isolates	Number of resistant isolates	% resistant isolates
S. aureus	1096	264 MRSA[4]	24.1% MRSA
E. faecium	364	136 VRE[5]	37.4% VRE
E. coli	2199	286 MDR[3]	13.0% MDR
K. pneumoniae	310	26 MDR[3]	8.4% MDR
P. aeruginosa	174	7 MDR[3]	4.0% MDR

[1] European Antimicrobial Resistance Surveillance Network.
[2] Bloodstream infections
[3] Multi-drug Resistant (resistant to 3 or more of the required antimicrobial classes)
[4] Meticillin-resistant *Staphylococcus aureus*
[5] Vancomycin-resistant enterococci

As is the case with MRSA, research has shown that infection due to multi-drug resistant Gram-negative organisms and vancomycin-resistant enterococci results in an increased length-of-stay in hospital and higher total hospital costs.[1] Over the last decade the main focus of research and antimicrobial development has been on the treatment of infection due to Gram-positive organisms, such as MRSA and VRE. Several new agents have been added to the Gram-positive arsenal including linezolid, daptomycin, tigecycline and ceftobiprole. Tigecycline remains the only newer agent with activity against some multi-drug resistant Gram-negative bacilli. However, a recent meta-analysis revealed decreased clinical and microbiological efficacy of tigecycline and advised to treat it as a 'last-resort drug'.[2]

Eradication of *Staphylococcus aureus* carriage can be achieved and is widely used by applying validated decontamination protocols. Although a few studies suggest that selective digestive decontamination (SDD) with non-absorbable oral antimicrobials can eradicate intestinal carriage of VRE and Gram-negative MDRO, this has been contradicted by other studies reporting that recolonisation can occur in the event that the patient becomes exposed to further antimicrobial treatment.[3]

The improvements achieved in the control of MRSA in Ireland and in other countries around the world show that adequate health policies and appropriately resourced infection control efforts can yield positive results in the control of multi-drug resistant organisms. The aim of this guideline document is to give recommendations for the prevention and control of MDRO, excluding MRSA in the healthcare setting (including hospitals and residential care settings).

To help control the spread of MDRO in the Irish healthcare setting, national and collaborative action is required. Sustained and effective control cannot be achieved if the infection control and antimicrobial stewardship efforts undertaken in one healthcare facility are counterbalanced by influx of MDRO from a neighbouring healthcare facility, which is not implementing equivalent infection control and antimicrobial stewardship efforts.

The Committee drawing up this guidance document was convened as a subcommittee of the Strategy for the Control of Antimicrobial Resistance in Ireland (SARI) national committee. Following the dissolution of the SARI national committee and the subsequent formation of the RCPI and HSE clinical care programme in healthcare associated infections (HCAI) and antimicrobial resistance (AMR), this Committee became a subcommittee of the RCPI clinical advisory group on HCAI.

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Objectives

- To produce national guidelines for the prevention and control of multi-drug resistant organisms (MDRO) in the Irish healthcare setting
- To reduce the prevalence of MDRO and thereby prevent serious infections (e.g., bloodstream infections, bone infection etc)
- To raise awareness of MDRO among healthcare workers
- To standardise laboratory detection of MDRO
- To advocate a national strategy for control of MDRO

Methodology

- The multi-drug resistant organisms which are addressed in this guideline include:
  1. Vancomycin-resistant enterococci (VRE)
  2. Resistant *Enterobacteriaceae*: Resistant *Enterobacteriaceae* described in this document include *Enterobacteriaceae* with transmissible resistance mechanism located on plasmids (ESBL, AmpC β lactamases and carbapenemases)
  3. Multi-drug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumanii*: Multi-drug resistant *P. aeruginosa* and *A. baumanii* described in this document include a bacterial isolate which is resistant to one or more agents in three or more different classes of antimicrobials that the isolate is expected to be susceptible to; e.g., β-lactam-inhibitor combinations, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.
- Principles outlined for the organisms listed above might be applicable to other multi-drug resistant organisms not specifically mentioned in this document.
- The terminology used in this document has been chosen by the Committee to reflect that which is most widely used in daily clinical practice. A list of acronyms and abbreviations as well as definitions used in this document is provided in Appendix 1 and Appendix 2.
- The Committee was convened in September 2010 and met on seven occasions to prepare a draft guidance document for consultation. After consultation the final version of the document was discussed over three additional meetings.
- This document intends to provide information for all categories of healthcare professionals.
- Due to the wide scope of organisms covered in this document and with the intention of delivering a 'user friendly' document, the Committee decided not to address treatment options.
- Although there is ample evidence in the literature supporting MRSA infection control measures, the same amount of evidence is not present for the control of all MDRO discussed in this document. The Committee therefore decided not to grade recommendations in this document. Recommendations are based on an analysis of the available epidemiology data for each MDRO type in Ireland and following consideration of published literature.
- As it was the Committee's intention that individual organism-specific chapters can be read in the context of the whole document as well as stand-alone documents, repetitions of recommendations applicable to different organisms were intentionally accepted.
- The aim of this guidance document is to provide information for the most frequently-encountered scenarios, but is not intended to be all-inclusive.
- The membership of the Committee drafting this guideline document was multi-disciplinary. Every effort was made to ensure that all relevant professional groups were represented on the Committee. Membership of the working group is listed in Appendix 3.

Antimicrobial Stewardship

There is ample evidence that widespread use of broad-spectrum antimicrobials leads to selective pressure which in turn, facilitates the proliferation of MDRO. While a comprehensive review of antimicrobial stewardship is beyond the scope of this guideline document, recommendations for control of MDRO must include attention

to judicious antimicrobial use.[1] A temporal association between formulary changes and decreased occurrence of a target MDRO was found in several studies especially those focusing on multi-drug resistance in Gram-negative bacilli.[2] Several classes of antimicrobials have been implicated in selecting multi-drug resistant Gram-negative bacilli. Promotion of prudent use of glycopeptides, such as vancomycin and teicoplanin, has been shown to reduce the prevalence of VRE in critical care units.[3] Therefore, rather than restricting the use of one particular class of antimicrobials, overall reduction of antimicrobial use is desirable.

Appropriate use of antimicrobials, also termed antimicrobial stewardship is a multi-factorial process and includes the following:

- Avoidance of inappropriate or excessive antimicrobial therapy in all healthcare settings – including hospital, residential care and community.
- Ensuring that antimicrobials are given at the correct dosage and for the shortest duration required for efficacy.
- Reducing the use of broad-spectrum antimicrobials, particularly third generation cephalosporins, fluoroquinolones and carbapenems.
- Limiting the use of glycopeptide antimicrobials to situations where their use is shown to be appropriate.
- Instituting antimicrobial stewardship programmes in all healthcare facilities.

More information on antimicrobial stewardship can be obtained from the 2009 'National Guidelines For Antimicrobial Stewardship in Hospitals in Ireland' [4] and the 2011 'Guidelines for Antimicrobial Prescribing in Primary Care in Ireland'.[5]

In November 2011, the Department of Health, Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) in England issued guidance for antimicrobial stewardship in English hospitals. This guidance coined the phrase 'Start Smart – Then Focus'.[6]

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1. Infection control measures for the control of multi-drug resistant organisms (MDRO) in the healthcare setting

1.1 Introduction

When MDRO are introduced into a healthcare setting, a number of factors aid the transmission and persistence of resistant strains in the environment. These include:

- The presence of vulnerable patients, such as those with compromised immunity from underlying medical or surgical conditions, those who have indwelling devices including endotracheal tubes, vascular catheters or urinary catheters[1]
- The reservoir of infected or colonised patients
- The selective pressure exerted by antimicrobial use
- The effectiveness of local infection prevention and control measures[1,2]

Transmission of MDROs tends to occur most frequently in acute care facilities, although all healthcare facilities may be affected. The severity and extent of disease caused by the resistant pathogens may vary by the population affected and also by the type of healthcare facility. Institutions may vary widely in terms of physical and functional characteristics, ranging from intensive care units in tertiary centres to long-term care facilities, and the approach to controlling the spread of a MDRO needs to be tailored to the needs of the population and the individual healthcare setting.[1]

Although successful control of MDRO has been documented using a number of different interventions, such as hand hygiene initiatives, enhanced education programmes, enhanced environmental cleaning, improved intra- and inter-hospital communication regarding identification of patients known to be colonised or infected with MDRO, it has not yet been possible to identify a specific intervention or combination of interventions which could be adopted by all healthcare facilities to limit the spread of a target MDRO. More research is needed in this area.[1]

1.2 Standard and Contact Precautions

In 1996, The US Centers for Disease Control & Prevention (CDC) issued guidelines recommending the use of Standard and Contact Precautions for MDRO thought to be clinically and epidemiologically significant.[1] These recommendations still apply although no studies to date have compared the effectiveness of Standard Precautions alone versus Standard and Contact Precautions for the control of MDRO spread.[1]
Standard Precautions are extremely important in limiting the spread of all transmissible pathogens, including MDRO and should be employed and adhered to by all staff at all times in all settings where healthcare is delivered.

Active surveillance cultures, such as screening for colonisation with a target MDRO may fail to identify colonised persons due to a lack of sensitivity of the screening method used, laboratory deficiencies or intermittent patient colonisation due to antimicrobial therapy.[1,3] For this reason, Standard Precautions must be used to limit transmission from potentially colonised patients. Hand hygiene is a vital component of Standard Precautions.

Contact Precautions are intended to prevent transmission of transmissible organisms, including MDRO whose spread is not interrupted by Standard Precautions alone and have the potential to contaminate the environment. Contact Precautions, in addition to Standard Precautions, should be routinely implemented in all acute healthcare facilities for any patient known to be infected with or colonised with an MDRO.[1]
The management of MDRO outside the hospital setting is discussed in section 1.14. A summary of Contact Precautions is included in Appendix 5.

1.3 Hand Hygiene

The contaminated hands of healthcare workers have long been implicated as a vehicle by which MDRO are transferred from person-to-person.[1,4] Effective hand hygiene has been shown to be the single most important measure in the prevention of spread of transmissible pathogens, including MDRO.[5] Current CDC guidelines recommend that hands should be decontaminated by washing with an antiseptic soap or waterless antiseptic agent such as a 70% alcohol handrub preparation.[6] Alcohol hand rubs (AHR) may be more convenient to use than other hand decontamination methods. However they should not be used as sole agents for hand hygiene in certain circumstances, including if hands are visibly soiled, where thorough hand washing with soap and water is preferable.[7] Current World Health Organisation (WHO) guidelines recommend that hand hygiene be performed in the following instances:[8]

1. Before touching a patient
2. Before clean/aseptic procedure
3. After body fluid exposure risk
4. After touching a patient
5. After touching a patient's environment

Hand hygiene should always be performed before donning and after removal of gloves. The importance of hand hygiene should be reinforced in an outbreak setting.[9]

National recommendations on hand hygiene were issued in 2005.[10] All staff working in the healthcare setting must receive appropriate training regarding hand hygiene opportunities and technique. This training must be repeated every one to two years with documentation of delivery of training and managers must ensure that staff are provided with the opportunity to attend training.[11]

Patients should be encouraged to practice good hand hygiene in an effort to reduce environmental contamination with MDROs. A hand hygiene information leaflet should be provided to the patient on admission to the healthcare facility.

1.4 Patient Placement and Priority for Isolation

Patients colonised or infected with an MDRO should be placed in individual single rooms with *en-suite* toilet facilities. When a sufficient number of single rooms is not available, priority for these rooms should be assigned according to a facility's HCAI strategy.[1,4]

Patient placement:
If single rooms are not available, patients carrying the same strain of MDRO may be cohorted in the same room after consultation with the local IPCT, ideally with dedicated nursing staff for the area.[1]

It is important that the quality of clinical care delivered to the patient should not suffer as a result of infection control interventions, such as placement in an isolation room.[9]

There should be adequate space around each bedspace to minimise the spread of infection. Many healthcare facilities fail to comply with the recommended specifications. National infection prevention and control building guidelines for acute hospitals in Ireland were issued in 2009.[12] These guidelines should be consulted when upgrading existing facilities or planning new units or hospitals.

Priority for isolation:
Factors that should be considered in determining isolation practices include:

- Healthcare facility type: Hospital versus long-term care facility
- Ward type: Non-acute, acute, critical care or other high-risk unit such as haematology, oncology or transplant ward, burns unit, neonatal intensive care unit (NICU)

- Facilities available for patient isolation: single rooms, *en-suite* toilet facilities, availability of dedicated commodes
- The nature of colonisation of the affected patient (whether the patient is likely to be a heavy disperser of the MDRO via uncontrolled secretions or excretions)
- Resistance pattern, virulence and potential transmissibility of the particular MDRO

A risk assessment should be performed by the IPCT and clinical team, taking into account the clinical needs of the patient, the background epidemiological picture and the risk category of the patient.
The highest priority for isolation should be given to those patients who have conditions which may facilitate transmission of an MDRO, i.e. those with uncontained excretions or secretions such as:

- Diarrhoea
- Draining wounds
- Incontinence of urine or faeces
- Copious respiratory secretions

It is not possible to be prescriptive for all circumstances as decisions need to be based on the local situation.[6] An isolation policy based on risk-assessment (e.g. Lewisham Isolation Prioritisation System-LIPS) has been implemented in a number of acute care facilities in the UK and Europe since 1999.[13] An 'isolation score' can be calculated based on the type of patient and the nature of the infecting organism.[14] While the system does not replace expert advice, it provides a framework to determine the priority of isolation. An excerpt from a recently revised version of the LIPS is included in Appendix 6.[15]

1.5 Personal Protective Equipment (PPE)

PPE refers to a variety of barriers used either alone or in combination to protect healthcare workers from contact with transmissible pathogens. These include single-use disposable gloves, aprons and long-sleeved gowns as well as facial protection for eyes, nose and mouth. PPE should be removed prior to leaving the isolation room and discarded into appropriate healthcare waste stream. Hand hygiene should always be the final step following removal and disposal of PPE.
Gloves: in addition to wearing gloves as outlined in Standard Precautions, gloves should be worn on entering an isolation room or cohort area and for all interactions that involve contact with the patient or items in close proximity to the patient (such as medical equipment, bed rails etc).[16]

- Gloves should be removed in the following circumstances:
  - After body fluid exposure risk
  - Before leaving the patient's environment (room or cohort bedspace)
- Gloves must be discarded between patients and must never be washed for re-use, as microorganisms cannot be reliably removed from glove surfaces and glove integrity may be compromised.
- It may be necessary to change gloves and perform hand hygiene during the care of a single patient in order to prevent cross-contamination of different body sites or before touching non-contaminated areas in the patient's environment.
- Gloves do not preclude the need for hand hygiene and this should always be performed after glove removal.[16]

Single use disposable aprons and long-sleeved gowns:
A disposable plastic apron and gloves should be donned before entering the room/cohort bed space of a patient infected or colonised with an MDRO. PPE should be changed between each patient in a cohort area and should be removed and discarded into appropriate healthcare waste stream prior to leaving the patient's room/bedspace, in order to prevent contamination of non-contaminated areas.[16] Hands should be decontaminated after PPE removal.

Aprons versus long-sleeved gowns
Aprons/long-sleeved gowns should be worn when contact with the patient and environment is anticipated. There is some evidence to suggest that the use of long-sleeved gowns may reduce contamination

of clothing of healthcare workers with MDRO, particularly during direct patient contact.[17,18] Healthcare workers should consider selecting long-sleeved gowns in preference to aprons if the level of anticipated environmental exposure may result in contamination of unprotected sleeves or arms when wearing an apron; or in situations where close physical contact with the patient is anticipated (e.g., paediatric setting, assistance with body care). The type of gown worn (i.e. whether fluid repellent) depends on the nature of the contact with the patient and the likelihood of exposure to an MDRO. There is considerable variation in the protection offered by gowns. Where extensive exposure to blood and body fluids is anticipated, fluid repellent gowns may be more appropriate and situations should be risk-assessed on an individual basis.[19]

Eye, nasal and mouth protection

Face masks and eye protection should be worn in accordance with Standard Precautions when performing splash-generating procedures, such as wound irrigation, oral suctioning, intubation, when caring for patients with open tracheostomies, where there is potential for projectile secretions and where there is evidence of transmission of MDRO from heavily colonised sites, such as an extensive burn wound. Masks are not otherwise recommended for healthcare workers carrying out routine care. Face masks should be single-use disposable and fluid resistant. Personal spectacles and contact lenses are not considered to provide adequate eye protection.[16]

1.6 Discontinuation of Isolation Precautions in Acute Hospitals

- Patients may remain colonised with MDRO for undefined periods of time and the appropriate duration of Contact Precautions for the types of MDRO discussed in this document has not been established. Shedding of MDRO may be intermittent and their presence may not always be detected by active surveillance cultures.[1]
- In general, it would seem advisable to continue Contact Precautions for all patients who have been previously infected with, or are known to be colonised with the MDRO addressed in this document for the duration of their admission.[1] On readmission rescreening is advised to facilitate an infection control risk assessment.
    - Carbapenemase-producing *Enterobacteriaceae*: No recommendations currently exist for the discontinuation of Contact Precautions during current or future admissions for patients colonised or infected with carbapenemase-producing carbapenem resistant *Enterobacteriaceae* (CRE). In consideration of the current epidemiology of carbapenemase-producing CRE in Ireland, it is recommended that patients known to be colonised or infected with carbapenemase-producing CRE should always be isolated on readmission and the decision to remove the patient from isolation should be taken following results of rescreening and IPCT risk assessment.
    - VRE: The 1995 Hospital Infection Control and Prevention Advisory Committee (HICPAC) guidelines for the prevention of transmission of VRE, suggested that Contact Precautions could be discontinued for patients known to be colonised with VRE after obtaining three negative stool/perianal surveillance cultures at weekly intervals. However, subsequent exposure of the patient to the selective pressure of further courses of antimicrobial therapy may lead to a recurrence of VRE colonisation as has been shown by a number of studies.[1] Therefore, the Committee decided for the purpose of this guideline document that patients found to be colonised/infected with VRE should be regarded as positive throughout their admission.
- The decision to discontinue patient isolation should always be made in conjunction with the IPCT and may need to be revisited in the event that the patient requires further antimicrobial therapy.

1.7 Cleaning of the Environment and Patient Care Equipment

The role of environmental reservoirs such as medical equipment and surfaces in the transmission of MDRO has been studied extensively.[1] Environmental contamination with MDRO is frequently due to a lack of adherence to policies and procedures for cleaning and disinfection.[1] While microbiological sampling of the environment is not recommended routinely, a number of studies have documented contamination of the environment with MDRO.[1, 21] Interventions which may reduce the risk of MDRO contamination of the environment include:

- Use of dedicated single-patient use non-critical medical equipment (blood pressure cuffs, thermometers etc.)
- Assignment of dedicated cleaning staff to areas where patients with MDRO are being cared for
- Increased cleaning frequency and enhanced attention to frequently-touched surfaces, such as bed rails, bed side chairs and door handles

Monitoring for compliance with local cleaning guidelines is important in controlling the transmission of MDRO in the healthcare environment.[1, 22] Although environmental screening is not routinely recommended, in the setting of ongoing transmission, it can be used to highlight deficient cleaning practices.[1,4] Even in the absence of known contamination with an MDRO, it should be hospital policy to clean the ward environment regularly and to audit cleaning in order to maintain appropriate standards of hygiene.[23] Daily cleaning of the isolation room with detergent and water should be sufficient with a terminal clean (i.e. cleaning and disinfection with for example a chlorine-releasing agent) being completed on transfer or discharge of the patient, in accordance with local hospital decontamination policy. Curtains should be changed at the time of terminal cleaning and according to local curtain change policy and particular attention should be paid to the cleaning of horizontal surfaces and dust-collecting areas such as radiators.[9]

Cleaning and disinfection of frequently-touched surfaces and equipment should be carried out on a more frequent schedule compared to that for minimal–touch areas. There is no evidence that one cleaning regimen is superior to another for eliminating the MDRO specified in this guidance. Medical devices (e.g., thermometers, sphygmomanometers, stethoscopes, blood glucose monitoring equipment) should be dedicated to single-patient use. If this is not possible, all devices should be decontaminated between patients in accordance with manufacturer's instructions and local policy. Wherever possible, consideration should be given to using disposable equipment.

During an MDRO outbreak, the entire ward environment may become heavily contaminated and may benefit from an enhanced cleaning schedule.[9] It is important that ward bed-pan washers be adequately maintained, especially when dealing with MDROs carried in faecal flora such as resistant *Enterobacteriaceae* and VRE. Where there are concerns regarding other MDR GNBs such as *Pseudomonas aeruginosa* or *Acinetobacter spp.*, efforts to reduce exogenous sources of these organisms should target moist environmental surfaces such as sinks and common-use devices with detergent cleaning and use of closed suctioning where possible.

Cleaning regimens should be in accordance with local hospital policy and should include the removal of organic material using a general purpose detergent.[6] It is essential that the proper amount, dilution and contact times for disinfectants are used consistently. Correct colour-coding system should be used for cleaning cloths or mops. Details of this system can be found in the National Hospitals Office Cleaning Manual for Acute Hospitals (2006).[24] Laundry should be treated as potentially infectious and managed as per Irish guidelines.[25]

1.8 Patient Movement and Transfer

The movement of patients with MDRO within a facility should be kept to a minimum to reduce the risk of cross-infection but this should not compromise other aspects of the patient's care.[6] Where patients need to attend departments for essential investigations or procedures, the receiving area should be notified of the patient's MDRO status in advance of transfer, and arrangements put in place to minimise contact with other patients and expedite the patient's journey through that department. Staff should adopt Contact Precautions when caring for the patient.[9] When a patient colonised or infected with an MDRO is transferred to another hospital or healthcare facility, the clinical team responsible for the patient should inform the receiving clinical staff of the patient's MDRO carriage status.

Surgical/invasive procedures:

- Patients colonised or infected with an MDRO do not need to be put last on the procedure list, if the procedure is carried out in a conventionally-ventilated operating theatre, which provides a recommended minimum of 20 air changes per hour, and cleaning and disinfection can be carried out adequately during a procedure list. 'Contaminated' air will have been significantly reduced after approximately fifteen minutes.[26]

- Appropriate signage should be placed on the theatre door to alert staff to the use of Contact Precautions.
- The operating theatre and equipment not to be sterilised (e.g., operating table) should be cleaned and disinfected between patients.
- The patient should be cared for in a designated area within the recovery department using Contact Precautions.

1.9 Endoscopy

Although the risk of transmission of MDRO carried in the intestine via endoscopy is low, several endoscope-related transmissions of resistant Gram-negative bacilli have been reported.[27, 28] Healthcare facilities should ensure that relevant staff understand the risks and take adequate precautions regarding decontamination of reusable invasive medical devices such as endoscopes, the details of which can be found in the publication "HSE Code of Practice for Decontamination of Reusable Invasive Medical Devices."[29] Special care should be taken to disinfect or protect delicate equipment used with endoscopes, such as cameras.[29]

1.10 Education of Patients, Staff, Visitors and Carers

A patient who is found to be newly-colonised or infected with an MDRO should be informed about his colonisation/infection status by the clinical team with appropriate documentation in the patient's healthcare record. The patient should be provided with information regarding the MDRO in question and advice regarding the prevention of transmission of the MDRO to other patients. An information leaflet should be given to the patient. All patients should be encouraged to perform hand hygiene after using the toilet and before meals. Visitors to the patient, as well as healthcare workers visiting the ward from other departments should be alerted to check with ward nursing staff for instructions prior to entering the room/cohort bedspace of a patient known to be colonised or infected with an MDRO.

1.11 Decolonisation

Decolonisation involves administration of treatment to patients colonised with a specific MDRO to eradicate carriage of that organism. Most healthcare facilities limit the use of decolonisation to those patients colonised with MRSA, where evidence exists for this intervention. Although attempts have been made to develop regimens for the decolonisation of patients with other MDRO, such as VRE, few have been successful. Currently there are no recommended regimens available for the routine decolonisation of patients harbouring MDRO other than MRSA.[1]

1.12 Healthcare Workers and MDRO

The bowel is the most frequent site of carriage of VRE and resistant Gram-negative bacilli. Asymptomatic carriage of these organisms by healthy individuals (including healthcare workers) is unlikely to cause them to become ill. To our knowledge, there have been no published reports to date, implicating staff bowel carriage of MDRO as a source of patient colonisation or infection with the MDRO discussed in this document.

Routine screening of healthcare workers for bowel carriage of MDRO is not considered to be helpful, may cause distress to healthcare workers, and is not generally recommended. Screening of healthcare workers for carriage of MDRO has on occasion been carried out in the context of an outbreak, as part of a multi-faceted epidemiological investigation, but its value is unproven.

In the absence of a decolonisation regimen with proven efficacy, the decision to screen healthcare workers for the organisms discussed in this document should only be undertaken following multi-disciplinary input and expert advice of an occupational health physician and an infection prevention and control professional. If healthcare workers found to be colonised with MDRO adhere strictly to Standard Precautions (including hand hygiene) and Contact Precautions where indicated, this should be effective in limiting the spread of MDRO in the healthcare environment.

1.13 Intensified Interventions to Prevent MDRO Transmission

A decision to employ additional MDRO control measures may arise from surveillance data and assessments of the risk to patients in various settings such as:

- When an MDRO is identified from even one patient in a unit or facility with a highly vulnerable patient population (ICU, NICU, Burns Unit) that had not previously encountered that MDRO.[1]
- There is failure to decrease the prevalence or incidence of an MDRO despite effective implementation of appropriate infection control interventions to limit transmission.

A risk assessment of the situation should be carried out along with an evaluation of the measures already in place. This requires input from the IPCT and the support of management and clinical staff in the healthcare facility.[9] Additional measures may include a combination of interventions including administrative, educational, surveillance, intensive antimicrobial stewardship as well as enhanced infection control measures as mentioned below. Various combinations of the above control measures have been shown to reduce MDRO in healthcare settings although their effectiveness has not been studied in clinical trials.[1]

Enhanced infection control precautions may include:

- Consider assigning dedicated nursing and ancillary staff to the care of patients with the MDRO. Some facilities may consider this option once transmission of an MDRO within the healthcare facility has been detected.
- Education of all staff, including cleaning staff, should be intensified.
- Cleaning and disinfection performance should be supervised and inspected, with particular attention to frequently-touched surfaces in the immediate patient environment. Cleaning and disinfection processes should be audited regularly and results reported back to cleaning staff, ward staff and hospital management.
- Environmental microbiological sampling may be considered when there is epidemiologic evidence that an environmental source may be associated with ongoing MDRO transmission. This may be of particular relevance when dealing with organisms associated with environmental reservoirs such as VRE and MDR-*Pseudomonas aeruginosa*.
- If there is failure to halt the spread of an MDRO within a ward or unit, it should be closed to admissions to limit further spread of the MDRO and to facilitate deep cleaning. This decision should be taken by senior hospital management on the advice of the IPCT or outbreak management team.
- Where there is failure to eliminate an environmental reservoir despite enhanced cleaning and disinfection, consideration may be given to the use of novel decontamination techniques, such as hydrogen peroxide vapour. This has been used successfully in the environmental management of *Clostridium difficile* and MRSA outbreaks. The main drawback with this technology is the need to vacate and seal clinical areas during use, which may be impractical where there are few single rooms and large multi-bedded wards.[30,31]

1.14 MDRO Infection Prevention and Control Measures for Settings Outside of Hospitals

MDRO-colonised patients in long-term care facilities

Patients colonised with an MDRO may be encountered in healthcare facilities outside of the hospital setting, including long-term care facilities (LTCF), such as nursing homes and residential care centres. Alternatively, they may be cared for in their own home. Good communication structures between acute and long-term care facilities are essential for appropriate initiation of infection control measures upon patient transfer.
A resident who is colonised with an MDRO should not be declined admission to a LTCF or have their admission delayed on the basis of colonisation status. However, strategies should be in place to control the spread of such organisms.

In general, residents of LTCF would have a lower risk of developing invasive infections than hospitalised patients. The management of residents of LTCFs who are colonised with an MDRO is quite different to that in the hospital setting. The implementation of infection control precautions at a level required in the hospital setting may have adverse psychological consequences for the nursing home resident, where the facility is also

their home.[30] However, all healthcare facilities should endeavour to prevent transmission of MDRO. Residents of LTCFs are frequently hospitalised. There may be frequent transfer of MDRO-colonised patients between LTCFs and hospitals. All healthcare facilities, whether hospitals or non-acute facilities should have an infection control programme in place, ideally incorporating the following:

- A process for monitoring infection control problems, including outbreaks of MDRO
- Education of health care staff in infection control practices, to include Standard and Contact Precautions and hand hygiene training.
- A programme for the development and updating of policies and procedures.
- Dedicated formal access to microbiology and infection prevention and control advice
- Dedicated formal access to occupational health services
- An active antimicrobial stewardship programme

Standard Precautions should be implemented by all healthcare workers when dealing with all patients in all healthcare facilities regardless of whether they are infected or colonised with an MDRO.[32]
Hand hygiene should be performed as discussed in chapter 1.3.

The decision to isolate a resident must be considered carefully and should take into account the infection risks to other residents, the presence of risk factors that increase the likelihood of transmission, and the psychological effects of isolation on the colonised or infected resident. Before isolating a resident, a plan to review the need for ongoing Contact Precautions must be in place. The following scenarios may arise:

a) Relatively healthy independent residents colonised with an MDRO:
Standard Precautions should be sufficient, ensuring that single-use disposable gloves and aprons are used when dealing with uncontrolled secretions, draining wounds, stool, ostomy bags or tubes and pressure ulcers.

b) Ill dependent residents OR residents with uncontrolled secretions/excretions OR residents suffering from an infection caused by an MDRO:
Contact Precautions are recommended in this situation. Single room accommodation is preferable, if available. If single rooms are not available, cohorting of patients known to be colonised or infected with the same MDRO is acceptable. If cohorting is not possible, then those residents colonised/infected with an MDRO should be placed in a room with a resident considered to be at low risk for acquisition of an MDRO (i.e. not immunocompromised, not on antimicrobials, without open wounds, drains or urinary catheters) or those who have an anticipated short duration of stay.

The mobile resident who is incontinent, confused and perhaps wandering, poses a particular infection control problem when colonised with an MDRO. Decisions regarding the best precautions to use for a patient with an MDRO may need to be made on a case-by case basis.[32]

Other aspects of control of MDRO in LTCFs include:

- Maintaining a list of residents infected/colonised with an MDRO
- Monitoring microbiology culture results of specimens sent to the local microbiology laboratory
- Communication of information relating to the status of an MDRO colonised resident to other receiving or transmitting facilities where indicated, such as upon referral to hospital or other healthcare facilities
- Ensuring adequate environmental cleaning

If the spread of an MDRO within a LTCF is not controlled by the infection control precautions mentioned above, intensified infection control measures may be required and expert advice should be sought.

MDRO-colonised patients in the home
Good communication between hospitals, MDRO-colonised patients, their families and general practitioners is essential. Patients should be informed that the risk to healthy family members is extremely low. Standard Precautions, hand hygiene and normal cleaning are sufficient as infection control measures in the home. Single-use patient care equipment should be used where possible. The amount of re-usable patient care

equipment brought into the home should be limited. Where possible, dedicated patient care equipment should be used, which should remain in the patient's home until they are discharged from the home-care service. Where equipment cannot be left in the patient's home (stethoscopes), they should be cleaned and disinfected before leaving the patient's home. Alternatively, the item of equipment should be placed in a plastic bag for transport to another site for cleaning and disinfection.[1] A leaflet providing general advice for patients discharged from hospital has been published by the Royal College of Physicians of Ireland in 2011.[33]

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2. Vancomycin-Resistant Enterococci

2.1 Recommendations for the control of VRE

Laboratory detection of VRE from clinical samples

- Enterococci should be tested for glycopeptide resistance according to EUCAST or CLSI standardised protocols, whenever susceptibility testing is indicated.

Laboratory detection of VRE from screening samples

- Rectal swab or faeces is the recommended specimen for the purpose of surveillance for VRE. Specimen taken from other sites (e.g. urine, drain fluids) may also be suitable for surveillance purposes.
- Considering the local epidemiology and available resources, active surveillance cultures should be undertaken on the following patient groups:
  - Patients admitted to high risk areas (ICU, haematology/oncology, transplantation) with weekly screening thereafter.
  - Patients known to be previously VRE positive, upon re-admission to hospital to facilitate an infection control risk assessment.
  - Patients transferred from another Irish hospital or patients transferred from any hospital abroad.
  - Where appropriate, 'at risk' patients who have been contacts of known VRE positive patients during an outbreak of VRE.

During an outbreak setting, environmental screening may be considered, targeting frequently-touched surfaces, such as bed rails, bed side chairs and door handles.

Infection prevention and control in the hospital setting

- In hospitals, patients with VRE should be considered to be colonised for the duration of their admission. No further screening specimen need to be taken.
- Ideally, every patient who is colonised or infected with VRE should be isolated in a single room with *en-suite* facilities. Contact Precautions should be applied. If limited isolation facilities are available, a local risk assessment should be undertaken in conjunction with the IPCT (e.g., Lewisham Isolation Prioritisation System: See Appendix 6).

Infection prevention and control in settings outside hospitals

- Isolation of a resident with VRE is generally not required in LTCF (see chapter 1.4 patient placement and priority for isolation).
- Routine screening for VRE is not recommended for LTCF.

2.2 Background

Enterococci form part of the normal flora of the human gastrointestinal tract. The genus includes over 17 species, of which *Enterococcus faecium* and *Enterococcus faecalis* are the most prevalent cultured from humans, accounting for greater than 90% of clinical isolates. Other species implicated in human infection include: *E. durans*, *E. raffinosus*, *E. avium* and *E. gallinarum.* Enterococci are important healthcare-associated pathogens. Enterococci have intrinsic resistance to many antimicrobials such as cephalosporins and macrolides and thus have a selective advantage in the healthcare setting where the frequent use of such agents facilitates their emergence.[1] Acquired resistance, most commonly to amoxicillin, aminoglycosides (high level resistance) and glycopeptides is increasing.

Glycopeptides such as vancomycin and teicoplanin have been the treatment of choice for invasive infections due to *E. faecium* as these organisms are frequently resistant to amoxicillin. For the purposes of this document, the term VRE is used to describe enterococci that exhibit resistance to glycopeptide antimicrobials.
Initial reports of VRE first emerged from England and France in 1988 and from the United States in 1989.[2] Emergence of enterococci with acquired resistance coincided with an increase in the global usage of glycopeptides for the treatment of infections caused by MRSA, resistant coagulase negative staphylococci and

Clostridium difficile. The major reservoir of vancomycin resistance is *E. faecium*. Vancomycin-resistant *E. faecalis* is still uncommon.

Six phenotypes of vancomycin resistance termed vanA, vanB, vanC, vanD, vanE and vanG have been described.[3]

The three major phenotypes are vanA, vanB and vanC.

- vanA is the most commonly encountered resistance mechanism. Enterococcal isolates that have acquired this mechanism of resistance exhibit high level resistance to both vancomycin and teicoplanin.
- vanB isolates have variable resistance to vancomycin and remain susceptible to teicoplanin. Both vanA and vanB phenotypes are typically associated with mobile genetic elements (transposons).[1]
- The vanC phenotype is constitutively present in *E. gallinarum* and *E. casseliflavus*; these genes confer relatively low resistance levels to vancomycin and are not transferable.
- To date, the vanD, vanE and vanG phenotypes have only been described in a few strains of enterococci.

In Europe the use of avoparcin, a glycopeptide antimicrobial used as a growth promoter for livestock has been proposed to explain the epidemiology of VRE. Until banned by the European Union in 1997, avoparcin had been used in several European countries and provided a selective pressure for the emergence and spread of vancomycin resistance genes.

2.3 Epidemiology

In 2010, the majority of countries (22 of 28) participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) reported vancomycin resistance rates of less than 10% in enterococcal BSI isolates. Ireland is the country with the highest proportion of vancomycin resistance in enterococcal BSI isolates in Europe, reporting a rate of 39% in 2010, followed by Portugal 24% and Greece 23%.[7] In the US, the National Healthcare Safety Network (NHSN) reported in 2008 a vancomycin resistance rate among *E. faecium* isolates from healthcare-associated infections of 80%.[8]

Ireland has contributed resistance data for enterococci to EARS-Net since 2002 with all clinical microbiology laboratories participating. Worryingly, Ireland has been the country with the greatest percentage of VRE isolated from patients with BSI in Europe since 2008 (Figure 1).

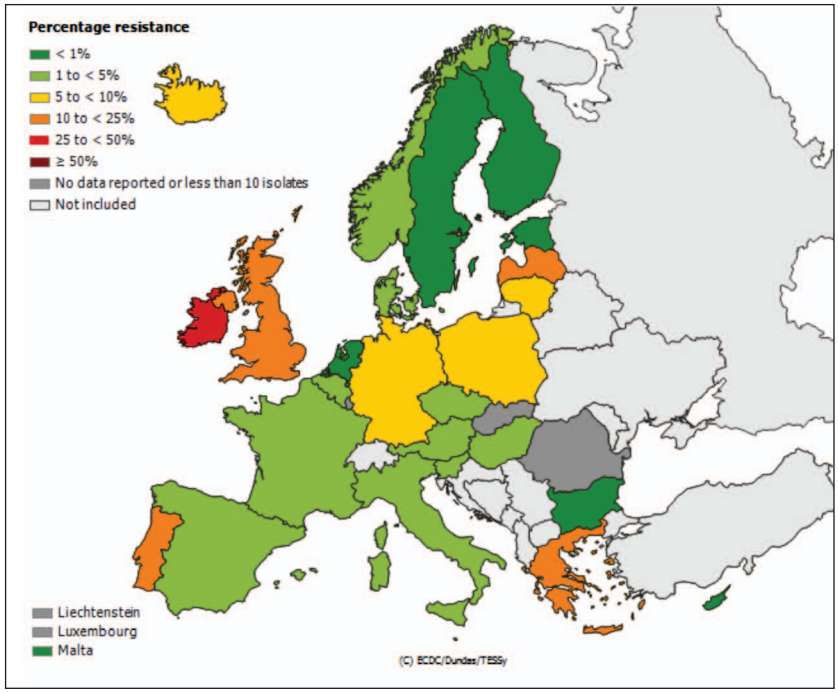

Figure 1: Vancomycin resistance among *E. faecium* BSI isolates reported to EARS-Net in 2010. Map downloaded from ECDC's TESSy database on 14/12/2011: http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx

The proportion of *E. faecium* that are resistant to vancomycin has increased from 11% in 2002 to 37.4% in 2011 (Figure 2). In addition, the true burden of disease caused by VRE in Irish hospitals is likely to be significantly greater than that reflected by analysis of BSI data alone. An anonymous surveillance screening study conducted in an Irish tertiary referral hospital during 2010 identified a VRE carriage rate of nearly 40% among 200 in-patient stool specimens (Wrenn C, personal communication). Although 10-20 times more patients are colonised than infected with VRE, this data suggests that VRE is endemic to the Irish hospital setting. To halt or diminish the rise of VRE in Irish hospitals, national VRE surveillance and epidemiological typing studies are urgently required.

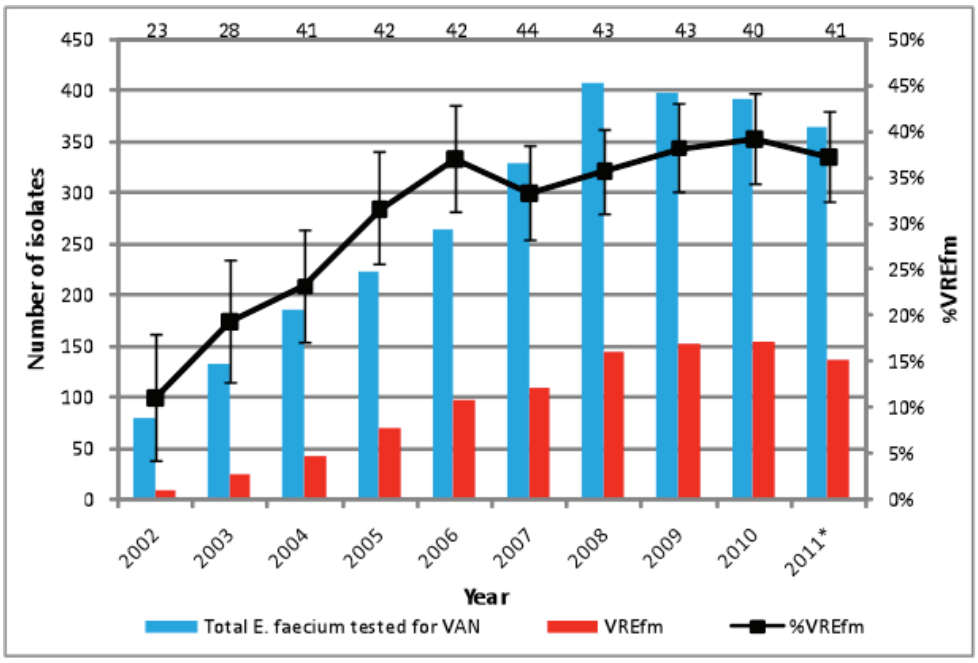

Figure 2: Trends for *E. faecium* by time period: by year for 2002–2011*: Total numbers of *E. faecium*/VREfm and percentage VREfm with 95% confidence intervals.
The numbers of participating laboratories by year-end are indicated above the bars. VAN, vancomycin; VREfm, vancomycin-resistant *E. faecium*
*2011 data are provisional as of 31st March 2012, data from www.hpsc.ie

Risk factors and mode of transmission

The gastrointestinal tract is the most important reservoir for VRE. The patient's environment can subsequently become contaminated with VRE, particularly when patients have diarrhoea. Enterococci can survive for long periods on environmental surfaces, a factor which contributes to transmission.

The most frequent mode of transmission is via the hands of healthcare workers.[9, 10] Hands are easily contaminated during the process of care-giving or from contact with environmental surfaces in close proximity to the patient. Enterococci may contaminate the environment around a patient and long-term persistence in the environment for several weeks has been described.[11] Environmental contamination is increased when patients have diarrhoea.[12, 13]

Increased rates of VRE usually occur within environments where there is heavy use of glycopeptides, for example in renal, liver, haematology, oncology, transplant and critical care units. VRE have frequently been associated with outbreaks in these settings.[3, 14] Prior receipt of antimicrobials to which enterococci are intrinsically resistant to (e.g., cephalosporins, macrolides) and readily acquire resistance to (e.g., fluoroquinolones) as well as glycopeptide use have also been described as risk factors for acquisition of VRE.[11, 14] Proximity to a patient with VRE and prolonged length of hospital stay has been associated with VRE.[15] Malignancy, receipt of enteral feeding, gastric acid suppression, central vascular catheters, increased morbidity, as measured by increased Acute Physiology and Chronic Health Evaluation (APACHE) score, renal failure, mechanical ventilation, neutropenia, organ transplantation and haematological malignancy have been identified through various studies as independent risk factors for VRE colonisation.

2.4 Clinical Significance

Gastrointestinal colonisation with VRE may persist for long periods of time and serves as a reservoir for transmission of VRE to other patients.[16]

VRE infection develops in VRE-colonised patients, with the ratio of infected to colonised patients being dependent on the specific patient population (i.e. healthy, immunocompetent individuals are at lower risk of infection).

Vancomycin resistance has been shown to be an independent predictor of death in enterococcal BSI.[17] In a study comparing the prognosis of patients with vancomycin-resistant versus vancomycin-susceptible enterococcal BSI, clinical failure was higher for patients with VRE BSI (60% versus 40%, *P* <0.001).[18] All-cause mortality was also higher for patients with VRE BSI (52% versus 27%, P < 0.001).[18] In another study, patients with VRE bacteraemia had longer in-hospital stays and costs than those with vancomycin-susceptible enterococcal BSI.[19] Despite similar severity-of-illness scores, survival was lower in patients with vancomycin-resistant versus vancomycin-susceptible enterococcal BSI (24% versus 59%, P <0.009). In 62% of the patients with VRE BSI, death was related to infection.[19]

It has been suggested that the high mortality rates of VRE BSI could be due to the fact that the patients who develop VRE infections have a more complicated medical course, and therefore are at higher risk of dying. Regardless, VRE has become a common cause of HCAI and treatment of VRE infections is becoming increasingly more challenging due to the emergence of resistance. Adherence to strict IPC measures, surveillance, and prudent antimicrobial prescribing remain the most effective methods of control.

VRE and Vancomycin-resistant *Staphylococcus aureus (VRSA)*
The *vanA* gene readily spreads among enterococci. To date, there have been only sporadic cases of *Staphylococcus aureus* isolates harbouring the *vanA* gene resulting in emergence of vancomycin-resistant *Staphylococcus aureus (*VRSA). Twelve VRSA infections have been reported in the US up to 2010.[20, 21] All of the isolates carried the *vanA* gene, found in vancomycin-resistant enterococci. The possibility of horizontal transfer between patients and establishment of endemic VRSA strains is of extreme global concern. No transfer of VRSA between patients has been documented so far.

2.5 Laboratory Detection

Enterococci are easily grown on a variety of culture media. Any media containing 5% animal blood can be used. After 18-24 hour incubation at 35-37°C, colonies are 1-2 mm and appear α, β or non-haemolytic on horse blood agar. Most enterococci express the group D Lancefield antigen, however some cross-react with Lancefield group G antiserum. Enterococci grow well in the presence of bile and may be differentiated from other streptococci by rapid hydrolysis of aesculin in the presence of bile. Enterococci are also PYRase positive which further differentiates them from *Streptococcus bovis* and *Streptococcus gallolyticus.*

Detection from clinical samples
National recommendations on whether all enterococcal isolates should be screened with vancomycin susceptibility testing differ. Australian guidelines advise testing all enterococcal isolates with vancomycin[22], others recommend that enterococci from clinically relevant sites and according to local policies should be tested.[23-24]

The conclusion of this committee is that enterococci should be tested for glycopeptide resistance according to EUCAST or CLSI standardised protocols, whenever susceptibility testing is indicated. Identification of enterococci to species level is not routinely required if antimicrobial susceptibility testing is performed. Colonies which appear resistant to vancomycin should be further investigated.[25] Minimum inhibitory concentrations (MIC) can be determined using agar gradient dilution or broth microdilution.[26] Automated systems are also available for both identification and susceptibility testing of VRE.

Identification of VRE to species level aids in confirming whether an isolate has intrinsic (vanC) or acquired resistance (*van*A or *van*B). Genotyping of the van gene can be performed using molecular methods,[27] however phenotypic methods such as MIC testing by gradient methods have been shown to have 100% sensitivity and

specificity for identification of the van type when compared to a PCR-based genotypic method.[28] Typically vanA phenotypes have vancomycin MICs of 64-1000mg/L and teicoplanin MICs of 16-512mg/L, whereas vanB phenotypes have vancomycin MICs of 4 –32mg/L and teicoplanin MICs of 0.5-1.0mg/L.

Detection from screening samples
Historically, agars such as Bile Aesculin Azide (BEA) agar, Slantez and Bartley agar and Kanamycin aesculin azide agar were used to screen patients for VRE colonisation. Such agars selectively recover enterococci. However, with the emergence of VRE, vancomycin has been incorporated into the media e.g., BEAV (BEA with 6 or 8 mg/mL of vancomycin) or alternatively, a vancomycin disc was placed on media to screen for VRE. Enterococci that exhibit intrinsic resistance to vancomycin also grow on these screening media (*E. gallinarum* and *E. casseliflavus).* Acidification of methyl-α-D-glucopyranoside (MGP) can be used for differentiating enterococci intrinsically resistant to vancomycin, from vancomycin-resistant *E. faecalis* and *E. faecium.*[29] Chromogenic media have been developed for VRE. These media incorporate enzymatic substrates and antimicrobial agents for the rapid detection and identification of VRE.[30] Isolates which are presumptive VRE on these media must be identified to species level and vancomycin resistance must be confirmed.

Molecular assays have been developed to rapidly screen for VRE. However, routine clinical microbiology laboratories may not have access to the equipment or finances for molecular screening.[31-32] A study comparing a molecular assay for vanA/vanB genes with conventional culture methods showed that there were disparate results. This was due to the fact that the *vanB* operon is naturally occurring in obligate anaerobes, e.g., *Clostridium* spp.[33] It is therefore recommended that molecular assays are used as a rapid screening tool but positive results must be confirmed using conventional techniques. Gradient MIC testing in combination with a screening agar are a cost-effective way to identify the vanA and vanB phenotypes in *E. faecium* and *E. faecalis.*[29]

Detection from environmental screens
Many outbreak investigations have shown that the environment can be heavily contaminated with VRE and environmental screening might be indicated as part of an outbreak investigation.[33] Studies have indicated that VRE can survive for long periods of time on inanimate surfaces and that occupancy of a room previously occupied by a VRE-positive patient is a risk factor for VRE colonisation.[34] *E. faecalis* can survive up to five days on an inanimate surface (e.g., handrail), while *E. faecium* can survive for up to seven days.[12]

Environmental recovery rates of traditional swabbing methods, e.g., cotton or rayon, are poor. Flocked nylon swabs can enhance the recovery by up to three times compared with a rayon/cotton swab.[35] The tip of the swab should be moistened in sampling solution (e.g., nutrient broth) before swabbing the surface. Swabs should then be inoculated in brain heart infusion (BHI) broth overnight at 35°C and subcultured on a selective media.[36] All presumptive VRE isolates should be identified to species level and vancomycin resistance confirmed.

2.6 Infection Prevention and Control

Detailed information on infection control measures within and outside the acute hospital setting is provided in Chapter 1 of this document. A summary of Contact Precautions is given in Appendix 5. Guidance for an infection control risk assessment is given in Appendix 6.

Active surveillance cultures for VRE
Screening patients for rectal carriage of VRE using active surveillance cultures increases VRE detection rates approximately three-fold above detection rates from clinical specimens alone.[37] Most studies reporting on the use of active surveillance cultures have used these in combination with other infection prevention and control interventions.[38] Experience from an Irish tertiary care referral centre showed that active surveillance cultures in combination with additional infection prevention and control interventions can control VRE colonisation and subsequent BSI.[39]

Considering the high proportion of VRE among enterococcal BSI, the colonisation pressure for VRE in Irish hospitals and the vulnerability of high-risk patients to VRE infection, the Committee advises active surveillance cultures on admission and weekly screening thereafter for patients admitted to high-risk units (ICU, haematology/oncology and transplantation).

In 2011, a survey of 37 Irish hospitals, incorporating 44 critical care units, reported that active surveillance cultures for VRE were performed in only 40% of critical care units (K. Burns, personal communication). Local hospital epidemiology and local risk factors may identify additional high-risk groups for VRE infection, where establishment of active surveillance cultures may prove useful.

Decolonisation

Long-term success of VRE decolonisation strategies has not been proven.[11, 38] The Committee recommends that patients with VRE should be considered to be colonised for the duration of their hospital admission. Screening does not need to be repeated on patients found to be positive during the same admission. Screening should be repeated upon readmission to the hospital to facilitate an infection control risk assessment. While awaiting screening results, patients should ideally be isolated and Contact Precautions implemented.

Enterococci with intrinsic vancomycin resistance

E. gallinarium and *E. casseliflavus* carry the vanC gene and cannot readily transfer this resistance gene to other enterococcal isolates. From a laboratory perspective, isolates with intrinsic resistance will grow on screening media and exhibit elevated MICs to glycopeptides. Where laboratories can reliably differentiate between intrinsic and acquired vancomycin resistance, Contact Precautions do not need to be implemented for patients with vanC isolates.

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3. Resistant *Enterobacteriaceae*

3.1 Recommendations for the control of Resistant *Enterobacteriaceae* in healthcare settings

Laboratory detection of resistant *Enterobacteriaceae* from clinical samples

- *Enterobacteriaceae* should be tested for 3rd generation cephalosporin resistance according to EUCAST or CLSI standardised protocols, whenever susceptibility testing is indicated.
- Laboratories should incorporate phenotypic methods for the detection of ESBLs as part of standard susceptibility testing procedures.
- Laboratories should consider the need and the feasibility for detection of plasmidic AmpC enzymes based on local epidemiology and available resources.
- Laboratories should ensure prompt notification of their local IPCT when ESBL/plasmidic AmpC producing *Enterobacteriaceae* are isolated.
- Invasive *Escherichia coli* and *Klebsiella pneumoniae* infections (isolated from blood or cerebrospinal fluid) are notifiable infections.

Laboratory detection of CRE (carbapenem resistant *Enterobacteriaceae*) from clinical samples

- Where antimicrobial susceptibility testing is indicated, laboratories should test *Enterobacteriaceae* isolates from all anatomical sites with at least one carbapenem.
- It is recommended that alert criteria for the suspicion of CRE be incorporated into the test system(s) of the laboratory.
- All laboratories should aim to gain competency in identification and phenotypic preliminary analysis of CRE isolates.
- All CRE isolates with presumptive phenotypic identification of carbapenemase production should be sent for molecular confirmation of resistance mechanism to a reference laboratory.
- The IPCT should be promptly informed as soon as carbapenem resistance is suspected or detected and this should never be deferred pending reference laboratory confirmation.
- Invasive infections with carbapenemase-producing CRE are notifiable. An enhanced CRE patient surveillance form should be completed for every patient who is identified as being colonised or infected with carbapenemase-producing CRE, once confirmed by molecular analysis (Appendix 12).

Laboratory detection of resistant *Enterobacteriaceae* from screening samples

- A rectal swab or faeces is the recommended specimen for the purpose of surveillance for resistant *Enterobacteriaceae*. Additional specimens taken from other sites (e.g., urine, swabs from skin breaks or manipulated sites) may also be suitable for surveillance purposes.
- Taking the local antimicrobial resistance epidemiology and available resources into account, patients admitted to high risk areas (ICU, haematology/oncology, organ transplantation) should be considered for routine surveillance for the carriage of resistant *Enterobacteriaceae* (including ESBL, plasmidic AmpC and CRE) on admission and weekly thereafter.
- In addition routine CRE screening is advised for the following at-risk patient groups
    a. Any patient with a history of admission for more than 48 hours to a named Irish healthcare facility reporting an outbreak of CRE in the past 12 months – See http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCRE/ScreeningforCREinIreland/ for latest list of named healthcare facilities.
    b. Any patient with a history of admission for more than 48 hours to a foreign healthcare facility in the past 12 months.
    c. Any patient transferred/repatriated from a healthcare facility in any foreign country.

d. In a patient who has attended either (i) an Irish healthcare facility reporting a CRE outbreak or (ii) a foreign healthcare facility for less than 48 hours or as a day case, the decision to perform CRE screening should be undertaken by the local IPCT following risk analysis.

e. Any patient previously identified as colonised or infected with CRE, upon readmission to hospital

- In an outbreak due to resistant *Enterobacteriaceae,* protocols should be in place for screening patients epidemiologically linked to patients positive for resistant *Enterobacteriaceae*.
- In a situation where carbapenemase-producing CRE has been isolated from a clinical specimen in an index case, rectal surveillance for CRE is recommended for patients epidemiologically linked to the index case within the ward/unit. If there is evidence of CRE cross-transmission, weekly surveillance in the unit should be performed until no further cases have been identified and the local IPCT is satisfied that cross-transmission has ceased.
- Patients found to be colonised with resistant *Enterobacteriaceae* do not need to be rescreened during the same admission. On readmission to a healthcare facility rescreening is advised to facilitate an infection control risk assessment, considering local circumstances. In consideration of the current epidemiology of carbapenemase-producing CRE in Ireland, it is recommended that patients known to be colonised or infected with carbapenemase-producing CRE should always be isolated on readmission and the decision to remove the patient from isolation should be taken following results of rescreening and IPCT risk assessment.
- Screening of healthcare workers for carriage of resistant *Enterobacteriaceae* is rarely indicated unless on the advice of a multi-disciplinary expert group (including occupational health physician and IPCT). Healthcare worker screening may be advised in exceptional circumstances, such as ongoing transmission of resistant *Enterobacteriaceae,* despite the implementation of active control measures.
- Patients should be informed of their positive status for colonisation or infection with a resistant *Enterobacteriaceae* upon laboratory confirmation of ESBL isolates and upon molecular confirmation of plasmidic AmpC or carbapenemase-producing CRE isolates. This information should be recorded in the patient's healthcare record. The patient should be provided with an information leaflet (Appendix 9, 10).
- During an outbreak, environmental screening targeting frequently-touched surfaces may be considered.

Infection prevention and control recommendations in the hospital setting for patients with ESBL/plasmidic AmpC and/or CRE

- Patients carrying resistant *Enterobacteriaceae* (ESBL, plasmidic AmpC and suspected/confirmed CRE) should be isolated in single rooms with *en-suite* toilet facilities using Contact Precautions.
- If the availability of isolation facilities is limited, a risk assessment should be carried out in conjunction with the IPCT. Priority for isolation should be given to patients with diarrhoea, faecal/urinary incontinence, copious respiratory secretions and draining wounds.
- There is insufficient evidence on decolonisation regimens for resistant *Enterobacteriaceae*. Attempts to decolonise patients are therefore not recommended.
- Rectal colonisation of healthcare workers with resistant *Enterobacteriaceae* has not yet been implicated in transmission. Healthcare workers found to be colonised with resistant *Enterobacteriaceae* should adhere to Standard Precautions, including optimal hand hygiene practices at all times.

Additional infection prevention and control recommendations in the hospital setting for CRE-positive patients

- As most cases of infection or colonisation with carbapenemase-producing CRE in Ireland are currently sporadic, any patient with suspected CRE in a clinical or surveillance specimen should be isolated with strict application of Contact Precautions, pending reference laboratory confirmation of carbapenemase production.
- Any patient considered to be at risk of CRE carriage (see above) should ideally be isolated with the application of Contact Precautions, pending the results of the rectal screening swab.
- It is recommended that dedicated staffing be arranged for the direct care of patients colonised or infected with carbapenemase-producing CRE. If the implementation of such a staffing arrangement is limited by local resource constraints, a risk assessment should be performed by the IPCT in conjunction with the clinical team and hospital management.

Infection prevention and control recommendations for resistant *Enterobacteriaceae* in settings outside of the hospital

- Isolation of a resident with resistant *Enterobacteriaceae* is generally not required in LTCF (see chapter 1.4 Patient placement and priority for isolation).

3.2 Background

Enterobacteriaceae is a term used to describe groups of Gram-negative bacilli that commonly live in the gastrointestinal tract and includes organisms such as: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundii*. β-lactams are a group of antimicrobials that comprise some of the most commonly used agents for treatment of infection, such as penicillins, cephalosporins, monobactams and carbapenems. The production of enzymes, known as β-lactamases by *Enterobacteriaceae* is a key mechanism for the development of resistance to the various types of β-lactam antimicrobials. Today many β-lactamases exist, including extended spectrum β-lactamases (ESBL), AmpC β-lactamases and carbapenemases.[1-3] These enzymes have varying spectra of hydrolytic activity and are frequently located on mobile genetic elements, known as plasmids, enhancing their transmissibility.

Resistant *Enterobacteriaceae* described in this document include broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC β-lactamases) and carbapenem-resistant *Enterobacteriaceae* (CRE), which encompasses carbapenemase producers and combinations of broad spectrum β-lactamases with loss of bacterial cell permeability or porins.

It is important to consider that antimicrobial resistance is a continuously evolving process. The emergence of CRE is clearly associated with increasing carbapenem use and the resulting selective pressure. The increasing carbapenem use is due to an increase in ESBL and AmpC-producing *Enterobacteriaceae*.[4] Therefore, to halt spread of CRE, it is crucially important to also address the spread of ESBL and AmpC-producing *Enterobacteriaceae*.

Broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC)

The first plasmid-mediated β-lactamase in *Enterobacteriaceae*, TEM-1, was described in the 1960s and had a narrow spectrum of hydrolytic activity directed mainly against penicillins. Since then, β-lactamase variants with expanded spectra of activity have been increasingly reported, and are known as extended spectrum β-lactamases (ESBLs).[1] ESBLs are generally located on plasmids, and are therefore easily spread between bacteria. ESBLs confer resistance to a range of β-lactam antimicrobials including broad spectrum third- and fourth-generation cephalosporins such as cefotaxime, ceftazidime, cefpodoxime and cefepime.[1] They may also confer resistance to monobactams, such as aztreonam and to β-lactam/β-lactamase-inhibitor combinations such as amoxicillin-clavulanate and piperacillin-tazobactam. Antimicrobial susceptibility test results require careful interpretation, as despite *in vitro* susceptibility, the therapeutic usage of third generation cephalosporins and β-lactam/β-lactamase-inhibitor combinations may lead to treatment failure.

A second group of broad spectrum β-lactamases are AmpC β-lactamases.[2] In contrast to ESBL enzymes, AmpC enzymes are commonly found on chromosomes of many clinically relevant species within the *Enterobacteriaceae* family, such as *E. coli*, *Enterobacter* spp., *Citrobacter freundii*, *Serratia marcescens, Shigella* spp., *Providencia stuartii* and *Morganella morganii*. Expression of chromosomal AmpC enzymes in many *Enterobacteriaceae* is usually low but inducible in response to exposure to β-lactam antimicrobials. Inducible AmpC enzymes confer resistance to β-lactams such as amoxicillin, amoxicillin-clavulanate, cefoxitin and cephalothin. Mutations can occur in the genes regulating the expression of AmpC β-lactamases, resulting in persistent over-production of these enzymes.[2] Such organisms are frequently referred to as AmpC hyper-producers or AmpC derepressed mutants. Derepressed mutants are frequently resistant to cephalosporins as well as other β-lactams such as aztreonam and piperacillin.

In recent years increasing numbers of AmpC β-lactamase genes have been mobilised onto plasmids, which are subsequently transferred to species such as *K. pneumoniae*. Plasmidic AmpC enzymes represent yet another group of transferable resistance determinants, but are so far, less frequently reported than ESBLs. Both ESBL and AmpC enzymes confer resistance to third generation cephalosporins, the term 3GC-resistance is sometimes used to encompass both resistance mechanisms.

Carbapenem resistant *Enterobacteriaceae* (CRE)

As a result of increasing resistance to various groups of β-lactams due to ESBLs and AmpC enzymes, there is increasing reliance on carbapenems for the treatment of infections caused by *Enterobacteriaceae* and other Gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. Carbapenems include meropenem, ertapenem, imipenem and doripenem. Over the last decade, there has been an alarming rise in the reports of carbapenem resistant *Enterobacteriaceae*.[3]

There are two major forms of carbapenem resistance in *Enterobacteriaceae:*

- The production of a broad spectrum β-lactamase enzyme (carbapenemase) that cleaves the carbapenem antimicrobial rendering it irreparably damaged and ineffective.
- The combination of broad spectrum β-lactamase (ESBL/AmpC) production with decreased permeability of the bacterial cell wall for the antimicrobial due to porin loss.

The term CRE as used in this document encompasses *Enterobacteriaceae* with both resistance mechanisms, although emphasis has been put on the laboratory detection and infection control measures recommended for carbapenemase producers, as these enzymes are located on plasmids, like ESBL/plasmidic AmpCs, and therefore have an enormous potential for dissemination.

The majority of CRE are also resistant to other commonly used groups of antimicrobials such as fluoroquinolones and aminoglycosides. Consequently, clinicians are increasingly depending on less commonly antimicrobials such as colistin and tigecycline in the treatment of infections caused by CRE.

3.3 Epidemiology

Broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC)

Since the 1980s, ESBLs have been increasingly detected in *Enterobacteriaceae*.[1,5] ESBLs have disseminated worldwide.[1] Over the last 15 years in Europe, CTX-M ESBLs have become the predominant type of ESBLs, occurring mainly in *E. coli.* ESBLs are widely disseminated in both hospital and community settings.[6]

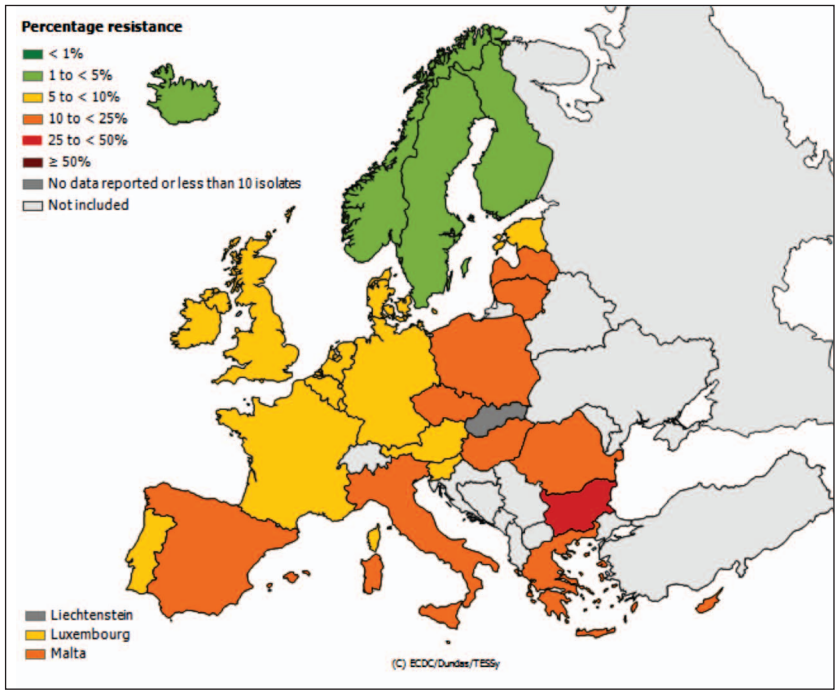

Figure 3: 3rd generation cephalosporin resistance among invasive *E.coli* BSI isolates reported to EARS-Net in 2010. Map downloaded from ECDC's TESSy database on 14/12/2011: http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx

In Ireland, ESBLs have been well documented in hospitals and nursing homes.[5] CTX-M enzymes have become the predominant ESBL type in Ireland.[5] The number of ESBL-positive BSI isolates has been continuously increasing over the last decade (Figure 4). As a significant proportion of infections caused by ESBL-positive

Enterobacteriaceae originate in the community setting, the true burden of disease is likely to be significantly greater.[7] A study investigating ESBL rectal carriage in intensive care, haematology/oncology and solid organ transplant patients at an Irish tertiary care hospital revealed an ESBL carriage rate of 10% (Niamh O'Connell, personal communication). This finding is similar to data from other European countries reporting that 3-9% of patients admitted to critical care units carry ESBL-positive *Enterobacteriaceae*.[8]

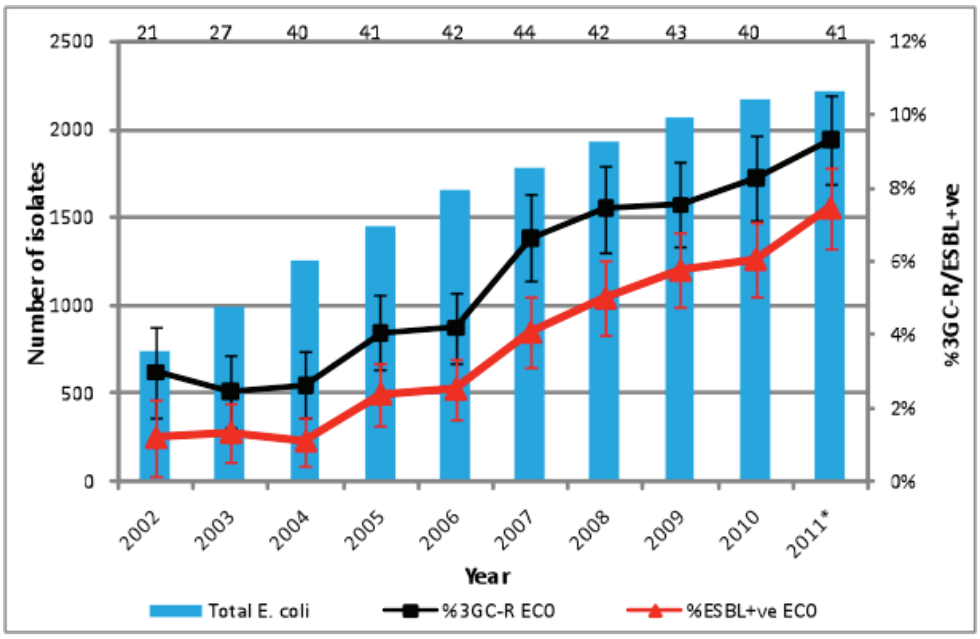

Figure 4: Trends for ESBL/3GC-R (3rd generation cephalosporin resistance) *E. coli* –total number of *E. coli* isolates and percentage positivity (with percentage 3GC-resistance for comparison) with 95%CIs
*2011 data are provisional as of 31st March 2012
The numbers of participating laboratories by year-end are indicated above the bars.
Data from www.hpsc.ie

Plasmidic AmpC enzymes are found worldwide and are predominantly detected in *K. pneumoniae*, *E. coli*, *Salmonella* spp. and *K. oxytoca*. In Ireland, plasmidic AmpC enzymes have also been reported in *Enterobacteriaceae* such as *Salmonella* spp. and *K. pneumoniae*.[9,10]

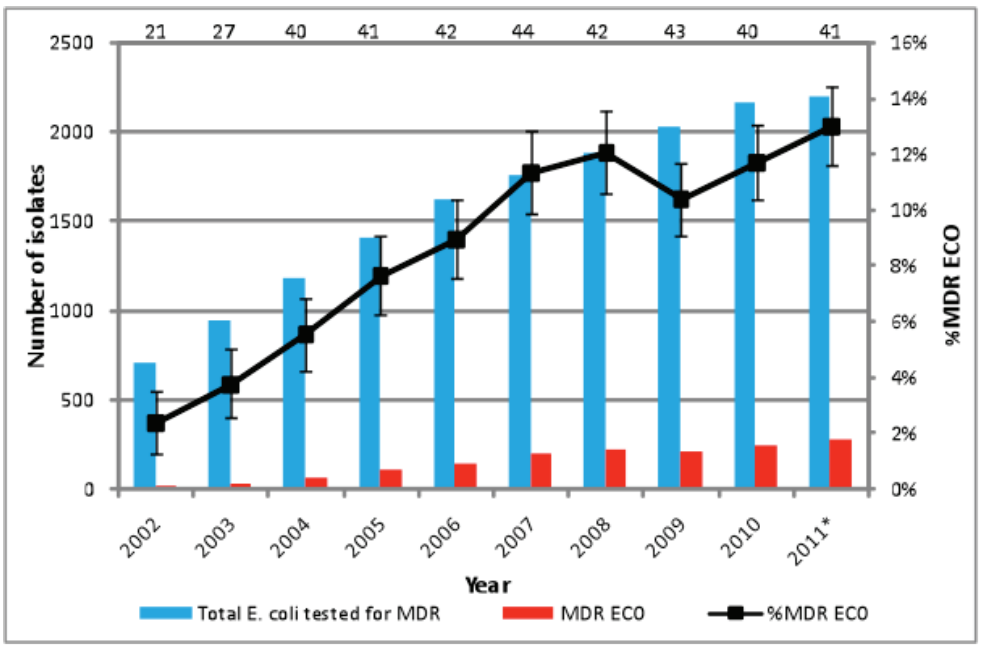

Figure 5: Trends for multi-drug resistant (MDR) *E. coli* –total numbers of *E. coli* and MDR isolates tested, and percentage positivity with 95%CIs
*2011 data are provisional as of 31st March 2012
The numbers of participating laboratories by year-end are indicated above the bars.
Data from www.hpsc.ie

In 2010, EARS-Net reported that 10.6% of all *E. coli* isolates from invasive infections were MDR. In Ireland, the proportion that were MDR increased from 11.7% in 2010 to 13.0% in 2011, which was the highest annual proportion reported to date (Figure 5).

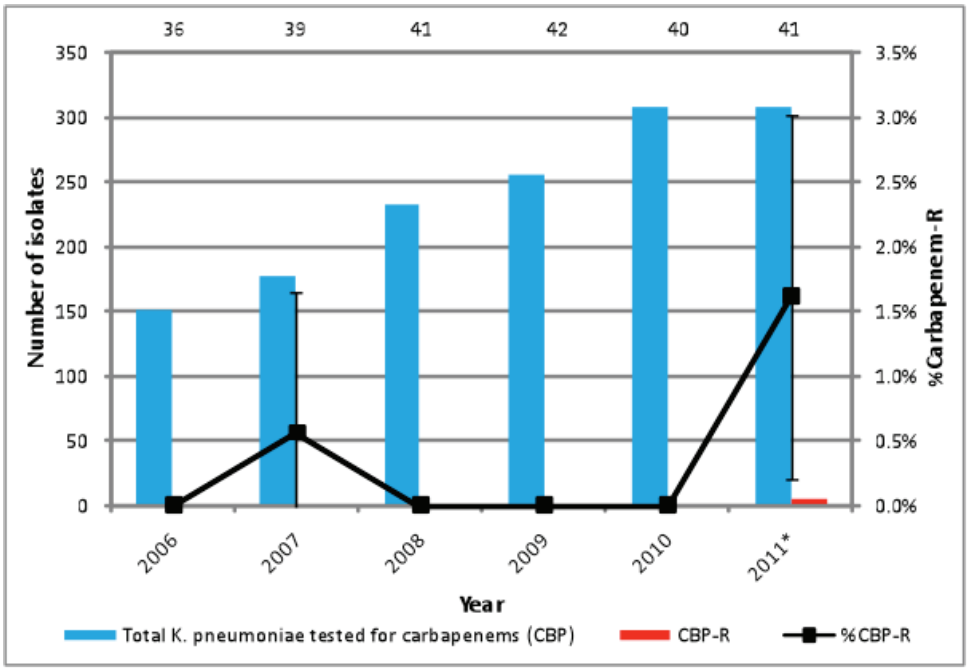

Figure 6: Invasive CRE isolates reported in Ireland: Trends for carbapenem resistant *K. pneumoniae* – total numbers of *K. pneumoniae* and carbapenem resistant isolates tested, and percentage positivity with 95%CIs
*2011 data are provisional as of 31st March 2012
The numbers of participating laboratories by year-end are indicated above the bars.
Data from www.hpsc.ie

In 2011, six invasive CRE isolates were reported, four of which were confirmed as carbapenemase-producing *Enterobacteriaceae* (3 OXA-48, 1 KPC)(Figure 6). Two isolates were carbapenem resistant due to combined ESBL+/- AmpC +/- porin loss/impermeability.

Carbapenem resistant *Enterobacteriaceae* (CRE)

Carbapenem resistance generated by the combination of ESBL/AmpC production and porin loss has been reported for several years. This resistance mechanism is not transferred to other bacterial strains, although resistant strains can be passed between patients.

Carbapenemases are a diverse group of broad spectrum β-lactamases. The most commonly encountered carbapenemases are:

- *Klebsiella pneumoniae* carbapenemase (KPC)
- New Delhi metallo-β-lactamase (NDM)
- Verona Integron-encoded metallo-β-lactamase (VIM)
- Oxacillinase (OXA)

A worrying aspect is the rapidity of international dissemination of carbapenemases, as exemplified by the importation of NDM-1 from the Indian subcontinent to the United Kingdom and other European countries as well as the global importation of KPC from the United States to various continents.[11,12] The rapid spread of these carbapenemases is usually mediated by transfer of plasmids between strains or species and/or clonal dissemination of certain strains.[11,12]

In Europe, Greece is considered endemic for CRE, but significant problems of CRE dissemination have also been reported in other European countries such as Italy, Poland, France, Spain and the UK (Figure 7).[13,14]

Although found predominantly in patients from the UK, NDM-1-producing *Enterobacteriaceae* have also been reported in other European countries such as France, Germany and Scandinavian countries.[4,13] OXA-48 has been reported in various regions including the Middle East, India, Europe and North Africa.[13]

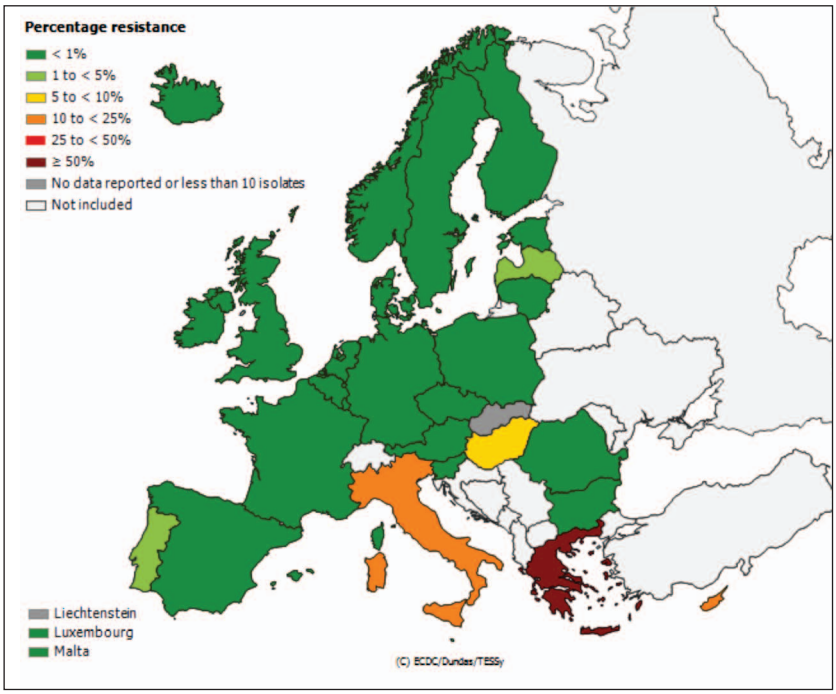

Figure 7: Carbapenem resistance among invasive *K. pneumoniae* isolates reported to EARS-Net in 2010. Map downloaded from ECDC's TESSy database on 14/12/2011: http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx

First data on carbapenem resistance among invasive *K. pneumoniae* isolates were reported from EARSS/ EARS-NET in 2005, when Greece already reported 28% carbapenem resistance. In 2010 Greece reported 49% carbapenem resistance among invasive *K. pneumoniae* isolates. Of note, Italy's resistance rate increased from 1.3% in 2009 to 15% in 2010.

CRE have also been encountered in Irish hospitals since 2009. Whereas only sporadic cases had been reported in 2009 and 2010, the epidemiology of CRE changed significantly in Ireland in 2011.[15] During 2011, CRE was reported from 36 patients in eight Irish hospitals, with four hospitals reporting CRE outbreaks. In January 2011, an outbreak with KPC producing *K. pneumoniae* was reported in the mid-west, with documented interhospital spread. During spring 2011, an outbreak of OXA-48 *K. pneumoniae* occurred in a tertiary hospital in Dublin.[16] Other hospitals have also reported sporadic cases of KPC-, OXA-48-, and VIM-producing *K. pneumoniae* [17] as well as VIM-producing *E. cloacae* (Boo TW *et al*., personal communication). The first case of NDM-1 producing *K. pneumoniae* detected in Ireland was notified in summer 2011.[18] In June 2011, a one-month prevalence survey was conducted in 40 Irish critical care units. Patients were screened weekly for rectal carriage of carbapenemase-producing CRE. CRE was not detected in any of the 40 participating units during this study. [19]

Risk factors and mode of transmission

Common risk factors for acquisition of resistant *Enterobacteriaceae* include:

- Exposure to broad spectrum antimicrobials, such as cephalosporins, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and carbapenems.[1,20-22]
- Prolonged hospitalisation
- ICU admission
- Presence of vascular catheters
- Urinary catheterisation

The gastrointestinal tract is the most likely site for asymptomatic colonisation with resistant *Enterobacteriaceae*. In one report, only two of 14 patients with gastrointestinal colonisation of CRE had positive cultures for CRE from clinical samples.[23] Hence, active surveillance cultures for rectal carriage of CRE can increase the detection rate, although the sensitivity of rectal surveillance swabs has not been determined.

Numerous outbreaks of ESBL and carbapenemase-producing *Enterobacteriaceae* have been described in various healthcare settings; regional, inter-regional and international spread of such organisms has also been reported.[7,13,24,25]

A significant number of infections originating in the community have been reported for CTX-M and NDM-1 producing *Enterobacteriaceae*, highlighting the enormous challenge the medical community is facing to try to contain their spread. [7,26]

Contaminated hands of healthcare workers have been implicated in hospital outbreaks due to resistant *Enterobacteriaceae*. There is no evidence that rectal colonisation of healthcare workers contributes to transmission.[27] Although resistant *Enterobacteriaceae* have been detected in the hospital environment, the role of environmental contamination in hospital outbreaks has been less defined in comparison to VRE.[28]

3.4 Clinical Significance

Members of the *Enterobacteriaceae* group are the most frequent cause of bacterial infections in patients of all ages. The most frequent sites of infection encountered are UTI, intra-abdominal sepsis, surgical site infections and BSI.
There are fewer therapeutic options for the treatment of infections caused by resistant *Enterobacteriaceae* as these organisms are often resistant to other classes of antimicrobials such as aminoglycosides and fluoroquinolones.[1,3,6] Carbapenems are currently the treatment of choice for serious infections caused by ESBL-producing and AmpC-hyperproducing organisms, but the increasing reliance on carbapenems for the treatment of infections by these organisms adds to the selective pressure for the emergence of carbapenem resistance. The significant therapeutic and infection control implications and challenges posed by ESBL and AmpC producing *Enterobacteriaceae* underscore the need for routine laboratory surveillance in clinical isolates. Most *Enterobacteriaceae* producing carbapenemases are resistant to carbapenems *in vivo*.[11,12] Therapeutic options for CRE infection are severely limited. The resistance profiles of most strains leave only a few antimicrobial agents such as tigecycline, fosfomycin and colistin available as potential therapeutic options. However, non-susceptibility or resistance to these antimicrobials is increasingly reported in CRE.[11,12,29] Some CRE strains may have carbapenem minimum inhibitory concentrations (MICs) that fall within the susceptible range according to CLSI or EUCAST breakpoint criteria; the clinical significance of carbapenemases in such strains is still unclear.[30]

Infections caused by resistant *Enterobacteriaceae* are associated with significantly increased risk of mortality.[7] Mortality rates associated with infections caused by CRE range from 38-57%.[30]

3.5 Laboratory Detection

Detection of resistant *Enterobacteriaceae* from clinical specimens
Optimal laboratory detection of resistant *Enterobacteriaceae* from clinical and surveillance specimens of patients is crucial for informing therapeutic decisions as well as for timely and effective implementation of infection control measures. As therapeutic options can be very limited, particularly in the case of CRE, the Committee recommends that every effort should be undertaken by Irish laboratories to identify resistant *Enterobacteriaceae*.

Broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC)
The inhibition of many ESBL enzymes by clavulanic acid forms the basis of the development of phenotypic methods for ESBL detection in the diagnostic laboratory.[31,32] Current EUCAST and CLSI guidelines do not recommend routine testing for ESBL production to guide treatment decisions. Nevertheless, the Committee recommends the testing of *Enterobacteriaceae* for ESBL production for accurate surveillance and appropriate infection control measures.

There are currently no CLSI, EUCAST or other widely-accepted standardised phenotypic methods for the detection of plasmidic AmpC enzymes. Molecular methods remain the current gold standard for the detection

of plasmidic AmpC enzymes.[2] Laboratories who wish to perform phenotypic detection of the above may refer to Appendix 7 for details of some of the more commonly used methods described in the literature.

Carbapenem resistant *Enterobacteriaceae* (CRE)

Although CRE outbreaks have been reported in Ireland, at the time of writing this guidance, CRE does not yet appear to be endemic in Ireland. In order to prevent CRE from becoming endemic in Ireland, prompt laboratory detection of CRE in any clinical isolate and identification and targeted active surveillance cultures of patients deemed to be 'at-risk' of CRE rectal carriage is crucially important. The information provided below summarizes the published literature at the time of writing this document. As CRE screening is a rapidly evolving area, the Committee recommends to access information provided by reference services regularly to obtain the latest details on laboratory screening protocols.

While other mechanisms such as combinations of ESBL/AmpC plus porin loss may be implicated, carbapenem resistance in *Enterobacteriaceae* may be due to the acquisition of plasmid-mediated carbapenemases.[3] There are differences in the carbapenem breakpoint criteria between CLSI and EUCAST. Carbapenem resistance rates may thus vary according to the respective criteria adopted by the individual laboratories. Both CLSI and EUCAST committees have made the recommendation of reporting susceptibility testing results at face value for therapeutic decision-making.[31,32] Some CRE strains have carbapenem MICs that fall within the susceptible category.[12,33] Criteria less stringent than CLSI or EUCAST breakpoint criteria have been proposed and are detailed in Appendix 7. The clinical significance of isolates with carbapenem MICs below clinical breakpoints has not been established. Some CRE strains can exhibit pronounced inoculum effect with regards to carbapenem MICs, particularly with imipenem and with broth-based susceptibility test methods.[22,34] To improve the sensitivity of detecting carbapenemase producers, this Committee recommends the use of screening breakpoints (for carbapenemase production) that are distinct from the EUCAST/CLSI clinical breakpoints, which are intended for therapeutic decision-making. Meropenem is preferred to ertapenem or imipenem as an indicator for carbapenemase production (see below). It is recommended that alert criteria for the suspicion of CRE be incorporated into the test system(s) of the laboratory.

Antimicrobial agent	**Proposed alert criteria for suspicion of carbapenemase production in *Enterobacteriaceae***	
	Zone diameter breakpoints (mm)	**MIC interpretative standard (mg/L)**
Ertapenem[a]	≤ 24	≥ 0.5
Imipenem[b]	≤ 22	≥ 1
Meropenem	**≤ 23**	**≥ 0.5**

[a]Ertapenem is a less specific indicator for carbapenemase production than meropenem or imipenem. Isolates, particularly *Enterobacter* spp., with cephalosporin and ertapenem non-susceptibility may have combination of AmpC hyperproduction and porin loss; [b]elevated imipenem MICs for *Proteae* (*Proteus* spp., *Providencia* spp., and *Morganella morganii*) may be due to mechanisms other than the production of carbapenemases.

In some laboratories, carbapenem susceptibility testing may not be routinely performed in *Enterobacteriaceae* isolates from certain clinical samples, particularly urine specimens. Notably, a significant proportion of isolates from patients with CRE were from urine specimens.[11,12] A European working group has recently recommended susceptibility testing of *Enterobacteriaceae* from all anatomical sites with at least one carbapenem.[13] Most carbapenemase-producing *Enterobacteriaceae* are also resistant to cephalosporins.[11,12] The potential pitfall of using cephalosporin (such as cefpodoxime) as a surrogate indicator for carbapenem resistance is the failure to detect OXA-48-producing strains, which can be susceptible to cephalosporins unless co-producing ESBLs.[35]

Several phenotypic tests have been described for the detection of carbapenemase production in *Enterobacteriaceae*. The two most commonly used methods are the modified Hodge test (MHT) (also known as the cloverleaf test) and inhibitor-based synergy tests. These tests are described in greater detail in Appendix 7. These phenotypic tests can be used by laboratories to further analyse organisms with elevated carbapenem MICs for potential carbapenemase production. As none of the above phenotypic methods have universally accepted interpretive standards, the use of molecular methods such as end-point or real-time PCR for the detection of carbapenemase genes has been recommended for isolates suspected of carbapenemase production.[33,36,37]

Detection of resistant *Enterobacteriaceae* from surveillance specimens

Broad spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL and AmpC)

Selective chromogenic agar media are currently the most widely used media for the detection of ESBL-producing *Enterobacteriaceae* from rectal swabs or faecal samples. Most of these commercially available media contain cefpodoxime for the selection of ESBL producers. The chromogenic media also allow for the presumptive identification of ESBL-producing *Enterobacteriaceae* isolates, although confirmatory identification of isolates as well as standardised susceptibility testing are still warranted to characterise the resistance phenotype. Both ChromID ESBL (BioMérieux) and Brilliance ESBL (Oxoid) media have been shown to produce sensitivity and specificity of ≥95%, respectively, for the isolation of ESBL-producing *Enterobacteriaceae*.[38]

Carbapenem resistant *Enterobacteriaceae* (CRE)

For detection of CRE carriage, surveillance cultures of rectal swabs or faecal specimens can be performed.[30,36,39] Manipulated site swabs such as from skin breaks or vascular catheter sites can also be considered as part of CRE screening.[36] Various CRE screening protocols have been reported in the literature, and the merits and pitfalls of four of these protocols are discussed in greater detail in Appendix 7. In the absence of a universally accepted screening method, the Committee advises the use of MacConkey or CLED agar with the placement of an ertapenem or a meropenem (10µg) disc within the inoculum, with or without prior culture in enrichment broth, as an acceptable method. Using this method, a carbapenem inhibitory zone diameter of ≤ 27mm has been used as a criterion for identifying colonies that warrant further analysis.[40]

Environmental screening for CRE detection is not usually indicated; no standardised method has been recommended.

3.6 Infection Prevention and Control

The limited therapeutic options for infections caused by resistant *Enterobacteriaceae*, as well as their propensity for outbreaks and global dissemination, underscore the importance of surveillance and infection control measures in tackling the spread of MDRO. Detailed information on infection control measures within and outside the acute hospital setting is provided in chapter 1 of this document. A summary of Contact Precautions is given in Appendix 5. Guidance for an infection control risk assessment is given in Appendix 6.

Broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and plasmidic AmpC)

The value of active surveillance cultures for the control of resistant *Enterobacteriaceae* has been established for the outbreak setting. The role of active surveillance cultures for control in the endemic setting has not been clearly defined in the literature. A study from France reported a reduction of ESBL *Enterobacteriaceae* using infection control interventions including active surveillance cultures. A previous publication concluded that rectal screening may not be needed in non-epidemic situations due to low prevalence of ESBL-producing *Enterobacteriaceae* carriers on admission and failure to detect carriers with their screening methodology.[41,42] Rectal colonisation is a known risk factor for infection due to ESBL-producing *Enterobacteriaceae*.[43]

In June 2011, a meeting of European Centre for Disease Prevention and Control (ECDC) experts on CRE prevention also recommended control of the spread of ESBL-producing *Enterobacteriaceae*. Failure to control ESBLs will result in ongoing requirement for high use of carbapenems with associated effects on risk for CRE. In consideration of the rising numbers of infections with ESBL-producing *Enterobacteriaceae* in Ireland and of the risk of developing infection after acquisition of these MDRO, this Committee advocates consideration of screening for rectal carriage of ESBLs for patients admitted to high-risk areas (ICU, haematology/oncology, transplantation).

Carbapenem resistant *Enterobacteriaceae* (CRE)

Patients with unrecognised carriage of CRE can serve as reservoirs for continuing cross-transmission in the healthcare setting and may be a potential source of healthcare-associated outbreaks. CRE may not be isolated from clinical specimens in patients with asymptomatic faecal carriage.[23] Screening for CRE colonisation in patients using rectal surveillance cultures was an integral component of successful programmes for the containment of CRE spread in outbreak settings.[24,25,44]

In settings with sporadic occurrence or outbreaks of CRE, the screening of all patients with epidemiological links to the index cases is recommended.[30,36,39] Patients with epidemiological links include patients in the same unit or who have been cared for by the same healthcare workers. Subsequent weekly active surveillance has also been recommended until no new cases of CRE colonisation or infection have been detected in the affected units or wards.[36,39]

Screening is also recommended for other high-risk patients including patients with known histories of CRE colonisation or infection and patients with previous admission for more than 48 hours to healthcare facilities (including hospitals, dialysis units, or longterm care facilities) with known CRE endemicity or ongoing outbreaks.[30] An ECDC risk assessment on the spread of CRE published in September 2011 recommends active surveillance of all cross-border patient transfers.[4] At the time of writing this document, the Irish epidemiology of CRE is becoming worryingly similar to other European countries, where sporadic occurrence was followed by single hospital outbreaks with subsequent spread to regional and national centres. In an attempt to prevent CRE from becoming endemic in Ireland, the Committee therefore advocates routine CRE screening of the following at-risk patient groups:

- Any patient with known history of CRE colonisation or infection.
- Any patient with a history of admission for more than 48 hours to a named Irish healthcare facility reporting an outbreak of CRE in the past 12 months – See http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCRE/ScreeningforCREinIreland/ for latest list of named healthcare facilities.
- Any patient with a history of admission for more than 48 hours to a foreign healthcare facility in the past 12 months.
- Any patient transferred/repatriated from a healthcare facility in any foreign country.
- If the patient has attended an Irish healthcare facility reporting a CRE outbreak or a foreign healthcare facility for less than 48 hours or as a day patient, the decision whether to perform CRE screening should be made upon local risk assessment.

In March 2011, carbapenemase-producing CRE became notifiable in Ireland under the category of an "Unusual cluster or changing pattern of illness that may be of public health concern". Upon revision of the Infectious Diseases Legislation in September 2011, medical practitioners and clinical directors of diagnostic laboratories are required to notify all cases of invasive infection caused by carbapenemase-producing CRE, upon reference laboratory confirmation to the relevant Medical Officer of Health. In April 2011, an enhanced patient CRE surveillance form was developed, which should be completed for every patient from whom carbapenemase-producing CRE is isolated, regardless of whether the patient is infected or colonised (See Appendix 12). In the event that suspected CRE (awaiting reference laboratory confirmation) is implicated in an outbreak, it is advised that this be notified as soon as possible and that all appropriate outbreak control measures are established immediately (see chapter 1.13).

In settings with endemicity of CRE or with ongoing outbreaks, further escalation of control measures may be required at hospital and national levels in addition to the above surveillance measures.[30,39] Details of recommended laboratory procedures for active surveillance are given in Appendix 7.

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4. Multi-drug Resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa*

4.1 *Acinetobacter* species

4.1.1. Recommendations for the control of multi-drug resistant *Acinetobacter baumannii*

Laboratory detection of *Acinetobacter* spp. from clinical samples

- MDR *Acinetobacter* isolates which are clinically significant should undergo molecular analysis to identify the prevailing mechanisms of resistance (see Appendix 8).

Laboratory detection of *Acinetobacter* spp. from screening samples

- Clinical specimens suitable for the purpose of surveillance for *Acinetobacter baumanii* include respiratory secretions, nasal, pharyngeal, antecubital, perineal, or wound swabs.
- In addition to standard media, modified Leeds *Acinetobacter* medium can be used for screening samples.

Infection prevention and control

- Upon isolation of a MDR *Acinetobacter* species from a clinical specimen, the patient should be isolated in a single room with *en-suite* toilet facilities using Contact Precautions. Single-use disposable long-sleeved gowns should be worn by healthcare workers if physical contact with the patient is anticipated.
- If limited isolation facilities are available, a risk assessment should be carried out in conjunction with the IPCT.
- A review of culture results of clinical specimens taken from other patients on the ward/unit should be considered to assess for secondary cases.
- Active surveillance for *Acinetobacter* carriage may be considered when a patient is transferred from healthcare facilities in regions known to have higher rates of MDR-*Acinetobacter* e.g., Southern European states, Asia, South America.
- Active surveillance with targeted screening of patients has been employed successfully and is recommended in an outbreak setting. Routine screening of patients is not justified outside of an outbreak setting.
- During an outbreak, environmental screening should be considered in consultation with the IPCT, targeting reusable medical equipment, frequently-touched surfaces and horizontal dust collecting surfaces.
- Deep terminal cleaning to include air vents/filters, humidifiers and shared use medical equipment is recommended to diminish the environmental reservoir.

4.1.2. Background

Acinetobacter species comprise Gram-negative coccobacilli which are ubiquitous in the environment. The predominant pathogenic species constitute a minority of all known *Acinetobacter* species. These are *Acinetobacter baumannii*, *Acinetobacter genomic species 3*, and *Acinetobacter genomic species 13TU*.[1] In practice, these species are often grouped together using the term 'Baumannii group'. This reflects the difficulty in accurately sub-speciating strains when utilising commercial identification systems.[2]

Over the last 15-20 years, *Acinetobacter* species have emerged as important healthcare-associated pathogens.[3] They frequently cause persistent outbreaks within and across healthcare facilities, and also are adept at developing resistance to multiple antimicrobial agents.[4,5]

4.1.3 Epidemiology of MDR *Acinetobacter* spp.

The prevalence of nosocomial infections caused by *Acinetobacter spp*. has increased steadily in recent years worldwide. International clones have been identified and multiple groups have reported prolonged outbreaks involving inter-hospital transmission and indeed, international transmission.[1,4-6] Combined with increasing rates of infection, a consistent rise in rates of non-susceptibility to antimicrobials, specifically carbapenems, is particularly concerning.

In the UK, large widespread outbreaks of carbapenem-resistant *Acinetobacter* spp. have occurred since 2000.[4] A number of different clones were identified affecting multiple hospitals. Between 2004 and 2008, the rates of non-susceptibility to meropenem rose from 13 to 29% respectively. In 2008, non-susceptibility of *Acinetobacter* spp. to other classes of antimicrobials was reported at: aminoglycosides 20%; ciprofloxacin 30%; ceftazidime 70%; cefotaxime 89%; piperacillin/ tazobactam 50%.[7] Within Europe, the highest resistance rates have been reported in Mediterranean regions including Greece, Turkey, Italy and Spain.[8]

Similarly in the US, data on healthcare-associated infections indicate that 65-75% of *Acinetobacter* spp. isolates are multi-drug resistant, and that carbapenem non-susceptibility rose from 9% in 1995 to 57% in 2008.[9]
Data from Ireland on *Acinetobacter* is somewhat limited. One Irish university hospital identified 114 *Acinetobacter* spp. isolated from clinical specimens over a 30 month period between 2005 and 2007.[2] Automated methods identified 77 as *A. baumannii*, however with molecular methods, the predominant species was actually *A. genomic species 3*. Of 114 isolates, 11% were carbapenem resistant. All these carried a carbapenemase gene (OXA-23) with an upstream promoter insertion sequence (IS*Aba1*).

Mode of Transmisssion
Acinetobacter species are ubiquitous in the environment and non-pathogenic species comprise part of the normal human skin flora. In the hospital setting, colonised patients and their environment represent the reservoir for pathogenic *Acinetobacter* species. Transmission is predominantly via contact between patients, patients and staff or via shared use of medical equipment.[10-11] Widespread environmental contamination by *Acinetobacter* spp. has been identified during outbreak investigations and *Acinetobacter* species are particularly adept at surviving for long periods of time in the environment. Aerosolized transmission secondary to colonisation of air-conditioning units and extractor fans has also been implicated.[12]
Risk factors for colonisation and infection due to *Acinetobacter* spp. include:

- Intensive care admission
- Prolonged hospital stay
- Use of invasive devices
- Use of broad-spectrum antimicrobials[13]

4.1.4 Clinical Significance
Ventilator-associated pneumonia (VAP) is the most common infection observed due to *Acinetobacter*. In some countries, rates of VAP due to *Acinetobacter* approach 5-10%.[14] *Acinetobacter* species are accepted causes of infection in patients with burn injuries, wounds, surgical sites and more recently have been recognised in infections complicating injured military personnel. *Acinetobacter* species represent a minority cause of BSIs in the United Kingdom and in the United States.[8,15] However, crude mortality figures attributable to *Acinetobacter* infection vary considerably and have been reported to range from 34-67%. Intrinsic to this mortality rate is the consequence of inadequate empirical therapy when managing infections subsequently identified as caused by *Acinetobacter*. Infection and to a lesser extent colonisation have also been independently associated with higher morbidity, costs and prolonged hospitalisation.[16]

4.1.5 Laboratory Detection
Detection of *Acinetobacter* spp. from clinical specimens
Acinetobacter species grow well on standard culture media in routine use. They can be identified to the genus level as Gram-negative, catalase-positive, oxidase negative, non-fermenting coccobacilli. Accurate subspeciation remains difficult and laborious. Automated methods are frequently unable to differentiate between the three species of clinical significance. In addition phenotypic methods to identify mechanisms of resistance are unreliable. Thus, there has been an increasing use of molecular methods both for speciation, and for detection of particular resistance gene determinants. Such methods are also of central importance for epidemiological purposes in an outbreak setting.

Resistance mechanisms comprise both intrinsic gene sequences, coding for resistance elements and sequences acquired via mobile genetic elements/plasmids from other Gram-negative bacteria[1]. Such mechanisms include:

- β-lactamases: the most prevalent being AmpC cephalosporinases and OXA-carbapenemases
- porin alterations

- multi-drug efflux pumps
- aminoglycoside modifying enzymes
- mutations within the fluoroquinolone target sites

Detection of *Acinetobacter* spp. from screening specimens

Identifying colonised patients via active surveillance cultures is hampered by low sensitivity. Screening has variably involved use of nasal, pharyngeal, antecubital or perineal swabs.[10,11] Doi *et al* described increased sensitivity for isolation of *Acinetobacter* species when employing a sponge method for screening compared with the standard use of swabs from the buccal mucosa or groin.[17]

4.1.6 Infection Prevention and Control

Acinetobacter spp. typically causes prolonged outbreaks that may progress to become endemic in healthcare facilities. They also display a striking ability to persist over time causing overlapping and/or serial outbreaks. Prompt identification and implementation of strict Contact Precautions is central to averting the establishment of widespread environmental contamination. Environmental screening has recovered *Acinetobacter* spp. from reusable medical equipment such as infusion pumps, ventilators, portable ultrasound equipment, horizontal dust collecting surfaces, air filters, humidifiers and vents. In an outbreak setting, ward closures have been necessary to interrupt transmission and enable satisfactory eradication of the environmental reservoir. [10-12, 18] Screening to identify colonisation is recommended in an outbreak setting. Screening of patients repatriated from foreign healthcare institutions should also be considered, especially if transferring from Southern European States, Latin America or Asia.[1] Detailed information on infection control measures within and outside the acute hospital setting is provided in Chapter 1 of this document. A summary of Contact Precautions is given in Appendix 5. Guidance for an infection control risk assessment is given in Appendix 6.

4.2 *Pseudomonas aeruginosa*

4.2.1 Recommendations for the control of multi-drug resistant *P. aeruginosa*

Laboratory detection of *P. aeruginosa* from clinical samples

- Under consideration of local epidemiology and resources, molecular analysis of MDR isolates is recommended to detect plasmid-borne, transmissible resistance mechanisms in MDR isolates (see Appendix 8).
- Invasive *P. aeruginosa* infections (isolated from blood or cerebrospinal fluid) are notifiable.

Laboratory detection of *P. aeruginosa* from screening samples

- Screening utilises swabs of the oro-pharynx, nose, axilla, or rectum and/or clinical specimens such as respiratory secretions or stool samples.

Infection prevention and control

- Upon isolation of MDR *P. aeruginosa* from a clinical specimen, the patient should be isolated in a single room with *en-suite* toilet facilities using Contact Precautions.
- If limited isolation facilities are available, a risk assessment should be carried out in conjunction with the IPCT.
- A review of the culture results of clinical specimens sent from other patients on the ward/unit should be undertaken to assess for secondary cases.
- Active surveillance cultures for carriage of *P. aeruginosa* are not generally recommended, but may be appropriate in an outbreak setting.
- During an outbreak, environmental screening should also be considered targeting moist environmental surfaces such as sinks, frequently touched items (door handles) and shared medical equipment. Deep terminal cleaning of the clinical area is recommended to eradicate the environmental reservoir.

4.2.2 Background

Pseudomonas aeruginosa is a Gram-negative bacterium existing widely in the environment. It is present in diverse environmental settings (e.g. aquatic environments and soil) and is also known to colonise plants, animals and humans. *P. aeruginosa* is primarily described as an opportunistic pathogen causing disease in compromised hosts, for example patients in intensive care settings, patients with chronic lung disease and immunocompromised patients.

4.2.3 Epidemiology

P. aeruginosa represents a nosocomial pathogen of considerable importance.[3] In addition to the high prevalence of infection, rising rates of antimicrobial non-susceptibility and the troubling characteristic of the emergence of resistance during therapy further hinder efforts to successfully control infections due to this pathogen.[19]

Non-susceptibility rates of *P. aeruginosa* to many classes of antimicrobials have remained broadly stable across Europe and the US; however a worrying trend of increasing non-susceptibility to carbapenems has been observed worldwide.

In 2010, the frequency of MDR *P. aeruginosa* amongst BSI isolates in Europe was estimated to be 15%.[20] The highest rates of MDR *P. aeruginosa* were reported from Greece (42.5%), and the Czech Republic (29%). With regards to the worrying trend of increasing resistance to carbapenems, this remains highest in Southern and Eastern European states. Overall 16 of 28 countries reported that 10% or more of the *P. aeruginosa* isolates were resistant to carbapenems. Countries with the highest non-susceptibility to these agents include Greece (43%), Cyprus (29%) and Bulgaria (31%).

Data from BSI surveillance in the UK between 2001 and 2006 report that 2.5 - 4% of all BSI isolates were *P. aeruginosa*.[21] Non-susceptibility rates remained broadly stable with the exception of carbapenems. Non-susceptibility to meropenem increased from 5.7% to 10%. Isolates from an ICU setting demonstrated statistically higher rates of non-susceptibility to imipenem and piperacillin/tazobactam. US estimates of MDR amongst *P. aeruginosa* stood at 18% in 2000, 21% in 2003, and most recently 17% in 2008.[22,23]

In 2011, 184 *P. aeruginosa* BSI isolates were submitted from Ireland to EARS-Net, 4.0% of which were defined as multi-drug resistant (2010, 6.5%) (Figure 8). This compares favourably with the 2007 MDR rate of 12.5%.[20] Indeed, non-susceptibility proportions of *P. aeruginosa* to all classes of antimicrobials have decreased over this time period. Non-susceptibility rates to the predominant classes of agents in 2011 were as follows: piperacillin/tazobactam 3%; ceftazidime 8%; carbapenems; 8%; ciprofloxacin 13%; and gentamicin 6.5%.

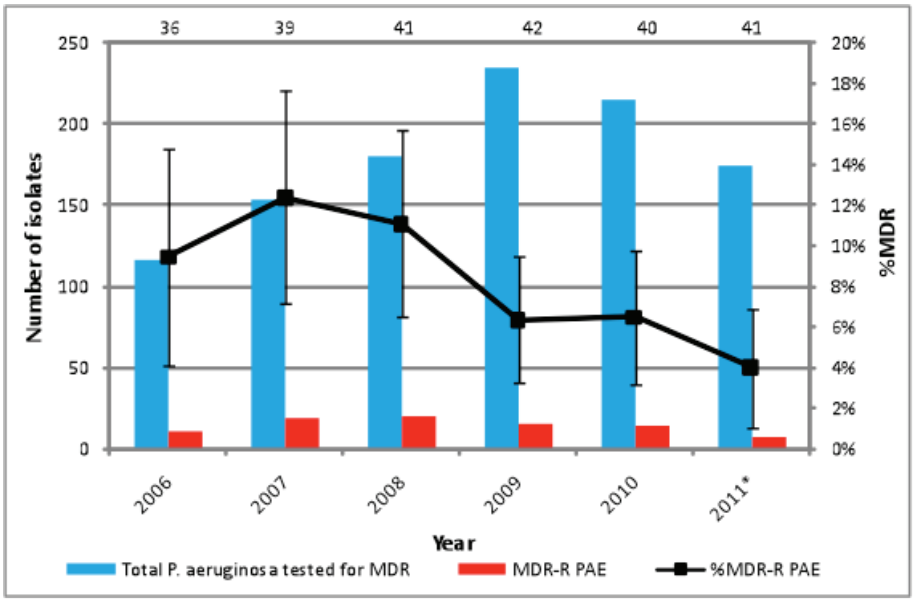

Figure 8: Trends for MDR *P. aeruginosa* –total numbers of MDR-R *P. aeruginosa* and percentage positivity with 95% CIs; * the numbers of participating laboratories by year-end are indicated above the bars

Data from www.hpsc.ie

Mode of Transmission

P. aeruginosa rarely colonises healthy non-hospitalised individuals. However up to 50% of hospitalised patients exhibit colonisation within the gastrointestinal or respiratory tracts.[24] *P. aeruginosa* can also survive for prolonged periods in moist environments such as taps, sinks and respiratory equipment and has a propensity to form biofilm, which facilitates further the success of this organism as a hospital pathogen.[25,26] Cross-

transmission in hospital occurs predominantly via patient-to-patient, staff-to-patient or environment-to-patient contact.

Specific cohorts, such as patients with cystic fibrosis exhibit higher colonisation rates with *P. aeruginosa*. Up to 80% of cystic fibrosis patients eventually become chronically colonised with *P. aeruginosa*. Surveillance of sputum microbiology in this cohort also suggests that the rates of MDR *P. aeruginosa* are increasing from 11.6% in 1995 to 17% in 2008.[27] Transmission of resistant strains among cystic fibrosis patients has also been documented.

Risk factors for colonisation with *P. aeruginosa* include:

- Breaches in the mucocutaneous barrier (eg. trauma, burns, surgery, invasive devices)
- Immunosuppression
- Chronic lung disease
- Use of broad-spectrum antimicrobials (particularly fluoroquinolones and carbapenems).[24,28]

A major obstacle to efforts to control rates of *P. aeruginosa* infection is that infection can arise both endogenously or exogenously. Reports have differed on the predominant source of *P. aeruginosa* infection in hospital settings. Some have concluded that the majority of incident ICU cases of colonisation and/or infection occurred as result of exogenous transmission.[29] Others have concluded that the endogenous source predominates.[25]

4.2.4 Clinical Significance

P. aeruginosa is reported to be the second most common cause of healthcare-associated pneumonia in the US, causing 14-16% of cases.[3, 30] European surveillance data for ICUs reports *P. aeruginosa* as the causative pathogen for 23-30% of cases of VAP, 19% of UTI and 10% of BSI. Equivalent figures from ICUs in the US are VAP 21%, UTI 10%, and BSI 3% respectively.

Infection due to MDR *P. aeruginosa* is associated with increased morbidity and mortality, prolonged length of stay, and increased costs. In particular, inappropriate empirical therapy in the context of MDR infection has been independently associated with both prolonged bacteraemia and higher morbidity and mortality.[31]

4.2.5 Laboratory Detection

Detection from clinical specimens

P. aeruginosa is easily isolated and identified in the laboratory. Antimicrobial resistance can be assessed using standard disc diffusion or commercial automated methods. Molecular methods have been employed to identify specific resistance mechanisms.

By virtue of the relative impermeability of its outer membrane, *P. aeruginosa* is intrinsically resistant to many antimicrobials. In addition, multiple separate resistance mechanisms have been identified, which combined contribute to the resistant phenotypes observed in clinical settings.[22] The mechanisms may be inherent to the bacterium or acquired via mobile genetic elements/plasmids.

Inherent resistance mechanisms described in *P. aeruginosa* include:

- AmpC cephalosporinase production
- Fluoroquinolone target site mutations e.g. *gyrA* (DNA gyrase gene), and *parC* (topoisomerase IV gene)
- Aminoglycoside modifying enzymes
- Efflux pump upregulation
- Modification of outer membrane porins.

Acquired resistance mechanisms have been widely described in *P. aeruginosa* with the acquisition of β-lactamases, extended spectrum β-lactamases, metallo- β-lactamases, and KPC carbapenemases all reported. High-level aminoglycoside resistance can occur following acquisition of genetic elements encoding an enzyme capable of methylation of 16S rRNA.

Detection from screening specimen

Active surveillance cultures of patients for carriage of MDR *P. aeruginosa* has been undertaken in ICU outbreak settings.

Environmental screening should be targeted towards moist environments, e.g. sinks and showers, frequent touch items (e.g., door handles), and shared medical equipment. Detailed guidelines addressing environmental sampling have been published by CDC and HICPAC.[32]

4.2.6 Infection Prevention and Control

Patients identified with MDR *P. aeruginosa* should promptly be placed in isolation with Contact Precautions. Additional precautions may be required depending on local risk assessment. Strict compliance with infection control measures is required to limit cross-transmission and environmental contamination. Patient screening for carriage using active surveillance cultures has been employed in ICU settings to successfully reduce infection rates but is not routinely recommended.[26-28] Screening has involved use of swabs of the oro-pharynx, nose, axilla, and rectum, and screening stool samples or respiratory specimen. Efforts to reduce the exogenous source of *P. aeruginosa* in the hospital can target sinks and common use devices (e.g., infusion pumps, ventilation equipment), adherence to appropriate use of sinks and adequate cleaning protocols in relation to sinks/sanitary equipment is important.

Detailed information on infection control measures within and outside the acute hospital setting is provided in Chapter 1 of this document. A summary of Contact Precautions is given in Appendix 5. Guidance for an infection control risk assessment is given in Appendix 6.

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Appendices

Appendix 1: Abbreviations used in the document

AMR:	Antimicrobial resistance
BSI:	Bloodstream infection
CDC:	Centers for Disease Control & Prevention
CLSI:	Clinical Laboratory and Standards Institute
CRE:	Carbapenem-resistant *Enterobacteriaceae*
EARS-Net:	European Antimicrobial Resistance Surveillance Network
ECDC:	European Centre for Disease Prevention and Control
EUCAST:	European Committee on Antimicrobial Susceptibility Testing
3-GC:	Third generation cephalosporin
HCAI:	Healthcare associated infections
HDU:	High dependency unit
HPSC:	Health Protection Surveillance Centre
HICPAC:	Hospital Infection Control and Prevention Advisory Committee
ICU:	Intensive care unit
IPC:	Infection prevention and control
MIC:	Minimum inhibitory concentration
MRSA:	Meticillin resistant *Staphylococcus aureus*
NHSN:	National Healthcare Safety Network
NICU:	Neonatal intensive care unit
PPE:	Personal protective equipment
RCPI:	Royal College of Physicians of Ireland
SARI:	Strategy for the Control of Antimicrobial Resistance in Ireland
VAP:	Ventilator-associated pneumonia
VRE:	Vancomycin-resistant enterococci
WHO:	World Health Organisation

Appendix 2: Definitions used in the document

MDRO: The term multi-drug resistance as used in these guidelines describes a bacterial isolate which is resistant to one or more agents in three or more different classes of antimicrobials that the isolate is expected to be susceptible to; e.g., penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.

Infection: the presence of MDRO in tissues or body fluids along with signs and symptoms of infection (either locally or systemically) or the presence of MDRO in normally sterile body sites or fluids (usually but not necessarily with symptoms of infection).

Colonisation: the presence of MDRO in body fluids or tissues (e.g., gastrointestinal tract, urine, or sputum) without clinical signs of infection.

Isolation: Placement of a patient in a single room with *en-suite* toilet facilities.

Isolation room: an isolation room indicated for the MDRO discussed in this guidance document is a single room with *en-suite* toilet facilities, including dedicated washing/bathing facilities for the patient. There should be a separate hand-washing sink and an alcohol hand rub dispenser at the entrance to the room.

Appendix 3: Membership of the working group

Dr Breida Boyle
Consultant Microbiologist, St. James's Hospital, Dublin.

Dr Teck Wee Boo
Consultant Microbiologist, Galway University Hospital, Galway.

Dr Karen Burns
Consultant Microbiologist, Health Protection Surveillance Centre & Beaumont Hospital, Dublin.

Dr Meaghan Cotter
Consultant Microbiologist, Mater Misericordiae University Hospital, Dublin.

Ms Judith Davitt
Infection Prevention & Control Nurse, Galway University Hospital, Galway.

Ms Carmel Fallon
Infection Prevention & Control Nurse, Health Services Executive West.

Ms Caitriona Finnegan
Senior Laboratory Scientist, Mid-western Regional Hospital, Limerick.

Dr Eithne Mc Carthy
Consultant Microbiologist, Galway University Hospital, Galway.

Mr Stephen Murchan
Surveillance Scientist, Health Protection Surveillance Centre, Dublin.

Dr Deirdre O'Brien
Consultant Microbiologist, St. Vincent's University Hospital, Dublin.

Dr Daire O'Shea
Specialist Registrar in Infectious Diseases, St. James's Hospital, Dublin.

Dr Kirsten Schaffer
Consultant Microbiologist, St. Vincent's University Hospital, Dublin (Chair).

Ms Cara Wrenn
Senior Medical Scientist, St. Vincent's University Hospital, Dublin.

Appendix 4: Consultation and Review Process

The draft document was circulated to the following professional bodies:

- Irish Society of Clinical Microbiologists
- Infectious Diseases Society of Ireland
- Irish Infection Prevention Society
- Academy of Medical Laboratory Science of Ireland
- Irish Antimicrobial Pharmacists Group
- Clinical advisory group on healthcare associated infections of the Royal College of Physicians of Ireland (RCPI)
- Irish Patients Association through representation on the RCPI clinical advisory group
- Occupational Health Physicians

The draft document was also sent to Dr Anna-Pelagia Magiorakos in the European Centre for Disease Prevention and Control.

Appendix 5: Summary of Contact Precautions for a healthcare facility

Patient placement

- Patients known to be colonised/infected with an MDRO should be admitted to a single room with *en-suite* toilet and shower facilities and an additional handwash sink where possible.
- An *en-suite* room is preferable, but if one is not available, a commode should be dedicated for each patient's individual use.
- If a single room is not available, patients colonised or infected with the same organism may be cohorted in 2, 4 or 6 bedded rooms.
- Patients with diarrhoea or incontinence are at a higher risk of spreading MDRO known to colonise the intestinal tract (e.g., VRE, CRE) and should be given priority for isolation rooms.
- Where placement in an isolation room or cohorting is not possible, consider the patient population when determining patient placement. Contact IPCT for advice before patient placement.
- The door to the patient's room should be kept closed to minimise spread to adjacent areas, unless it is likely to compromise patient care.
- The appropriate signage should be placed on the outside of the door to alert healthcare workers of the need to apply Contact Precautions.

Hand hygiene and Personal protective equipment (PPE)

- Hand hygiene should be performed using soap and water or alcohol hand rub on entering the room/bedspace prior to touching the patient. It is imperative to perform hand hygiene again before or on exiting the isolation room or bedspace.
- Gloves are required, as outlined for Standard Precautions for potential contact with blood or body fluids. In addition, as part of Contact Precautions, they should be donned prior to entering an isolation room or bed space for all interactions that may involve contact with the patient or potentially contaminated areas in the patient's environment.
- Gloves should be removed on completion of a task and before leaving the patient's single room or bed space.
- Hand hygiene should be performed before donning and immediately upon removal of gloves with an antimicrobial or waterless antiseptic agent.
- Hand hygiene is performed in other circumstances as outlined in "Guidelines for hand hygiene in Irish healthcare settings" and in the 'WHO 5 moments for hand hygiene'.
- Adequate handwashing facilities and alcohol hand rub should be available for staff and visitor hand decontamination before and after touching the patient or after touching the patient's environment.
- A single-use disposable plastic apron should be donned before entering the room/bed space. A non sterile disposable gown is advised if close patient contact is anticipated; advice on this should be obtained from local infection control personnel. The apron or gown should be removed before exiting the room/bed space.
- Surgical masks and eye protection may occasionally be necessary for healthcare workers, such as when performing splash-generating procedures.

Visitors

- Visitors to the isolation room or ward and staff from other wards and departments (e.g., physiotherapists, radiographers, other medical teams, students etc) should only enter after permission and instruction from the nurse in charge. An information sheet detailing Contact Precautions should be displayed prominently.

Cleaning and decontamination of environment and patient-care equipment

- Local policies for environmental cleaning and equipment decontamination, waste and linen management should state the necessary standards and should be applied rigorously.

- Hospital wards should be cleaned regularly as part of a general programme of hospital hygiene.
- Instruments or equipment should preferably be single-use disposable.
- Multiple-patient-use items should be decontaminated appropriately before use on another patient, in accordance with local policy and manufacturer's instructions.
- The room in which a patient with an MDRO has been cared for should be disinfected after the patient's discharge with a chlorine releasing agent, such as hypochlorite, with special attention to frequently touched areas, horizontal surfaces and dust-collecting areas (e.g., ventilation grids). Curtains should be removed and laundered if not single-use disposable curtains. Pillows and mattress covers should be checked for damage.
- After an outbreak or incident of MDRO colonisation or infection the ward environment (including clinical and non-clinical areas) must be cleaned thoroughly to reduce environmental contamination.
- Documents including the nursing notes and patient's chart should not be taken into an isolation room.
- Only essential equipment and supplies should be taken into the patient's room. Stockpiling of supplies inside the room should be avoided.

Linen

- All linen from patients infected with or colonised with MDRO should be considered to be contaminated/ infected, including bedding and adjacent curtains. Linen should be removed from the bed with minimal agitation and should be further managed in accordance with local policy and national guidance, where provided.

Re-usable bedpans and urinals

- Dedicated bedpans or urinals are not required, provided that the human waste washer disinfector is in working order.

Crockery and cutlery

- No special precautions are necessary with this equipment.

Healthcare waste

- The majority of waste from a room where a patient has a MDRO should be considered non- risk waste e.g. gloves and aprons, unless contaminated with blood or infectious body substances.

Patient movement and transport

- When a patient with an MDRO is transferred to another hospital, the clinical team responsible for the patient should inform the receiving clinical staff of the patient's MDRO carriage status.
- During actual transportation between departments, it is important to maintain patient confidentiality.
- As the patient is not normally in direct contact with surrounding environmental surfaces or the staff members clothing during transportation, single-use disposable aprons or gloves are not required unless directed by Standard Precautions.
- Unnecessary equipment and linen should be removed before transporting patients.
- Patients on stretchers should be covered by a clean sheet before leaving the ward.

Ambulance transportation

- Ambulance staff should adhere to Standard Precautions with all patients.
- To minimise the risk of cross infection with any infectious agent, ambulance staff should use an alcohol based hand rub after contact with all patients, as part of Standard Precautions.
- If ambulance transfer is required, the ambulance service should be notified in advance of any infection risk by the responsible ward staff.
- In general, the patient may travel with other patients, unless the patient is deemed at high risk of transmission of MDRO (e.g., diarrhoea, discharging lesions which cannot be covered with an impermeable dressing) or if the other patients requiring transport are especially vulnerable, e.g., immunocompromised or upon recommendation of the IPC team.

- To minimise transfer of MDRO to other patients who may be at risk, ambulance staff should wear a single use disposable plastic apron and gloves and minimise patient contact where possible.
- Blankets and sheets should be placed into an alginate bag and then into a laundry bag after transport of patient.
- Local areas of patient contact (e.g., chair and stretcher) should be cleaned and disinfected as per local decontamination policy.
- After patient contact, protective clothing and gloves should be removed and hands decontaminated using an alcohol based hand rub.
- Fumigation and prolonged airing of the ambulance is not necessary.

Deceased patients

- The infection control precautions for handling deceased patients are the same as those used in life. Any lesions should be covered with impermeable dressings. Plastic body bags are not necessary, but may be employed as part of general practice in accordance with Standard Precautions for all patients.

Adapted from and excerpts from "Guidelines for the control and prevention of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities "*J Hosp Infect* 2006;63S: S1-S44

Appendix 6: Practical Guidance for Decision Makers on Isolation

The decisions regarding isolation of patients in the hospital setting may be difficult due to the limited resources available and competing priorities. In the absence of widely used accepted evidence-based guidelines, the scoring system below (adapted from the Lewisham Isolation Prioritisation System - LIPS) may help in the risk assessment process. The LIPS was developed in 1999 as a scoring system based on factors likely to influence transmission. It was modified by one of the original authors in 2009, following extensive feedback from users.

The Lewisham Isolation Prioritisation Scoring System (LIPS)[§]

Criteria	Classification	Score	Comment
ACDP category*	2	5	
	3	10	
	4	40	
Route	Air-borne	15	
	Droplet	10	
	Contact	5	Includes faecal-oral transmission
	Blood-borne	0	
Evidence of transmission	Published	10	
	Consensus or likelihood	5	
	No consensus or likelihood	0	
	No evidence	-10	
Significant resistance	Yes	5	Eg MRSA,VRE,MDR-GNB
	No	0	
High susceptibility of other patients with serious consequences of infection	Yes	10	Specific for various infection and patient populations
	No	0	
Prevalence	Sporadic	0	
	Endemic	-5	This reflects the burden of infection in the hospital and cohort measures may be more applicable.
	Epidemic	-5	See above
Dispersal	High risk	10	This includes diarrhoea, projectile vomiting, coughing, infected patients, confused wandering patients
	Medium risk	5	
	Low risk	0	
Total score			

Advisory Committee on Dangerous Pathogens (ACDP); Extended spectrum β-lactamases (ESBL); Meticillin resistant *Staphylococcus aureus* (MRSA); Vancomycin resistant enterococci (VRE); Multi-drug-resistant Gram-negative bacilli (MDR-GNB)

[§] Adapted from Jeanes A and Gopal R (1999) Lewisham Isolation Priority System (LIPS).University Hospital Lewisham and Jeanes A and Macrae B (2011) *British Journal of Nursing* 20(9):540-544

* Advisory Committee on Dangerous Pathogens (ACDP)- "approved list of biological agents", http://dh.gov.uk/ab/ACDP

Example for a patient with an MDRO such as VRE:

Patient colonised with VRE who is incontinent of faeces on a haematology/oncology ward in a hospital with endemic VRE	**Score**
ACDP=2	5
Route=contact	5
Evidence of transmission=published	10
Significant resistance=yes	5
High susceptibility of other patients with serious consequences of infection=yes	10
Prevalence=endemic	-5
Dispersal=high risk	10
Total score	**40 = High priority for isolation**

Appendix 7: Laboratory phenotypic methods for the detection of β-lactamases in *Enterobacteriaceae*

1. Laboratory detection of ESBL-producing *Enterobacteriaceae*

1.1 CLSI recommendation on *Enterobacteriaceae* ESBL screening (CLSI, 2011)

ESBL screening is recommended for *E. coli* and *Klebsiella* species only**;** and septicaemia isolates of *P. mirabilis*:

- Test with both cefotaxime and ceftazidime; or with cefpodoxime alone
- Perform ESBL confirmatory tests on isolates that are non-susceptible to any of the above cephalosporins, plus identification to genus/species level of the respective isolates.

1.2 EUCAST recommendation on *Enterobacteriaceae* susceptibility testing (EUCAST, 2011)

ESBL screening is recommended for all *Enterobacteriaceae.* Firstly, susceptibility testing is performed either with both cefotaxime and ceftazidime, or with cefpodoxime alone. ESBL confirmatory tests are then performed on isolates found to be non-susceptible to any of the above i.e. cefotaxime, ceftazidime and cefpodoxime. Cefpodoxime was found to be the most sensitive substrate for the detection of ESBLs.[1]

1.3 How to screen with indicator

The indicator cephalosporins should be included in primary susceptibility testing done e.g. standardized disc diffusion testing using either CLSI / EUCAST guidelines. Most automated systems such as Vitek and Phoenix currently incorporate ESBL detection tests or strategies and are an alternative to the present recommendations.[2]

Species identification is necessary to allow proper interpretation of results. CLSI recommend the following antimicrobials, and corresponding zone diameters and breakpoints for the screening of ESBLs

Antimicrobial and disc content	**Zones below may indicate ESBL production (mm)**		**Growth at or above the MIC concentrations may indicate ESBL production (mg/L)**	
	CLSI *E.coli* & *Kleb* spp	**EUCAST All *Entero-bacteriaceae***	**CLSI *E.coli* & *Kleb* spp**	**EUCAST All *Entero-bacteriaceae***
Cefpodoxime 10 µg	≤17	< 21	≥4	> 1
Cefpodoxime 10 µg, *P.mirabilis* (CLSI)	≤22		≥1	
Ceftazidime 30µg, CLSI Ceftazidime 10µg, EUCAST	≤22	< 19	≥1	> 4
Cefotaxime 30 µg, CLSI Cefotaxime 5µg EUCAST	≤27	< 18	≥1	> 2
Ceftriaxone 30 µg	≤25	< 20	≥1	> 2
Aztreonam 30 µg	≤27	< 24	≥1	> 4

Please refer to the most current EUCAST and CLSI reference documents for any changes to the above breakpoints.

1.4 Confirmatory tests for ESBLs

Enterobacteriaceae isolates resistant to any indicator cephalosporin in the screening tests outlined above should be subjected to confirmatory tests. Confirmation of ESBL production depends on demonstrating synergy between clavulanate and those indicator cephalosporin(s) to which the isolate was initially found resistant. Three methods can be used:

(i) **Double disc tests.** A plate is inoculated as for a routine susceptibility test. Discs containing cefotaxime and ceftazidime 30 µg (or cefpodoxime 10 µg) are applied on either side of a disc containing co-amoxiclav 20+10 µg; and circa 25-30 mm away from it.

ESBL production is inferred when the zone of *either* cephalosporin is expanded by the clavulanate. Disc-approximation methods are difficult to perform and interpret since the susceptibility depends on the optimal distance between the discs, which can be difficult to achieve since this distance varies with the strain of *Enterobacteriaceae*.

(ii) **Combination disc methods**. These methods compare the zones of cephalosporin discs to those of the same cephalosporin plus clavulanate. Several formats are available commercially (eg. Oxoid, MAST). Presence of ESBL can be inferred according to the respective criteria set by the individual manufacturers.

(iii) **Gradient MIC ESBL strips** (eg. Etest, AB Biodisk). These tests have a cephalosporin MIC gradient at one end and a cephalosporin + clavulanate MIC gradient at the other. The presence of an ESBL is confirmed when (1) there is a ≥ 3 doubling decrease in the cephalosporin MIC in the presence of clavulanate (i.e. cephalosporin to cephalosporin/clavulanate MIC ratio of ≥8), or (2) a phantom zone or a deformation of the inhibition ellipse is present even if the above ratio is < 8. However a result is considered indeterminate when the MICs are outside the range of MICs of the respective Etest ESBL test strip and a MIC ratio cannot be calculated.

1.5 Quality control of Confirmatory tests

Quality Control of the cefpodoxime, cefotaxime and/or ceftazidime discs used in primary screening should be in accordance with standard CLSI recommendations, as appropriate.
Positive controls should be used to ensure the performance of ESBL confirmatory tests. Three ESBL-positive *E. coli* strains are available from the NCTC:

- CTX-M-15 (cefotaximase) NCTC 13353
- TEM-3 (broad-spectrum) NCTC 13351
- TEM-10 (ceftazidimase) NCTC 13352

CLSI recommends *K. pneumoniae* ATCC 700603 as an ESBL-producing QC control, as does AB Biodisk (Etest). Zones of the Ceftazidime and Ceftazidime + clavulanate discs for ESBL-positive *K. pneumoniae* should be ≥ 5 mm. Zones of the Cefotaxime and Cefotaxime + clavulanate discs for ESBL-positive *K. pneumoniae* should be ≥ 3 mm.

E. coli ATCC 25922 should also be used as a negative control in ESBL confirmation tests. Use of such controls is especially important when the cephalosporin and cephalosporin + clavulanate combination discs are from different batches, which may vary in original content or retained potency.
Differences in zone diameters between those of cephalosporin and of cephalosporin + clavulanate discs for ESBL-negative *E. coli* should be ≤ 2 mm.

1.6 Pitfalls and problems of phenotypic tests for ESBL production

ESBLs are harder to detect in other species of *Enterobacteriaceae* e.g. *Enterobacter* species, *Citrobacter freundii, Morganella morganii, Providencia* species and *Serratia* species with inducible chromosomal AmpC β-lactamases. The production of AmpC enzymes may be induced by clavulanate, which is in turn hydrolysed by the AmpC enzyme, thereby masking any potential synergy arising from inhibition of the ESBL.

- If ESBL tests are to be done on *Enterobacteriaceae* with chromosomal AmpC enzymes, an AmpC-stable cephalosporin (i.e. cefepime or cefpirome) should be utilised as the indicator compound in the synergy test. Cefepime/clavulanate Etests (AB Biodisk) and cefpirome/clavulanate combination discs (Oxoid) are available commercially, and should be used with these genera.

K. oxytoca: 10-20% of *K. oxytoca* isolates hyperproduce their class A "K1"chromosomal β-lactamase. These are resistant to cefpodoxime and often to cefotaxime, but not to ceftazidime.

- They may produce false-positive ESBL test results with cefotaxime or cefepime (but not with ceftazidime). K1 hyperproduction should be suspected if a *Klebsiella* isolate is indole-positive and has high-level resistance (very small zones) to piperacillin/tazobactam and cefuroxime, borderline susceptibility to cefotaxime, and full susceptibility to ceftazidime.

Acinetobacter spp., *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia:* ESBL tests are not specifically developed for these species and should not be used for them. False positive results with *Acinetobacter* are common owing to inherent susceptibility to clavulanate, whilst *S. maltophilia* may give positive results *via* inhibition of one of its chromosomal L-2 β-lactamase. ESBLs may occur in these genera (e.g. VEB-1 in *Acinetobacter* spp.), but are less commonly found than in *Enterobacteriaceae*.

2. Laboratory detection of AmpC-producing *Enterobacteriaceae*

2.1 Screening for AmpC-producing *Enterobacteriaceae*

Screening for AmpC production is usually performed on *Enterobacteriaceae* that do not carry chromosomal AmpC enzymes (such as *Klebsiella* spp., *P. mirabilis*, *Salmonella* spp.). Molecular methods are usually required to distinguish between plasmidic and chromosomal AmpC enzymes if the occurrence of both enzymes is a possibility (eg. in *E. coli* isolates), as phenotypic methods often cannot distinguish between the two types of AmpC enzymes.

Cefoxitin susceptibility in cephalosporin-resistant isolates is often used to distinguish between carriage of ESBL and of plasmidic AmpC enzyme in *K. pneumoniae*. While ESBL-producing *K. pneumoniae* isolates are usually susceptible to cefoxitin and the converse is true for AmpC-producing isolates, exceptions do occur. Certain TEM variants can confer cefoxitin resistance, as can isolates with porin loss.[3] While most types of plasmidic AmpC enzymes confer resistance to cefoxitin, ACC-1-producing strains are usually susceptible to cefoxitin.[4,5] An alternative approach is to perform phenotypic screening in cephalosporin-resistant isolates for both ESBL and plasmidic AmpC enzymes.

2.2 Phenotypic methods for detection of plasmidic AmpC enzymes

There are currently no standardised phenotypic methods for the detection of AmpC enzymes in *Enterobacteriaceae*, but several methods have been described including three dimensional assays as well as synergy testing using inhibitors.[6,7,8,9,10] Synergy testing using inhibitors such as cloxacillin or boronic acid derivatives is most frequently reported, while the substrates used in studies include cefoxitin, cefpodoxime, cefotaxime, and ceftazidime. Testing can be carried out using disc approximation, combined disc testing, or gradient MIC methods.[5,6,7,8,10,11] Discs or MIC strips may be available commercially (eg. Etest; Rosco Diagnostica), or can be made up using in-house methods. Diagnostic criteria vary according to the test method and to manufacturers' specifications. Details of individual methods can be obtained from the respective references quoted. Molecular methods remain the current gold standard for the confirmation of plasmidic AmpC production in isolates.

2.3 Pitfalls and problems of phenotypic tests for AmpC production

- Interpretation of disc-approximation test results may be difficult since the sensitivity depends on the optimal distance between the discs.
- Most phenotypic tests cannot reliably distinguish between plasmidic and chromosomal AmpC enzymes, so would be most applicable only to isolates which do not carry chromosomal AmpC enzymes, such as *K. pneumoniae*.
- Specificity is a concern for tests based on boronic acid derivatives as they may also inhibit certain class A β-lactamases.
- Due to the lack of standardised methodology and diagnostic criteria, the sensitivity and specificity of methods vary according to choice of substrate, choice of inhibitor and differences in diagnostic criteria.[11]

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3. Laboratory detection of Carbapenemase-Producing *Enterobacteriaceae*

3.1 Screening for carbapenemase-producing *Enterobacteriaceae*

Screening for carbapenemase-producing *Enterobacteriaceae* based on CLSI or EUCAST clinical breakpoints can potentially miss some isolates as their carbapenem MICs may result in the isolates being classified within the susceptible category.

Criteria less stringent than CLSI or EUCAST breakpoint criteria have been proposed, which include the following carbapenem MICs as cut-off values for suspicion of carbapenemase production: ertapenem MIC ≥ 0.5 mg/L; meropenem MIC ≥ 0.5 mg/L; imipenem MIC ≥ 1 mg/L or ≥ 2 mg/L.[1,2] It should be noted that elevated imipenem MICs in *Proteae* isolates (*Proteus* spp., *Providencia* spp. and *Morganella morganii*) may be due to mechanisms other than carbapenemase production. For the disc diffusion method, EUCAST proposed non-susceptibility cut-off values of ≤ 24 mm, ≤ 20 mm and ≤ 21 mm for ertapenem, imipenem and meropenem, respectively; while CLSI proposed non-susceptibility cut-off values of ≤ 22mm for the three carbapenems, respectively.[3,4] Less stringent zone diameter criteria for meropenem had been proposed for suspicion of carbapenemase production, with cut-off values ranging from ≤ 23mm to ≤ 27mm.[1,2] Ertapenem may be a more sensitive indicator of carbapenemase production in *Klebsiella pneumoniae* than meropenem or imipenem.[5] However, ertapenem is also less specific for carbapenemase production than meropenem or imipenem, since reduced ertapenem susceptibility can be due to a combination of either AmpC hyperproduction or ESBL production plus porin loss, particularly in *Enterobacter* spp.[6,1,2]

Some CRE strains can exhibit pronounced inoculum effect with regards to carbapenem MICs, particularly with imipenem and with broth-based susceptibility test methods.[7,8] Automated test systems may not reliably detect low-level imipenem resistance in CRE, reiterating the need for proper inoculum preparation for broth-based susceptibility methods.[7,5] Carbapenem susceptibility testing of KPC-producing organisms, particularly *K. pneumoniae*, using gradient diffusion method can also be problematic. Scattered inner colonies with the Etest (bioMerieux) method can make the determination of the inhibition zones difficult.[9]

All isolates where an automated or disc diffusion susceptibility test have indicated reduced carbapenem susceptibility should have a carbapenem MIC performed, to exclude technical errors and to limit the number of strains to be confirmed for carbapenemase production.

Carbapenem MIC results obtained by gradient MIC tests should be interpreted with caution because mutant colonies with higher MICs than the dominant population may be found in the inhibition ellipse. These colonies should be included when interpreting the MIC result in accordance with the manufacturer's instructions.

3.2 Phenotypic tests for carbapenemase production

Carbapenemase production in *Enterobacteriaceae* should be confirmed by molecular testing for carbapenemase genes. Laboratories who wish to undertake phenotypic tests can also consider the tests mentioned below.

3.2.1 Modified Hodge Test (MHT, Cloverleaf test)

The MHT is the only laboratory method currently recommended by CLSI for the detection of diffusible carbapenemases.[3] The carbapenemase diffuses from the producer into the surrounding agar and its ability to protect the susceptible *E. coli* strain on the same agar plate indicates the presence of a carbapenemase. A 1/10 dilution of a 0.5 McFarland broth suspension of *E. coli* ATCC 25922 is applied to a Mueller-Hinton agar plate and allowed to dry for 10 minutes. A meropenem (10 µg) disc is applied to the centre of the plate. Three to five colonies of the suspected carbapenemase producer are picked and streaked from the disc to the edge of the plate and incubated overnight at 35 ± 2 °C aerobically. Following incubation, the intersection of the edge of the inhibition zone and the streak of the test isolate is examined. Carbapenemase production by the test strain is inferred by the presence of a 'cloverleaf shaped' zone of inhibition at the intersection.

Quality control of the MHT should be performed as recommended with the following controls (CLSI, 2011):
Klebsiella pneumoniae ATCC BAA 1705 (KPC-positive).

Klebsiella pneumoniae ATCC BAA 1706 (resistant to carbapenem by mechanisms other than carbapenemase, Modified Hodge Test negative).

E. coli ATCC 25922 (carbapenemase-negative).

3.2.2 Synergy testing

Synergy testing in carbapenemase-producing *Enterobacteriaceae* is based on inhibition of carbapenemase activity by the addition of an inhibitor specific for a particular class of carbapenemase, i.e. synergy between the carbapenem and the inhibitor. It has the potential advantage over the MHT in differentiating between the various classes of carbapenemases in *Enterobacteriaceae*. The main current drawback is the lack of standardised methodology in synergy testing. Several formats of synergy tests exist:

Combination Disc Tests (CDTs). Combination disc can be prepared in-house by applying defined amounts of inhibitors to routine antimicrobial discs or are available commercially. Carbapenem inhibitory zones are measured in the presence or absence of the carbapenemase inhibitor. An increase in the zone diameter of the carbapenem disc plus inhibitor over the carbapenem disc alone above a defined threshold value implies the presence of carbapenemase production.

Double Disc Synergy Test (DDSTs) (disc-approximation method). A carbapenemase inhibitor is deposited on a blank disc placed a specified distance from a carbapenem disc on a MH agar inoculated with the test isolate. The potentiation of the carbapenem inhibitory zone by the carbapenemase inhibitor implies the presence of carbapenemase production.

Gradient MIC Test. These tests (eg. Etest MBL) have a carbapenem gradient at one end and a carbapenem plus inhibitor gradient at the other end. The presence of a carbapenemase producer is inferred when there is a ≥ 3 doubling dilution in the carbapenem MIC in the presence of the inhibitor (ie. a carbapenem to carbapenem/ inhibitor MIC ratio of ≥8).

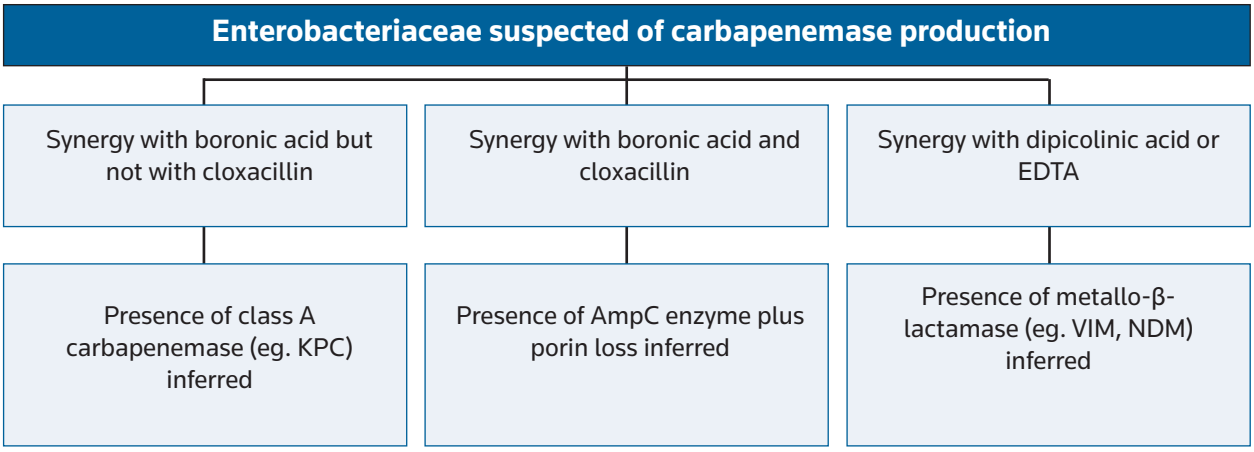

Figure 9: Suggested algorithm for synergy testing of suspected carbapenemase-producing *Enterobacteriaceae*[10, 11, 12]

3.2.2.1 Carbapenemase Inhibitors

Boronic acid derivatives. Boronic acid is an inhibitor of both class A and class C β-lactamases. It has been used in synergy testing for the detection of class A (KPC) carbapenemases and for detection of AmpC β-lactamase in *Enterobacteriaceae* in the formats of combination disc test as well as double disc synergy test. Carbapenem discs containing boronic acid are available commercially (eg. Rosco Diagnostica) or can be made up in-house. Details of in-house methods are reported in various studies.[1,10,13,14] Different concentrations of boronic acid derivatives have been used in the discs, ranging from 300 µg to 3000 µg; different cut-off values in zone diameter differences (≥ 4 mm and ≥ 5 mm, respectively) between carbapenem alone versus carbapenem plus boronic acid were also used in studies. [1,10,13,14]

Ethylene diamine tetra-acetic acid (EDTA) and dipicolinic acid (DPA). Both EDTA and DPA are chelating agents which bind the zinc molecules present in the active sites of MBLs to reduce their carbapenem-hydrolysing activities. Synergy testing for MBL detection exists in all three formats as mentioned above. Diagnostic tablets for combination disc testing (eg. Rosco Diagnostica) or gradient MIC strips (eg. Etest MBL) are commercially available; alternatively, EDTA- or DPA-containing discs can be made using in-house methods.[10,15,16] Different diagnostic criteria exist depending on the format of the synergy tests. False-positive MBL synergy test results

are less common with DPA than with EDTA in *Enterobacteriaceae*.[10] Gradient MIC tests may not be suitable for MBL-producing isolates with low carbapenem MICs.[12,15]

Cloxacillin and oxacillin. Both compounds are inhibitors of class C enzymes (AmpC). In conjuction with synergy testing with boronic acid derivatives, they can be used to distinguish between isolates producing class A carbapenemases (inhibited by boronic acid but not by cloxacillin or oxacillin) and those producing AmpC enzymes (inhibited by both boronic acid and cloxacillin/oxacillin). Discs containing these compounds are available commercially (eg. Rosco Diagnostica) or can be made up using in-house methods.[10,17] In the latter, 1000 µg of oxacillin and 750 µg of cloxacillin, respectively, have been suggested for synergy testing.[10,17]

3.3 Pitfalls and problems of phenotypic tests for carbapenemase production

- Interpretation of MHT results can be difficult and subjective especially for weak carbapenemase producers particularly for MBLs in *Enterobacteriaceae*.[1]
- The MHT is unable to differentiate between different classes of carbapenemases.
- A number of false-positive MHT results may be obtained especially with ertapenem and with isolates producing either higher amounts of AmpC-type cephaloporinases or CTX-M ESBLs plus porin alterations.[1] A study has found that the poor specificity may be overcome by additional synergy testing with oxacillin and boronic acid.[17] However, the findings have yet to be validated by other studies.
- The lack of standardised test methods is the major drawback in synergy testing. Different compounds, concentrations, and diagnostic criteria add to the difficulties in the interpretation of test results. However, good correlation of synergy test results has recently been found between an in-house test and a commercially-produced diagnostic method.[10]
- Interpretation of disc-approximation test results may be difficult since the sensitivity depends on the optimal distance between the discs.[1,15]
- MBL Etest may not be appropriate for MBL-producing organisms with MIC ≤ 4 ug/ml.[12,15]
- No specific inhibitor of Class D carbapenemases (eg. OXA-48) is available for laboratory diagnostic purposes so far.

3.4 Laboratory screening methods and protocols for CRE

Several laboratory screening methods and protocols for CRE have been reported in the literature.[7,8,18,19,20] For CRE surveillance in patients, rectal swabs and faeces are the usual recommended specimens to be taken.[6,12,21,22] Manipulated site swabs such as from skin breaks or vascular catheter sites can also be considered as part of CRE screening.[6] Four CRE screening protocols are summarised below:

Method 1:

This protocol is recommended by the Centers for Disease Control and Prevention (CDC), US. Briefly, rectal swab is inoculated in 5 ml of trypticase soy broth containing a 10-µg ertapenem or meropenem disc. Subculture of 100 µL of broth culture onto MacConkey agar is carried out following overnight incubation at 35°C in ambient air. The plate culture is examined following overnight incubation at 35°C in ambient air for representative colonies (lactose-fermenting colonies), followed by subculture of representative colonies of each morphological type onto non-selective media for isolation and further analysis including carbapenem susceptibility testing, species-level identification, and where indicated, genotypic/phenotypic tests. Full details of the protocol can be obtained from the CDC website at www.cdc.gov/ncidod/dhqp/pdf/ar/Klebsiella_or_Ecoli.pdf.

Method 2:

Direct inoculation of rectal swab onto MacConkey or CLED agar with the placement of ertapenem or meropenem disc (10 µg) on the inoculated plate, with or without prior culture in carbapenem-containing enrichment broth. Following overnight incubation, the plate culture is examined for appropriate colonies within the carbapenem zone of inhibition. Representative colonies are further analysed as in the latter steps in method 1. This protocol is recommended by the Health Protection Agency, UK (HPA, 2011).

Method 3:

Direct inoculation of rectal swab onto ChromID ESBL medium (bioMerieux). Following overnight incubation, the plate culture is examined for the presence of colonies, and representative colonies are further analysed as in the latter steps in method 1.

Method 4:
Direct inoculation of rectal swab onto CHROMagar KPC medium (CHROMagar). Following overnight incubation, the plate culture is examined for the presence of colonies, and representative colonies are further analysed as in the latter steps in method 1.

3.5 Comparison of laboratory screening methods for CRE

Each of the methods discussed in section 3.4 has merits as well as drawbacks with respect to CRE rectal surveillance in patients. The main disadvantages in method 1 are the longer turnaround times, higher threshold levels for detection, and significant proportions of false-positive results compared to other commonly used methods.[8,19]

Method 2, without prior culture in enrichment broth, has been found to be simpler, more sensitive and more specific than method 1.[19] However, the sensitivity and specificity of method 2 are dependent on the inhibitory zone criteria set by individual laboratories. Currently, there are no universally accepted inhibitory zone criteria for this method. Method 2 using an imipenem zone of ≤ 15 mm was found to be less sensitive than method 1, while method 2 using an ertapenem zone of ≤ 27 mm was more sensitive and specific than method 1.[8,19] Using CLSI or EUCAST zone diameter breakpoint criteria may not be sufficiently sensitive in CRE surveillance of rectal swab cultures, as carbapenem susceptibility in some *Enterobacteriaceae* isolates can exhibit significant inoculum effect.[7,8] It should also be noted that a significant proportion of false-positive results may still be obtained with method 2.[19] The main advantages of this method are that it is relatively inexpensive and simple to perform.

Methods 3 and 4 also have relatively simple methodologies but are more costly than methods 1 and 2. Method 3 using ChromID ESBL agar has the lowest limits of detection compared to the other methods.[8,18,23] However, ChromID ESBL agar does not distinguish between ESBL-producing and carbapenemase-producing colonies, and may not detect isolates producing OXA-48 carbapenemase (particularly those not co-producing ESBLs).[18] Method 4 using CHROMagar KPC agar is more specific in the detection of CRE than the other three methods, but it may not detect isolates with carbapenem MICs of < 4 mg/l.[18,20,24]

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Appendix 8: Laboratory Detection of Acquired Carbapenem resistance in *Acinetobacter* spp and *Pseudomonas aeruginosa*

There are no established phenotypic methods for the detection of carbapenemases in *Pseudomonas aeruginosa* and *Acinetobacter* spp. Detection of genes coding for carbapenem resistance by PCR give reliable results. However in the routine laboratory it is not practical to test all isolates for carbapenemase genes using PCR, therefore a screening algorithm is required. It has been suggested that MBL production is further investigated in *Pseudomonas* spp. and *Acinetobacter* spp. which are non-susceptible to carbapenems (imipenem and/or meropenem) and resistant either to ticarcillin, ticarcillin–clavulanate or ceftazidime.[1] This criterion has been extended to include all carbapenemases even though there are some differences in the breakpoints recommended by different institutions.[2]

The Modified Hodge Test has not been recommended for non-fermenting gram negative bacilli as it yields false negative results.[3] Further studies have shown that the sensitivity of the Modified Hodge Test for *P. aeruginosa* isolates with carbapenemase enzymes was as low as 78% and specificity of the test was 57%.[4]

MBL E-tests have been used to screen for MBL production in *P. aeruginosa* and have been credited with good sensitivity and specificity, although it has been repeatedly pointed out that specificity might be impaired by the possible intrinsic activity of EDTA.[2] Positive MBL E-test results should also be interpreted with caution in *Acinetobacter* spp. as false positive results have been reported due to presence of OXA-23.[5] For this reason dipicolinic acid has been investigated as an alternative. It was shown that 600 µg of 3-aminophenylboronic acid had a sensitivity and specificity of 97% for KPC enzymes, whereas 1000 µg of dipicolinic acid detected MBL enzymes with a sensitivity and specifity of 97% and 81% respectively in *P.aeruginosa*.[4] These discs are not currently commercially available.

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Appendix 9: ESBL patient information leaflet

Please refer to http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/EuropeanAntimicrobialResistanceSurveillanceSystemEARSS/ReferenceandEducationalResourceMaterial/Ecoli/PatientInformationLeaflet/File,13106,en.pdf

Appendix 10: CRE patient information leaflet

Please refer to http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCRE/Factsheets/File,12778,en.pdf

Appendix 11: VRE patient information leaflet

Please refer to http://www.hpsc.ie/hpsc/Publications/InformationLeafletsfortheGeneralPublic/File,13103,en.pdf

Appendix 12: CRE enhanced surveillance form

Please refer to http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCRE/SurveillanceForms/File,12671,en.pdf

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