OÉ Gaillimh
NUI Galway

The role of long-term care facilities in the dissemination of antimicrobial resistance

A Thesis Presented to the National University of Ireland, Galway

for the Degree of Doctor of Philosophy

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July 2014

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List of Abbreviations

6-APA	Penicillin nucleus 6-aminopenicillanic acid
Ami	Amikacin
Amp	Ampicillin
AMRO/ARO	Antimicrobial resistant organism
AR	Antibiogram- resistogram
ATCC	American Type Culture Collection
BJM	Bush Jacoby Medeiros
BSAC	British Society for Antimicrobial Chemotherapy
BURP	Based upon repeating patterns
°C	Degrees Celsius
CARDI	Centre for Ageing Research and Development in Ireland
Ca-MRSA	Community-associated meticillin-resistant *Staphylococcus aureus*
CC	Clonal complex
CDC	Centre for Disease Control and Prevention
Cdm	Cadmium acetate
CI	Confidence Interval
Cip	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CPD	Cefpodoxime
CPD/CV	Cefpodoxime plus clavulanic acid
CTX-M	Cefotaximase
D-Ala-D-Ala	D-alanyl-D-alanine
DIN	Deutsches Institut für Normung
E	Environmental
EARS-NET	European Antimicrobial Resistance Surveillance Network
Eb	Ethidium bromide
ECDC	European Centre for Disease Prevention and Control
EMRSA	Epidemic MRSA
Ery	Erythromycin
ESAC	European Surveillance of Antimicrobial Consumption

ESBL	Extended-spectrum β -lactamases
ESBL-EC	Extended-spectrum β -lactamases- producing *Escherichia coli*
ESBL-KP	Extended-spectrum β -lactamases- producing *Klebsiella pneumoniae*
ESBL-PE	Extended-spectrum β -lactamases- producing *Enterobacteriaceae*
ESRI	Economic and Social Research Institute
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal pathogenic *Escherichia coli*
FDA	Food and Drug Administration
FQREC	Ciprofloxacin-resistant *E. coli*
Fus	Fusidic acid
GEE	Generalised Estimating Equations
GP	General Practitioners
GUH	Galway University Hospitals
HA	Hospital/Healthcare-associated
HAI	Healthcare-associated infection
HALT	Healthcare-Associated infections in Long-Term care facilities
HA-MRSA	Healthcare / hospital-associated MRSA
HIQA	Health Information and Quality Authority
HPA	Health Protection Agency
HPSC	Health Protection Surveillance Centre
HSE	Health Service Executive
IPC	Infection prevention and control
Kan	Kanamycin
KPC	*Klebsiella pneumoniae* carbapenemase
Lin	Lincomycin
LTCF	Long-term care facility
LPS	Lipopolysaccharide
MC	Mercuric chloride
MRSA	Meticillin-resistant *Staphylococcus aureus*
MIC	Minimum Inhibitory Concentration
MLST	Multi Locus Sequence Type

Neo Neomycin

NHSS Nursing Home Support Scheme

N or n Number

NMRSARL National meticillin-resistant *Staphylococcus aureus* reference laboratory

OR Odds ratio

PAβN Phenylalanine arginine β-naphthylamide

PBP Penicillin binding protein

PCR Polymerase chain reaction

PFGE Pulsed field gel electrophoresis

PFP Pulsed field profile

pI Isoelectric point

Pma Phenyl mercuric acetate

PVL Panton-Valentine leukocidin

QRDR Quinolone resistance determining region

R Residents

RCH Roscommon County Hospital

S1-PFGE S1 nuclease pulsed field gel electrophoresis

SD Standard deviation

SHV Sulfhydryl variable

SCC*mec* Staphylococcus cassette chromosome *mec*

SFM Société Française de Microbiologie

SIR Swedish Reference Group for Antibiotics

SNP Single-nucleotide polymorphisms

spa Staphylococcal Protein A

Spc Spectinomycin

ST Sequence type

TEM Temoniera

Tet Tetracycline

Tob Tobramycin

UK United Kingdom

UPEC Uropathogenic *Escherichia coli*

USA	United States of America
UTI	Urinary tract infection
VRE	Vancomycin-resistant *Enterococcus*
VREfa	Vancomycin-resistant *Enterococcus faecalis*
VREfm	Vancomycin-resistant *Enterococcus faecium*
UTI	Urinary tract infections
UHG	University Hospital Galway
UPGMA	Un-weighted pair group method with arithmetic averages
WRG	Werkgroep Richtlijnen Gevoeligheidsbepalingen

Declaration

This work is submitted to fulfil the requirements of the degree of Doctor of Philosophy at the National University of Ireland, Galway.

No part of this thesis has been previously submitted at this or any other university.

Apart from due acknowledgements, it is entirely my own work.

Signed: _____________________________________

Date: _____________________________________

“Education is the most powerful weapon which you can use to change the world”

Nelson Mandela

Acknowledgements

As I am known by many as "little miss chatterbox" it may be difficult to keep this section brief. My four years spent doing this Ph.D. has been full of ups and downs and I have so many people to thank for reminding me that when things go bad, they can only get better. I can honestly say that this Ph.D. is my biggest achievement to date and has tested me mentally and physically but I am so glad that I have made it.

I have to give a very special thank you to my fantastic family. I may be biased, but I truly think that I have been blessed with the best parents in the world. Without the support and advice of my parents I do not know what career path I would have taken but I am so glad they nudged me in the Ph.D. direction. They made me believe that anything in life is possible once you believe in yourself. I would like to thank my mum for all the coffee, scones, and the continuous lighting of candles for me. Dad I remember you telling me that 'three D's' are necessary to succeed in anything. Well I have tried to remember to stick by those 'three D's' ever since - **D**edication, **D**etermination, and **D**iscipline. Thanks dad for the constant motivation.

I need to thank my very supportive brothers and sisters**:** Maria, Sara, Caroline, Donal, and Ciarán. You all have been amazing throughout, even when you were not on the same continent. Thankfully, for your sanity I have made it and you have all played your role in that, whether by a simple cheer me up phone call convincing me it will all be worthwhile or a well done phone call. I would like to thank the little rascals Brian, Jonathan, and Aoife for always bring a smile to my face and I am determined to make a scientist out of one of you yet. Also to Pa, you have always been there for me and have provided me with not just endless cups of coffee when you listened to me rant about the stresses of the Ph.D. but also with a tremendous amount of funny times during my Ph.D. to keep me going. To Noreen and Veronica, you both have been so good encouraging me since a child to go after the big things. Well here it is! I would also like to thank my grandparents Paddy and Sarah, and friend Vera for your support and prayers.

I would like to think all my amazing friends. Lou and Steph I can't believe it's over 21 years we know each other. Who would have thought that we would have stayed

besties for so long. Our travelling memories kept me going throughout and you both have always been there for a chat when I needed. I also need to thank my great college buddies - Kel, Irene, Shane, and Tash. Kel, our late evening coffees in the Huntsman kept me sane. Irene, our random nights always give me a laugh, and Shane, you always made time to help me when I asked. Natalie, you may be gone to Mexico but not forgotten. I remember us in India discussing where we would be in five years time. We'll have to see where my next journey leads me. I would also like to thank my school buddies Fiona, Ciara, Deborah, Sinead, Marina and Sharon. To all my friends, thank you for your patience and understanding and always providing me with laughter during the tough times.

Without trying to be gender biased, but I need to give a big thanks to 'the boys'. Firstly, to my special Paul who has stuck by me from the beginning of this Ph.D. right up till the end. You kept me laughing, calm and positive, making me forget all the stresses of my day. You have listened to me talking about ST131 and gels and never complained. You really are a star!! I would like to thank Miskell, John, Richie and Thomas. Not only have you all provided me with a place to stay and a kitchen table to study on but also with friendship.

I would like to all the Bacteriology and NSSLRL gang: Mary C, Sandra, Fiona, Siobhán, Carol, Tori, Mary W, Niall, Jean and Akke. You have provided me with friendship and advice. To Mary, what would I have done without our late night coffee breaks and you convincing me that it will be worth it in the end? To Fiona, our 'short' runs kept me going. To Sandra, without you being such a good teacher to me as an undergraduate and encouraging me to pursue research I don't know if I would be writing this thesis now. To Siobhán, our morning chats always started me off for the day on a good note. What will I do without you? To Niall and Jean, you have been such great friends to me throughout and always there for me offering support. I would like to thank Akke for providing advice for project design and statistics during my Ph.D. I would also like to thank Mark and Emer for our lunchtime chats and friendly banter. I feel that all of you have made my time in Bacteriology so memorable and I thank you all for your friendship and guidance.

I would like to thanks everyone who helped me out along the way, in particular all the staff in the microbiology laboratory in GUH. Tom, you have been so helpful to me throughout and I am so grateful. Belinda, you really are amazing at what you do and I thank you so much for always trying to make time for me. To Maura, you were always there for me when I needed you. To Elaine, you always provided me with friendly advice. I would also like to thank Fran, Anne, Jo, Breda, Collette, and Dave for always giving me a helping hand when needed.

I would also like to thank all my collaborators who helped me make the best of my Ph.D. I would especially like to thank Dr. Tim Dowling from NUIG, Dr. Brian O' Connell and Grainne Brennan from the NMRSARL, all the staff at the Department of Zoology Oxford, in particular Prof. Martin Maiden and Carina Brehony, Dr. Mark Holmes and Dr. Ewan Harrison from Department of Veterinary Cambridge, Prof. Mark Achmann and Dr. Zhemin Zhou from University of Warwick, Prof. Gordon Dougan from the Sanger Institute, Prof. Neil Woodford from the HPA, and to Prof. James R. Johnson and his colleagues at the VA Medical Center Minneapolis. I have learned so much by working with such talented and knowledge people and I am very grateful for your advice and support along the way.

Last, and certain not least, I would like my supervisors Prof. Martin Cormican and Dr. Dearbháile Morris for your fantastic leadership and guidance throughout my Ph.D. Martin, I remember when I first started my Ph.D. you walked into the Bacteriology office and you told Fiona that she was nearly there, Mary that she was getting there and turned to me and gave a little laugh and said that I had a long way to go yet. But, thanks to your help and support I am 'there'. You have provided me not just with mentorship with my studies but also invaluable life and career advice. I admire your love for research and dedication to your career and I feel that you are a great role model for any Ph.D. student and I am honoured that I was lucky to be one of them. To Dearbháile, you have been my supervisor and a friend and I always felt your door was always open for me whenever needed. I have learned so much from you and Martin during these last four years and you have given me so much confidence in myself. I am so glad that you saw the potential in me to be able to do this Ph.D.

This Ph.D. thesis is dedicated to my amazing parents and family for all the support over the years and for believing in me

Summary of Content

The dissemination of antimicrobial resistant organisms (AROs) is recognised as a global problem in healthcare-settings and in the community. There is limited data on the role long-term care facilities (LTCF) play in the spread of AROs and risk factors for acquisition in this vulnerable population. This Ph.D. was performed to address these issues and the findings are presented as five publications (I-V).

Paper I describes the comparison of ESBL-producing *E. coli* (ESBL-EC) collected from LTCFs, hospitals and the community in 2010. This paper supports clonal dissemination of *E. coli* ST131 in the West of Ireland, particularly in LTCFs. Paper II provides evidence of surface contamination with MRSA in a LTCF preceding occupation by residents, but concurrent with commissioning the facility by staff, and more widespread contamination of MRSA in the LTCF after occupation by residents in 2011. A selection of MRSA isolates recovered from the environment (2011-2013) and residents (2013) of a LTCF were further investigated by genotypic analysis (Paper III). The results described in Paper III illustrate a PVL-negative *spa* clonal group (*spa*-CC22), associated with ST22, predominating in both environmental and clinical isolates. The findings from Papers IV and V demonstrate a high prevalence (>50%) of ESBL-EC colonisation among LTCF residents over a one year period, which is attributed to the clonal spread of the ST131 *H30-Rx* subclone. The main risk factors identified for the acquisition of this subclone include antimicrobial usage and residents highly dependent on care by healthcare staff. Similar risk factors were reported for MRSA acquisition and a lower prevalence of MRSA colonisation in the LTCF was identified (>25%).

This Ph.D. research enhances our understanding of the role LTCFs play in the dissemination of AROs illustrating environment contamination with MRSA and a high prevalence of ESBL-EC and MRSA attributed to clonal dissemination in LTCFs.

Chapter 1

General Introduction

1.1 Antimicrobial development, mechanisms of action and resistance

The study of microbiology started with the discovery of bacteria in 1676 by Antonie van Leeuwenhoek. In 1888 E. de Freudenreich isolated a substance known as pyocyanase from bacterium *Bacillus pyocyaneus* that had antimicrobial activity. In 1928, Alexander Fleming noticed a mould growing on a plate which he had inoculated with *Staphylococcus aureus*. He observed a zone of *S. aureus* clearance around the mould, indicating inhibition of *S. aureus* by the mould. This mould was identified as a species of Penicillum. Howard Walter Florey and Ernst Boris Chain successfully isolated penicillin by freeze-drying the mould, and introduced the first effective antimicrobial into clinical practice. Florey, Chain, and Fleming received a Nobel Prize in Physiology or Medicine in 1945 for discovering penicillin and developing it for therapeutic use [1].

Further discoveries by others have resulted in the widespread use of antimicrobials since the 1940s to treat and prevent many different types of bacterial infections. Streptomycin was the first aminoglycoside discovered and introduced for the treatment of tuberculosis [2]. Subsequently, broad spectrum antimicrobials followed including tetracycline, kanamycin, gentamicin, tobramycin, amikacin and chloramphenicol. Quinolone development began in 1962 when naladixic acid was discovered and introduced for clinical use in 1967 [3]. Few new antimicrobials were introduced into the clinical setting from the 1970s to 1999 and majority were analogues of older antimicrobials. Since 2000, 22 new antimicrobials have been launched with 5 new classes of antimicrobials approved for clinical use including daptomycin, linezolid, retapamulin, fidaxomicin, and bedaquiline [4]. However, antimicrobials from all 5 new classes are limited to treatment of Gram-positive infections [4]. As of May 2013, there are 49 compounds and 6 β-lactam/β-lactamase inhibitor combinations under clinical evaluation [4].

Selection pressure of antimicrobial use in clinical therapy, aquaculture and farming promotes higher levels of antimicrobial resistance. All such settings may act as reservoirs for antimicrobial resistant organisms and resistance genes. Inappropriate disposal of antimicrobials also encourages antimicrobial resistance and the extent of this problem is difficult to determine due to the lack of reported data. The European Surveillance of Antimicrobial Consumption Network (ESAC-Net) monitors trends in antimicrobial consumption in the community and hospital setting throughout Europe. Surveillance results of antimicrobial resistance in Europe are recorded by the European Antimicrobial Resistance Surveillance Network (EARS-Net). There are various classes of antimicrobials and multiple mechanisms of resistance exist for each class. Antimicrobials can be divided into classes based on their chemical structure. Examples of such classes include the beta-lactam antimicrobials and fluoroquinolones which are further explained in Section 1.2 and Section 1.5 below. Use of any one particular antimicrobial may select for resistance to the single antimicrobial and also to numerous or all other antimicrobials in the corresponding class [5]. This concept has been referred to as the 'class effect' [5].

Antimicrobials can also be differentiated based on their mechanism of action:

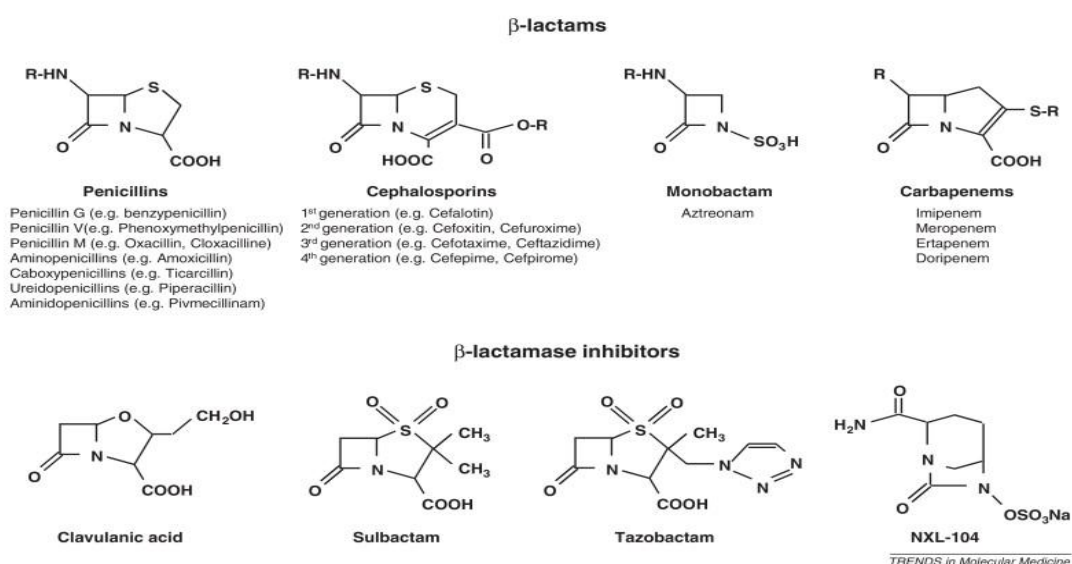
- Inhibition of cell wall synthesis e.g. beta-lactams, glycopeptides, fosfomycins
- Inhibition of DNA replication e.g. quinolones
- Inhibition of protein synthesis e.g. aminoglycosides, tetracycline, macrolides, lincosamides, chloramphenicol
- Inhibition of membrane activity e.g. polypeptides
- Folate pathway inhibitors e.g. trimethoprim/sulfamethoxazole

1.2 β-lactam antimicrobial agents

The β-lactam class of antimicrobial agents accounts for 60% of all antimicrobial use due to their efficacy and safety, ability to manipulate their structure, and their

broad spectrum of activity against both Gram-positive and Gram-negative bacteria [6]. The target site of the β-lactams is the penicillin binding proteins (PBPs) in the peptidoglycan of the bacterial cell wall. The β-lactam ring mimics D-Alanine-D-Alanine of the N-acteyl-muramic acid (NAM) pentapeptide [7]. This results in the PBPs using the β-lactams as the 'building blocks' of the cell wall synthesis, which in turn lead to acylation of the PBP preventing the cross-linking of the peptidoglycan wall [7]. Subsequently, the cell wall is weakened, increases in permeability and induces cell lysis. β-lactam antimicrobials are based on a 4 carbon ring structure (Figure 1.1) [8]. The β-lactam antimicrobials include penicillins, cephalosporins, cephamycins, monobactams and carbapenems [8].

Figure 1.1: Chemical composition of some β-lactam antimicrobials and β-lactam inhibitors. Reprinted from Trends in molecular medicine, 18/5, Nordmann P, Dortet L, Poirel L., Carbapenem resistance in *Enterobacteriaceae*: here is the storm!, 263-272., Copyright (2012), with permission from Elsevier [8]



- **Penicillins**

The penicillins consist of a 4-member β-lactam ring with a 5-member thiazolidine ring forming a penicillin nucleus 6-aminopenicillanic acid (6-APA) molecule [8]. An

N-*acyl* side chain is located at position 6. The penicillins differ by various modifications of the *N*-acyl side chain attached to the 6-APA molecule, reflecting difference in activity against certain bacteria. Penicillin G (benzylpenicillin) was initially the mostly widely used penicillin due to its ease of production and its activity against Neisseriae, many anaerobes, and Spirochaetes. It can be used to treat infections such as severe pyodermas caused by susceptible organisms, tropical ulcers, streptococcal pharyngitis, neurosyphilis, and impetigo caused by *Streptococcus pyogenes*. The above penicillins are susceptible to hydrolysis by β-lactamases produced by Gram-negative bacteria and are administered parenterally to avoid degradation by gastric acid. Penicillin V was later derived from penicillin G for oral administration [9].

Later examples of more extended spectrum-penicillins include ampicillin, piperacillin, amoxicillin which have activity against Gram-positive and Gram-negative bacteria including *Escherichia. coli*, *Shigella, Salmonella,* and *Proteus* [7, 9]. The penicillinase-resistant penicillins include flucloxacillin and dicloxacillin; narrow spectrum antimicrobials which have replaced meticillin for treatment of meticillin susceptible Staphylococcal infection [10]. Amoxicillin is the most common individual penicillin prescribed in European healthcare facilities according to a point prevalence study performed from 2011-2012 [11]. It is indicated for the treatment of infections in the respiratory tract, urinary tract, and skin and soft tissue infections [12].

- **Cephalosporins**

The first cephalosporin discovered was cephalosporin C, produced by the fungus Cephalosporium acremonium. They have a ring like structure similar to penicillins; however, cephalosporins contain a 6-member dihydrithiazine ring [8]. The cephalosporins are some of the most widely used antimicrobials in the clinical setting [11]. The classification of cephalosporins can be based on 'generations' 1-5

[13]. The higher the generation usually reflects the newer antimicrobials with a broader spectrum of activity and greater stability against β-lactamases.

The first generation comprises antimicrobials that are active against Gram-positive cocci including *S. aureus* (meticillin susceptible) and *Streptococcus* spp. [13, 14]. These antimicrobials remain in use today for the treatment of Gram-positive infections. Examples of first generation cephalosporins include cefazolin, cephalexin, cefadroxil, and cephalothin.

The second generation cephalosporins have increased activity against Gram-negative bacteria and remain active against Gram-positive bacteria; however, with a decreased activity against *S. aureus* for some second generation cephalosporins [13-15]. Examples of second generation cephalosporins include cefuroxime, and cefamandole.

The third generation cephalosporins have a wider spectrum of activity especially against Gram-negative species [14, 15]. These agents may not be active against *Enterobacter* due to intrinsic resistance mechanism such as AmpC β-lactamases [14, 16]. Due to the broad spectrum of third generation cephalosporins they are used routinely as a treatment for a wide variety of infections. Ceftazidime is active against *Psuedomonas aeruginosa* and is often prescribed for nosocomial infections; however, is less active against staphylococci and streptococci in comparison to other third generation cephalosporins [14, 17]. Cefotaxime and ceftriaxone are commonly used for the treatment of bacterial meningitis, respiratory tract infections, and urinary tract infections [14]. Examples of third generation cephalosporins include cefotaxime, cefoperazone, ceftriaxone, ceftazidime, and cefpodoxime.

The fourth generation cephalosporins such as cefepime and cefpirome have an expanded spectrum of activity due to increased ability to cross the outer membrane of Gram-negative bacteria, especially members of the *Enterobacteriaceae* and *P. aeruginosa*. The fourth generation have activity against Gram-positive organisms (particularly *S. aureus*) similar to that of ceftazidime and are not active against meticillin-resistant *S. aureus* (MRSA) and *Bacteroides fragilis* [18].

Fifth generation cephalosporins include ceftobiprole and ceftaroline are unlike other cephalosporins in that both are active against MRSA. Ceftobiprole is approved for the treatment of hospital-acquired (non ventilator) and community-acquired pneumonia [19]. Ceftobirole has a broad spectrum of activity including meticillin susceptible *S. aureus* (MSSA), MRSA, meticillin-susceptible coagulase-negative staphylococci, penicillin-resistant *Streptococcus pneumoniae, Enterococcus faecalis, Enterobacteriaceae* spp. *P. aeruginosa, Haemophilius influenzae* and *Moraxella catarrhalis* [19]. Ceftaroline is approved for the treatment of community-acquired pneumonia and skin infections and has a similar spectrum of activity to ceftobiprole [13, 19]. Both fifth generation cephalosporins are given as parenteral antimicrobial therapy [13, 19].

- **Monobactams**

Monobactam antimicrobials are synthetic compounds consisting of one β-lactam ring [8]. Aztreonam was the first monobactam used in the treatment process. Its poor affinity for penicillin binding proteins of Gram-positive bacteria and anaerobic bacteria make aztreonam limited to Gram-negative bacteria. Monobactams have little cross reactions with other antimicrobial classes, thus produce few hypersensitivity reactions [14].

- **Carbapenems**

The carbapenems differ from other β-lactam agents by the location of their hydroxyl-ethyl group and differ from the penicillins by a carbon atom instead of a sulphur atom in the five-member ring [8]. Carbapenems are broad spectrum β-lactams used for the treatment of both Gram-positive and Gram-negative bacterial infections and are also active against obligate anaerobes. Carbapenems are effective against streptococci and are more active than cephalosporins against staphylococci and Gram-negative bacilli. Imipenem and doripenem are most active against Gram-positive organisms, while meropenem, ertapenem, and doripenem are more active against Gram-negative organisms [20]. Imipenem must be administered with the enzyme inhibitor cilastatin to prevent degradation. Unlike imipenem, meropenem, ertapenem, and doripenem do not require cilastatin. The use of carbapenems has risen due to the increase in the prevalence of cephalosporin-resistant *Enterobacteriaceae* [8]. Carbapenems in clinical use include: imipenem, meropenem, ertapenem, and doripenem.

- **Beta-lactamase inhibitors**

β-lactamase inhibitors act by binding to the β-lactamases and preventing their action, allowing the co-administrated antimicrobial to be effective therapeutically. Clavulanic acid, tazobactam, and sulbactam are the three most clinically used β-lactamase inhibitors [11]. Clavulanic acid was isolated from *Streptomyces clavuligerus,* whereas both sulbactam and tazobactam are synthetic compounds [7]. The addition of clavulanate to both amoxicillin and ticarcillin results in a wider spectrum of activity for both Gram-positive and Gram-negative infections and combinations such as amoxycillin-clavulante are commonly prescribed to treat respiratory infections, particularly as they can be administered orally. New combinations such as cefpirome-clavulanate are being researched for the treatment of such infections, which would also help to reduce the use of carbapenems helping prevent further resistance [7]. β-lactam-β-lactamase inhibitor combinations include amoxicillin-clavulanate, ticarcillin-clavulanate, ampicillin-sulbactam, cefoperazone-sulbactam, and piperacillin-tazobactam [7].

1.3 Resistance to β-lactam antimicrobial agents

Bacteria are intrinsically resistant to different classes of antimicrobials and this form of resistance is not attributed to the human application of therapeutic antimicrobials or horizontal gene transfer. Intrinsic resistance in Gram-negative bacteria is commonly due to the Gram-negative outer membrane and the expression of efflux pumps reducing the concentration of antimicrobials [21]. Therefore some antimicrobials active against Gram-positive bacteria such as vancomycin are not active against Gram-negative bacteria and in this example it is due to the impermeability of the outer membrane [21]. Acquired resistance occurs due to the horizontal gene transfer of mobile genetic elements such as plasmids, transposons, and/or integrons containing antimicrobial resistant genes.

The predominant mechanisms of resistance to the β-lactam antimicrobial agents are:

1. Alteration of the target site due to a change in one or more penicillin binding protein (PBPs) causing a reduction or no binding of the antimicrobial to the target site.

2. Decreased permeability of the outer membrane due to porin loss and subsequent reduction in the penetration of the antimicrobial through the membrane

3. Efflux systems

4. Production of enzymes which inactivate the antimicrobial, e.g. beta-lactamases

1. Alteration of the target site due to a change in one or more penicillin binding protein (PBPs) causing a reduction or no binding of the antimicrobial to the target site

Penicillin binding proteins (PBP) are required for cross-linking the pentapeptides during cell wall synthesis and they are also the binding sites/target for β-lactam antimicrobials. PBPs are membrane-bound and cytoplasmic proteins. Bacteria contain four to eight PBPs, two to four of which are usually essential. They can be divided into two categories: high molecular mass PBP and low molecular mass PBP [22]. Modifications of the active site of PBPs results in lower β-lactam activity, thus increasing resistance [7]. Horizontal transfer of a PBP gene can result in β-lactam resistance in other bacterial species. β-lactam resistance due to changes in the active sites of PBPs has occurred in *S. pneumoniae*, *Neisseria meningitides,* and *S. aureus.*

2. Decreased permeability of the outer membrane due to porin loss and subsequent reduction in the penetration of the antimicrobial through the membrane

Gram-negative bacteria have a hydrophobic lipid outer membrane containing protein channels known as porins, which facilitate the transfer of nutrients and hydrophilic compounds such as antimicrobials into the cell. Hydrophilic antimicrobials, such as β-lactams**,** tetracycline, chloramphenicol, and fluoroquinolones, enter the cell via the porin channels. Alteration in the porin channels can lead to decreased permeability of the outer membrane to hydrophilic antimicrobials, thereby impeding the entry of antimicrobials into the periplasmic space. Resistance due to impermeability of the outer membrane has been observed in various species including *Klebsiella pneumoniae, Enterobacter* spp., *E. coli, Proteus* spp.*, P. aeruginosa,* and *Acinetobacter baumanii* [23-26]. Antimicrobial resistance due to impermeability of the outer membrane in conjunction with other resistance mechanisms such as the production of β-lactamases and efflux mechanisms has been reported [27].

3. Efflux

Antimicrobials can be exported out of the bacterial cell by efflux pumps. Efflux and porin loss are co-ordinated by the same regulation cascade; however, can be activated independently from each other [28]. There are five known families of efflux systems: the major facilitator super family (MFS); the ATP-binding cassette (ABC) family; the resistance-nodulation-division (RND) family; the multidrug and toxic compound extrusion (MATE) family; and the small multidrug resistance (SMR) family [29, 30]. A single organism can have multiple efflux systems from more than one of the 5 families and can also contain more than one efflux pump type from the same family [31]. The chromosomally encoded RND family of efflux pump systems are most common in Gram-negative bacteria and confer resistance to a wide variety of antimicrobial classes such as the β-lactams, including carbapenems, and third and fourth generation cephalosporins. Efflux pump systems such as the *E. coli* AcrAB-TolC effect the antibacterial activity of commonly prescribed antimicrobials such as chloramphenicol, fluoroquinolones, β-lactams, and tetracycline [31]. No efflux pump inhibitor is currently licensed for use. Lamers *et al.* (2013) showed that the use of the extended spectrum efflux pump inhibitor phenylalanine arginine β-naphthylamide (PAβN), also called MC-207,110, can increase outer membrane permeability and/or impair drug efflux, reducing the β-lactam (piperacillin, cefotaxime, ceftazidime) minimum inhibitory concentration (MIC) for *P. aeruginosa,* including over-expressing AmpC *P. aeruginosa* [32]. Kallman *et al.* (2003) demonstrated that cefuroxime resistance in an *E. coli i*solate was reduced by the efflux inhibitor PAβN [33]. The development of such inhibitors could prove important for future treatment regimens facilitating higher intracellular antimicrobial concentrations.

4. Production of enzymes which inactivate the antimicrobial, e.g. beta-lactamases

Beta-lactamases, the enzymes that inactivate β-lactam antimicrobials by hydrolysis of the β-lactam ring have been evolving for over 2 billion years with the number of

distinct, naturally occurring β-lactamases now exceeding 1,300 [34, 35]. Four groups of β-lactamases can be defined: penicillinases, cephalosporinases, extended-spectrum beta-lactamases (ESBL) and carbapenemases [8]. β-lactamases are found in Gram-positive bacteria and Gram-negative bacteria; however, are a major mechanism of resistance in Gram-negative bacteria. Production of β-lactamases is the most common and effective mechanism of resistance to β-lactam antimicrobials.

Classification of β-lactamase enzymes is commonly based on two schemes: the Ambler molecular Scheme and the Bush Jacoby functional Scheme. The Ambler molecular classification scheme divides the beta-lactamases into four groups based upon their amino acid sequences; serine groups A, C, and D and the metal-dependent group B [35]. The metal independent groups A, C, and D have an active site serine which binds to and inhibits the β-lactam ring of β-lactam antimicrobials by the production of an acyl enzyme. Group B is metal dependent as it requires a zinc ion active site to hydrolyse the β-lactams, are inhibited by metal ion chelators and can be divided into four classes (B1a, B1b, B2, B3) based on the amino acids involved in zinc ligands [35]. As gene sequencing became more accessible in the 1980's this led to the development of classification schemes combining both functional groups and molecular classes. Such a nomenclature system was developed by Bush, Jacoby, and Medeiros (BJM) in 1995 and further expanded in 2010 by Bush and Jacoby accounting for the newly described β-lactamases [36]. The functional groups are segregated by hydrolysis and inhibition characteristics [35, 36].

ESBLs are generally considered as enzymes that confer resistance to the penicillins, the monobactams, hydrolyse oxyimino-cephalosporins at 10% more than benzylpenicillin, and can't degrade β-lactamase inhibitors, cephamycins, and carbapenems [7, 37]. Initially, ESBLs were recognised as TEM and SHV mutants; however, the definition of an ESBL has become confusing over time with the identification of new enzymes from different evolutionary backgrounds with no

narrow spectrum parent enzyme (e.g. CTX-M, PER), mutations occurring in clavulanate resistant β-lactamases resulting in a broader spectrum of activity (e.g. OXA), and with the detection of β-lactamases with a typical ESBL profile but can hydrolyse carbapenems at a slow rate (e.g. GES), β-lactamases with a greater increased activity against cephalosporins than the parent enzyme but do not meet the 10% more than benzylpenicillin when hydrolysing oxyimino-cephalosporins (e.g. TEM-12), and extended-spectrum AmpC [37]. In addition, it has been questioned whether chromosomal encoded enzymes meeting the inhibition and hydrolysing criteria should be included as ESBL enzymes but no clear agreement has been made [37]. When defining an ESBL it is important to also state the ESBL class such as "TEM ESBL", "OXA ESBL" or "CTX-M ESBL" [37]. Although there is confusion regarding the definition of an ESBL, there is agreement that ESBLs are associated with significant clinical and economic costs. It has been reported that patients with ESBL bacteraemia often received inappropriate initial antimicrobial therapy, have a higher mortality rate by 21 days and have longer, more expensive hospital stays [38].

1.3.1 TEM β-lactamases

The first plasmid mediated β-lactamase, TEM-1, was described in the 1960s [39]. TEM-1 was named after the patient from whom the bacterium harbouring the enzyme was initially found, Temoniera. It has been estimated that TEM-1 is responsible for 90% of ampicillin resistance in *E. coli* [39]. This enzyme has an isoelectric point (pI) of 5.4 and is transposon mediated which has aided in its dissemination to various species of *Enterobacteriaceae, P. aeruginosa, Haemophilus influenzae*, and *Neisseria gonorrhoeae* [40]. Shortly after the discovery of TEM-1 its derivative TEM-2 was detected. TEM-2 differs from TEM-1 by one amino acid at position 39 where the substitution of lysine for glutamine occurs [41]. Position 39 is not located near the β-lactam binding site, therefore there is no substrate alteration [16]. Both TEM-1 and TEM-2 β-lactamases belong to Ambler class A and BJM group 2b β-lactamases.

TEM-3, the first TEM-type ESBL, was reported in France in the 1980s, differing from TEM-2 by two amino acid substitutions: lysine for glutamine at position 102 and serine for glycine at position 236 [42, 43]. These substitutions altered the substrate binding due to their location at the substrate binding site, thus an expanded substrate hydrolysis profile was formed for TEM-3 in comparison to TEM-2 and TEM-1. TEM-type ESBL enzymes belong to Ambler class A and BJM group 2be β-lactamases, with increased hydrolysis of oxyiminio- β-lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam). This group of enzymes have continued to diversify; however are predominately reported in *E. coli* and *Klebsiella* spp. and other members of the *Enterobacteriaceae*, varying in substrate and resistance profiles. There are now over 210 TEM β-lactamases described (www.lahey.org/Studies). Certain amino acid changes influence the hydrolysis of the expanded spectrum of antimicrobials [44]. TEM-derived ESBLs can also be found in *P. aeruginosa.* The dissemination of various TEM-type ESBLs within the hospital and the community setting worldwide has resulted in decreased antimicrobial therapeutic options [45].

1.3.2 SHV β-lactamases

The term SHV is derived from 'Sulfhydryl Variable', a description of this class of enzymes. The first SHV variant SHV-1 was described as Pit-2 by Pitton in 1972 and belongs to Ambler class A, functional group 2b [46]. SHV-1 is predominantly chromosomally encoded and is ubiquitous in *K. pneumoniae* [46].

The first SHV-type ESBL was reported in Germany in 1983 in a *Klebsiella ozaenae* isolate and was designated SHV-2 [16]. SHV-2 differs from SHV-1 due to amino acid substitutions at positions 238 (serine for glycine) and 240 (lysine for glutamate [26, 47]. SHV-type ESBLs belong to Ambler Class A, functional group 2be. SHV-type ESBLs have been reported in members of the *Enterobacteriaceae*, *P. aeruginosa* and *Acinetobacter* spp. [48-50]. There are currently over 180 SHV β-lactamases described (www.lahey.org/Studies).

1.3.3 OXA β-lactamases

OXA-type β-lactamases are Ambler class D group 2d enzymes that hydrolyse oxacillin and cloxacillin more efficiently than benzylpenicillin, are generally not inhibited efficiently by clavulanic acid; however may be inhibited by sodium chloride [7, 16]. OXA enzymes can be divided into 3 main groups based on the modified BJM Classification scheme:

- Molecular group D, functional group 2d are generally classified as hydrolysing cloxacillin or oxacillin, weak hydrolysis of cephalosporins, no carbapenemase activity, and may be resistant to the beta-lactamase inhibitors e.g. clavulanic acid. Examples of this group include OXA-1, OXA-2 and OXA-10 [16, 36]. However, reports have highlighted the need to recognise the OXA-1 enzyme as having the potential to produce ESBL activity [51]. It has been observed that isolates of *S.* Typhimurium with an OXA-1 β-lactamase can demonstrate resistance to cefepime (fourth generation cephalosporin) with significant inhibition by clavulanic acid [51]. A 39kb plasmid (pFEF39), with a P2 promoter containing a GGG triplet upstream of the -10 signal, has been reported to carry $bla_{\text{OXA-1}}$ and this plasmid has been found in multidrug-resistant isolates [51]. Similarly, the presence of $bla_{\text{OXA-1}}$ in clinical isolates of *E. coli* has been associated with resistance to co-amoxiclav [52].

- Molecular group D, functional group 2de demonstrates penicillinase and cephalosporinase activity, ESBL activity, and no carbapenemase activity [36]. OXA variants commonly recognised with ESBL activity are derived from OXA-10 and OXA-2 by amino acid substitutions [53]. Examples of function group 2de include OXA-11, OXA-14, and OXA-15 [36].

- Molecular group D, function group 2df generally have activity against cloxacillin or oxacillin and carbapenems and are frequently sensitive to inhibition by clavulanic acid. Examples include OXA-23 and OXA-48 [36].

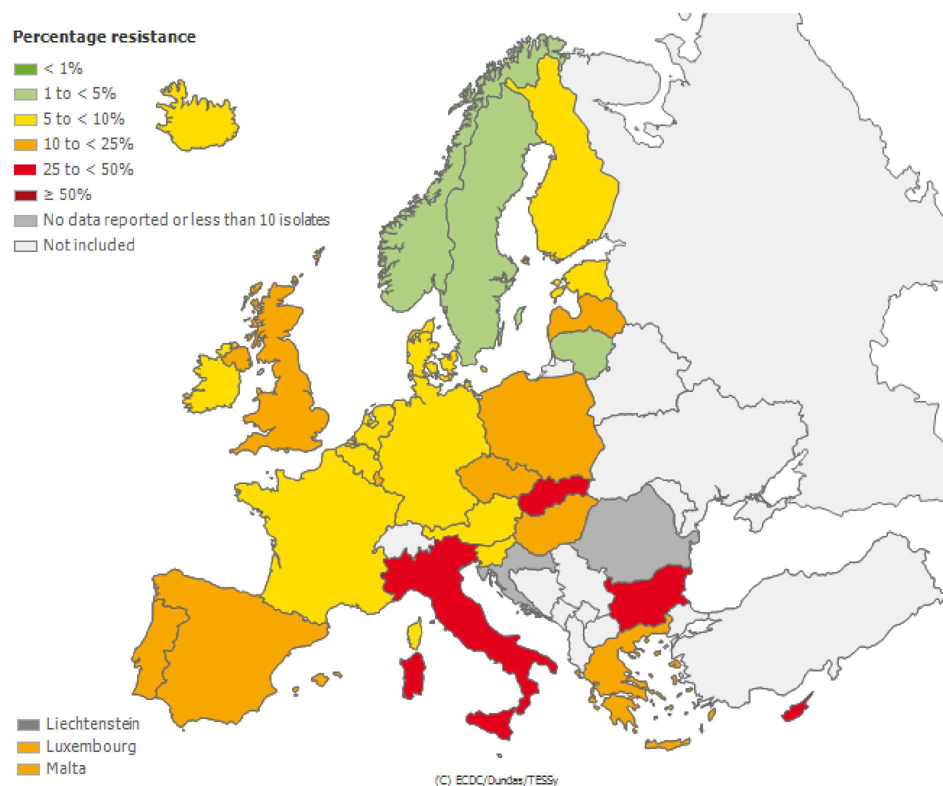
1.3.4 CTX-M extended spectrum β-lactamases

CTX-M enzymes preferentially hydrolyse cefotaxime (hence the designation CTX-M). The CTX-M β-lactamases belong to Ambler class A/ group 2be [54]. At present, there are over 150 CTX-M-type β-lactamases described (www.lahey.org/Studies). There are five groups of CTX-M β-lactamases differentiated by their amino acid sequences: CTX-M-1,-2,-8,-9 and -25. CTX-M β-lactamases share >94% amino acid homology within each group and $\leq$ 90% similarity between the various groups [55]. These enzymes are susceptible to β-lactamase inhibitors such as clavulanic acid and tazobactam. The CTX-M β-lactamases are believed to have arisen from the mobilisation of a chromosomal β–lactamase gene from the environmental *Kluyvera* spp. [56]. The CTX-M β-lactamases have been found in members of the *Enterobacteriaceae*, predominantly in *E. coli*, *Salmonella* spp*, K. pneumoniae* and *P. mirabilis*. The first reports of CTX-M β-lactamases emerged in the late 1980s from *E. coli* isolates in Germany and France [54]. The dissemination of this CTX-M class of enzymes is alarming as these enzymes are currently widespread in Ireland, have been rapidly increasing worldwide since 2000, and are now the most commonly reported ESBL enzymes [57-59].

The relative importance of CTX-M variants differs by region with CTX-M group 1, particularly the CTX-M-15 variant, being most common throughout the world [60-64]. CTX-M-1 and CTX-M-32 are also common throughout Europe [42]. CTX-M group 9, particularly CTX-M-14, has also been detected worldwide and is commonly reported in Spain, Portugal, France, the UK, and throughout Asia [42, 56, 64-66]. CTX-M group 2 has been the main CTX-M group identified in South America (e.g. Argentina), throughout Asia, Russia, Israel, and eastern European countries [42, 56]. The CTX-M group 8 (CTX-M-40 variant) has only been detected in the UK and the CTX-M group 25 (CTX-M-26 variant) has also been reported in the UK [42].

CTX-M enzymes are associated primarily with urinary tract infections and blood stream infections [67-69]. Spread of CTX-M enzymes, particularly CTX-M-15, are linked to association with specific epidemic clonal groups such as *E. coli* sequence type ST131 (typically of serotype O25b:H4) and specific epidemic plasmids, e.g. IncFII and are a major contributor to resistance to third generation cephalosporins (Figure 1.2) [70-72].

Figure 1.2: Percentage of invasive *E. coli* isolates with resistance to third generation cephalosporins in EU/EEA countries in 2012 (EARS-Net 2012).



1.4 Laboratory detection of extended spectrum β-lactamases

- **Screening rectal swabs for ESBL production**

Chromogenic media such as chromID ESBL (bioMérieux), Brilliance ESBL Agar (Oxoid Ltd) and CHROMagar ESBL (CHROMagar, Paris, France) are convenient for initial screening of rectal samples for ESBL-producing bacteria. Both chromID ESBL agar and Brilliance ESBL are ready to use, therefore do not require any additional supplements. The two chromogenic agars contain cefpodoxime to select for ESBL producers and to inhibit the growth of Gram-positive organisms [73]. chromID ESBL and Brilliance ESBL Agar contain two chromogens which are metabolised by β-glucuronidase and/or β-galactosidase enzymes facilitating bacterial identification [73].

- **Detection of ESBL production**

Antimicrobial susceptibility testing (AST) and interpretive criteria can be applied for the purposes of testing isolates for ESBL production. Breakpoints are used as the interpretative criteria for defining if an organism is susceptible or non-susceptible to a selection of antimicrobials. Various committees develop breakpoints for antimicrobials; however, the two methods most commonly implemented worldwide include those from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS, National Committee for Clinical Laboratory Standards). Other standards are set by the British Society for Antimicrobial Chemotherapy (BSAC) in the UK, the Deutsches Institut für Normung (DIN) in Gemany, Société Française de Microbiologie (SFM) in France, the Swedish Reference Group for Antibiotics (SIR), Werkgroep Richtlijnen Gevoeligheidsbepalingen (WRG) in the Netherlands, and the U.S. Food and Drug Administration [74]. Variations in breakpoints due to the lack of one consensus breakpoint system has led to confusion for microbiologists and manufacturers of antimicrobial susceptibility testing equipment worldwide. EUCAST and CLSI breakpoint standards will be referred to in the remainder of this thesis.

ESBL production can be detected based on non-susceptibility to oxyimino-cephalosporins, followed by confirmation tests [75]. EUCAST guidelines recommend using both cefotaxime (or ceftriaxone) and ceftazidime (or cefpodoxime) when evaluating ESBL production (Figure 1.3) [75]. Ceftazidime is reported as having high sensitivity for detecting TEM and SHV ESBLs, whereas cefotaxime has high sensitivity for detecting CTX-M enzymes, therefore both antimicrobials are recommended for confirmation of ESBL-producing *Enterobacteriaceae* [76]. CLSI guidelines recommend the use of more than one of the following antimicrobials for screening for ESBL production: cefpodoxime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone [75, 77]. EUCAST guidelines (2013) recommend broth dilution, agar dilution, disk diffusion or automated systems as the screening methods for ESBL production [75]. According to EUCAST guidelines (2013), it is important to detect the ESBL resistance mechanism for infection control and public health; however, ESBL production can't be used to solely to report an organism as being resistant [75].

- **Confirmation of ESBL production**

EUCAST and CLSI recommend disk diffusion methods, double disk synergy tests, broth microdilution or gradient testing with predefined concentrations of the antimicrobial (Etest) as phenotypic ESBL confirmation tests based on cephalosporin/clavulanic synergy [75, 77]. The combination disk diffusion method is performed by disk diffusion using two extended spectrum cephalosporins (cefotaxime, ceftazidime, cefepime) alone and in addition to clavulanic acid. A positive ESBL results is indicated if there is a $\geq$ 5mm zone of inhibition for the extended spectrum cephalosporin plus clavulanic acid in comparison to the extended spectrum cephalosporin alone [75].

EUCAST recommends performing the double disk synergy test using cefotaxime, ceftazidime and cefepime. This test involves applying a cephalosporin disc 20mm apart from a disc containing clavulanic acid (amoxicillin-clavulanic acid) [75]. A presumptive ESBL is indicated when the zone of inhibition around the disc

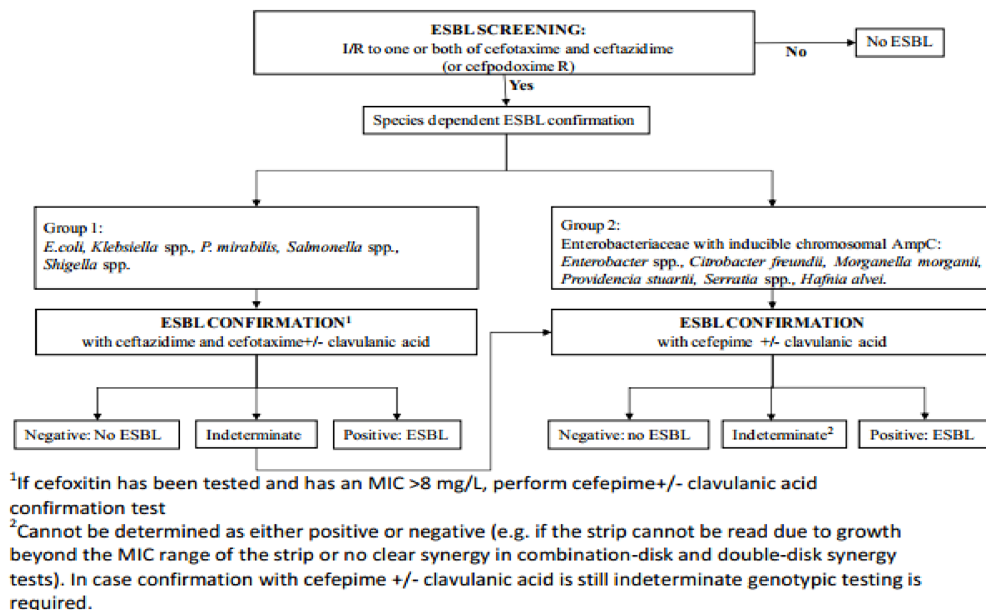
containing a cephalosporin is augmented in the direction of the disc containing clavulanic acid, demonstrating an inhibitory effect by clavulanic acid .

The ESBL Etest can be used to report an ESBL producing organism in the event of a $\geq$ 8 fold decrease of the MIC of the extended spectrum cephalosporin in combination with clavulanic acid compared to the MIC of the cephalosporin alone or if a phantom/ deformed ellipse occurs [75, 78].

Antimicrobial dilution assays are quantitative designed to determine the MIC of serial two-fold dilutions of cephalosporins (cefotaxime, ceftazidime, and cefepime) ranging from ranging from 0.25 to 512 mg/L, in the presence and absence of 4 ml/L clavulanic acid [75]. An ESBL producer is reported if there is an 8-fold reduction in the MIC of the cephalosporin in conjunction with clavulanic acid in comparison to the cephalosporins alone [75].

The confirmation methods outlined here are designed for ESBL-producing *E. coli* or *K. pneumoniae.* Confirming ESBLs in other bacteria such as *Enterobacter* is more challenging as clavulanate can induce production of AmpC β-lactamase and therefore may mask the ESBL if present. AmpC β-lactamases may be indicated by resistance to cephamycins such as cefoxitin. Cefepime is the recommended cephalosporin to use when confirming ESBL production in *Enterobacteriaceae* with a inducible chromosomal AmpC such as *Enterobacter* spp., *Citrobacter freundii, Morganella morganii, Providencia stuartii, Serratia* spp., *Hafnia alvei* (Figure 1.3) [75]. Cloxacillin is a good inhibitor of AmpC β-lactamases and may be used for detection of AmpC enzymes. Cefotaxime is not recommended for confirming ESBL production in *K. oxytoca* as it may yield a false positive result due hyperproduction of the chromosomal K1 or OXY-like β-lactamases [75]. Carbapenamase production or membrane permeability may also mask ESBL production.

Figure 1.3: Flow-chart for phenotypic detection of Extended Spectrum β-lactamases in *Enterobacteriaceae* based on recommendations by The European Committee on Antimicrobial Susceptibility Testing [75]



- **Molecular methods**

Molecular methods can be used to rapidly identify ESBL encoding genes. Such methods include the use of PCR and sequencing analysis, DNA microarray-based methods and enzyme-linked immunosorbent assays (ELISA) [73].

1.5 Fluoroquinolones

In 1962, the first quinolone, nalidixic acid, was synthesised and in 1967 it was introduced into clinical therapy [3]. The earlier quinolones were active against Gram-negative bacteria. In addition, the earlier quinolones were mainly used for the treatment of urinary tract infections (UTIs). Since then, a diverse group of synthetic compounds have been developed based on the 4-quinolone nucleus. In 1976, the first fluoroquinolone was developed, named flumequine, and the development of fluoroquinolones arose by the addition of fluorine to the 4-quinolone nucleus at position 6 which increased DNA gyrase inhibitory activity [3, 79]. In 1983, ciprofloxacin was synthesised [80]. Ciprofloxacin retains fluorine at

position 6, a 6 member piperazine ring at position 7 and a cyclopropyl group at position 1.

Fluoroquinolones act by inhibiting DNA replication targeting DNA gyrase and topoisomerase IV enzymes [56]. These enzymes are required for maintaining the supercoiled DNA helix during DNA replication and transcription [81]. The DNA gyrase consists of two subunits (GyrA and GyrB). The cellular function of DNA gyrase is to introduce negative supercoils into DNA, aiding the separation of DNA strands [81]. The topoisomerase IV consists of two subunits (ParC and ParE) required for the separation of the linked daughter chromosomes in the replication process [81]. The fluoroquinolones binds to and inhibits DNA gyrase and topoisomerase IV synthesis disrupting replication and transcription [3, 79, 81].

Ciprofloxacin has greater activity against Gram-negative and Gram-positive bacteria than its quinolone and fluoroquinolone predecessors and remains more active against *P. aeruginosa* than newly synthesised fluoroquinolones [80]. According to the studies performed by the European Centre for Disease Prevention and Control (ECDC) and the European Surveillance of Antimicrobial Consumption ciprofloxacin is the most frequently prescribed fluoroquinolone in Europe [11, 82]. Other commonly prescribed fluroquinolones include levofloxacin and ofloxacin [11, 82, 83]. Fluoroquinolones are primarily used in clinical medicine for treatment of urinary tract infections, sexually transmitted diseases, gastrointestinal infections, respiratory tract infections, skin infections, wound infections, pelvic infections, and also as prophylaxis for the prevention of UTIs [79, 83].

The development of fluoroquinolones has continued leading to new fluoroquinolones with activity against anaerobes and increased activity against Gram-positive bacteria, such as *S. pneumoniae,* and Gram-negative bacteria, in particular *Enterobacteriaceae*, *P. aeruginosa, H. Influenzae, Mycoplasma pneumoniae,* and *Legionella pneumophila* [3]. Since 2000, seven fluoroquinolones

have been approved for clinical practice for the treatment of Gram-positive and Gram-negative infections, and there are various fluoroquinolones currently undergoing clinical trials [4].

1.5.1 Resistance to fluoroquinolones

High levels of fluoroquinolone resistance have been attributed to three mechanisms that can have an individual or synergistic effect. Resistance to fluoroquinolone can occur due to the following:

1. Chromosomal mutations in the quinolone-resistance-determining region of *gryA*, *gryB*, *parC*, and *parE.*
2. Porin loss and over-expressing efflux pumps such as PmrA [84].
3. Plasmid-mediated quinolone-resistant determinants: the genes encoding protective proteins of *DNA gyrase qnr (qnrA, qnrB, qnrC, qnrD, qnrS),* the fluoroquinolone-modifying acetyltransferase gene *(aac(6')-Ib-cr),* and efflux pump gene *qep* [56, 85, 86].

Resistance to fluoroquinolones has become a major problem and continues to increase, particularly in *E. coli*. In Ireland in 2013, 25% of all *E. coli* bloodstream isolates were ciprofloxacin-resistant (Figure 1.4) [87]. The results from EARS-NET illustrate high rates of fluoroquinolone resistance throughout Europe in 2012, with an upward trend in fluoroquinolone resistance occurring in several other EU countries [88].

Figure 1.4: Trends for invasive infections caused by *E. coli* with resistance to third generation cephalosporins, ciprofloxacin/ofloxacin, gentamicin, and gentamicin/tobramycin/amikacin in Ireland from 2004-2013 [87] .

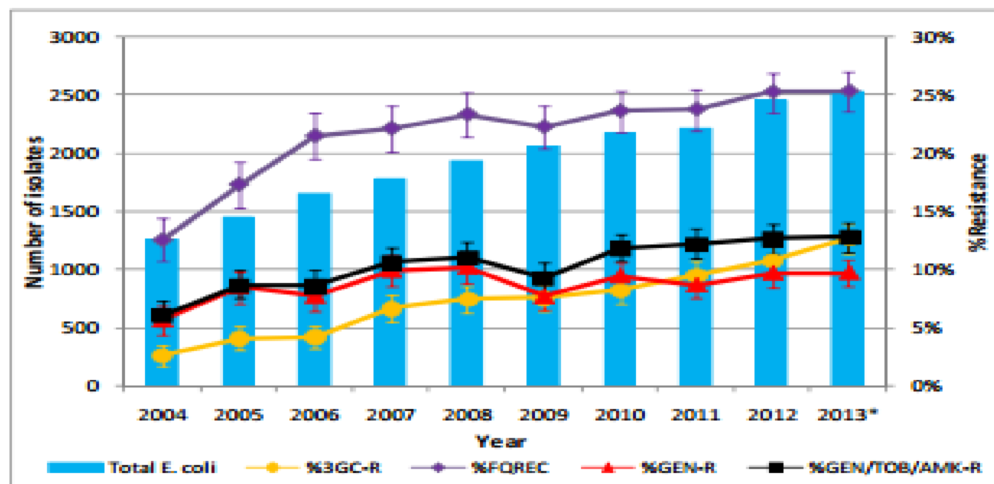


Figure 3. Trends for *E. coli* – total numbers of *E. coli* and percentage resistance to 3GCs, ciprofloxacin/ofloxacin (CIP/OFX), gentamicin (GEN) and gentamicin/tobramycin /amikacin (GEN/TOB/AMK) with 95%CIs

* Data for 2013 are provisional to the end of Q4

1.6 The *Enterobacteriaceae*

The *Enterobacteriaceae* are a common family of bacteria isolated in the clinical setting and are responsible for various types of infections including urinary tract infections, blood stream infections, food borne infections, and meningitis among others [89]. The *Enterobacteriaceae* comprise over 50 genera and more than 100 species. The four main species causing healthcare-associated infections (HAIs) in Europe are, in order of highest prevalence, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp. and *Proteus* spp. [11]. Members of the *Enterobacteriaceae* have been associated with 80% of Gram-negative bacilli isolated from clinical specimens and are a major cause of bloodstream infections [84]. The *Enterobacteriaceae* are enteric organisms commonly found in the gastrointestinal tract, Gram-negative rods, facultatively anaerobic, oxidase–negative, reduce nitrates to nitrites, ferment

glucose, catalase-positive (except *Shigella dysenteriae* type 1), and the majority are motile via flagella with the exceptions of *Shigella* and *Klebsiella.* Species of the *Enterobacteriaceae* can be differentiated in clinical laboratories based on growth factors, colonial morphology, and the use of various biochemical reactions. Matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) can be used to identify microorganism by examining the expression of their intrinsic proteins. Using MALDI-TOF MS, species are identified based on mass and intensity of primarily ribosomal proteins. The spectrum generated is compared to a database of reference spectra allowing microorganism identification.

1.7 *Escherichia coli*

Dr. Theodore Escherich first described *E. coli* in 1885 [84]. *E. coli* is one of the most studied species of bacteria. *E. coli* commonly colonises the lower part of the intestine of warm-blooded animals. Pathogenic variants of *E. coli* have been identified and this thesis will discuss extraintestinal pathogenic *E. coli* (ExPEC) and uropathogenic *E. coli* (UPEC). ExPEC are associated with urinary tract infections, sepsis, and newborn meningitis and UPEC is a common cause of urinary tract infections. Colonisation and infection can be associated with the expression of virulence and adhesion factors and the levels of expression can differ between pathogenic and non-pathogenic bacteria [90].

Lipopolysaccharide (LPS) molecules contain a central lipid A, a polysaccharide 'O' antigen and are located on the outer membrane of Gram-negative bacteria. The lipid A can induce a host response to infection which can led to fever, shock, and metabolic acidosis associated with severe sepsis [91]. Serological classification of *E. coli* is based on the identification of somatic (O) antigens, flagellar (H) antigens and capsular (K) antigens [92, 93]. In *E. coli* there are more than 180 different O antigens and more than 50 H antigens [94, 95]. Pathogenic *E. coli* possess virulence

factors including adhesins (e.g. fimbrial adhesion H), invasins (e.g. haemolysin), toxins [cytotoxic necrotizing factor 1 (cnf-1), secreted autotransporter toxin (sat), vacuolating autotransporter toxin (vat)], iron acquisition systems, defence against serum bactericidal reactions, multiple pili, mobile genetic elements (transmissible plasmids and the aerobactin system), flagella, and/or antiphagocytic surface properties (capsules). Approximately 80% of UTIs are due to uropathogenic UPEC causing cystitis and phyelonephritis [96]. Binding of UPEC to the uroepithelium is associated with fimbriae, particularly P fimbriae and type 1 fimbriae, which can be distinguished according to their binding receptors. P fimbriae bind to receptors on the P blood group antigens of human erythrocytes and uroepithelial cells, and type 1 fimbriae bind to cells that contain sugar mannose. *E. coli* expressing type 1 fimbriae can cause lower UTI, whereas P fimbriae are particularly associated with pyelonephritis and blood stream infection septicaemia [91, 97, 98]. Both P fimbriae and type 1 fimbriae have been reported to act in synergy to promote colonisation [99]. *E. coli* can also cause neonatal meningitis, septicaemia, and sepsis in wounds and abscesses. The K1 capsule antigen is recognised as a significant virulence factor of *E. coli* enhancing the survival of the bacteria by inhibiting phagocytosis and adhering to brain endothelial cells [100, 101]. It is found in approximately 80% of *E. coli* strains causing neonatal meningitis [102].

Bacteriuria is the presence of bacteria in the urine and may be asymptomatic or symptomatic. Treatment of bacteriuria depends on the clinical features presented by a patient and the risks associated [103]. An example of when asymptomatic bacteriuria should be treated would be when a pregnant woman presents with bacteriuria [103]. In this case a pregnant woman with bacteriuria should be treated as she may develop phyelonephritis later in pregnancy, whereas asymptomatic bacteriuria is common in older people and does not require treatment [103]. Patients with bacteriuria may present with clinical symptoms of cystitis (infection of the bladder) and/or phyelonephritis (infection of the kidney). Clinical features of cystitis may include dysuria, increased frequency of voiding, urgency to pass urine, and abdominal discomfort [89]. Symptoms such as fever, rigors, tiredness, loin pain,

and vomiting are associated with pyelonephritis. Urine that appears cloudy, strong-smelling and/or containing blood may indicate a UTI [89]. UTIs can be characterised as uncomplicated or complicated. Uncomplicated UTIs involve infection of the urinary tract without any underlying condition and the urethra appears normal. Complicated UTIs may involve renal problems, abnormalities in the urinary tract and may be linked with underlying conditions such as diabetes mellitus.

UTIs are the 3rd most frequently reported healthcare-associated infection (HAIs) and accounted for approximately 19% of HAIs in Europe and 15% of HAIs in Ireland, according to a recent European point prevalence study performed from 2011-2012 [11, 104]. It is estimated that 20-40% of women will have a urinary tract infection in their lifetime with a high incidence, accounting for up 6% of consultations in general practice [89]. *E. coli* is the predominant organism causing UTIs [105]. *E. coli* are also associated with other infections including surgical site infections, bloodstream infections, pneumonia/ lower respiratory tract infections, and gastrointestinal tract infections [11].

Urinary catheterisation is applied in a number of situations but most commonly among the elderly, particularly among residents of long term care facilities. Bacteriuria is common among individuals with a urinary catheter and is often asymptomatic. Prolonged catheterisation is associated with increased risk of catheter-associated symptomatic UTI and mortality [106, 107]. It is imperative that catheters are only used when necessary and are removed when no longer needed. Catheter-associated UTIs have a tendency to reoccur and are difficult to treat as the pathogen can form a biofilm on the catheter [108]. *E. coli* and *P. mirabilis* are the most common cause of catheter-associated UTIs [108]. Catheterised individuals should only be tested for bacteriuria and receive antimicrobial treatment if symptomatic of a urinary tract infection or a systemic infection [103].

1.7.1 *E. coli* O25b:ST131

E. coli sequence type (ST) 131 producing CTX-M-15 was first reported in 2008 [109-111]. However, recent studies published in 2012 have identified the earliest isolate of *E. coli* ST131 dating back to 1967 and observed that the earliest strain demonstrated fluoroquinolone susceptibility, followed by fluoroquinolone-resistant strains, and *E. coli* ST131 with associated ESBLs, predominantly CTX-M-15, emerged mainly in the 2000s [112]. This clonal group was designated OH serotype (O25:H4) and phylogenetic group B2 [109]. The success of *E. coli* O25b:ST131 is attributed to virulence, antimicrobial resistance, and transmissibility [113]. *E. coli* O25b:ST131 will be abbreviated to ST131 for the remainder of this thesis.

ST131 has disseminated worldwide and is associated with a wide variety of infections including UTIs, bacteremia, pneumonia, and wound infections [114-119]. ST131 has predominantly been reported from the hospital setting; however, in recent years it has also been reported in high prevalence in the community setting and long term care facilities (LTCFs) [120, 121]. LTCFs and hospitals act as ideal reservoirs due to their vulnerable population and the extensive use of antimicrobials in these settings. A study performed between 2004 and 2006 reported intestinal colonisation with ESBL-producing *E. coli* in 118/294 residents in 16 LTCFs in Belfast, 117/118 belonging to ST131 clone [61]. Similar findings of ST131 carriage among the elderly and LTCFs has been reported in Europe and the United States [119, 122-124].

It is important to identify the risk factors for acquisition of ESBL-producing organisms including the *E. coli* ST131 clonal group. Various studies have reported urinary catheterisation, prior antimicrobial use, hospitalisation, disabilities, and being elderly as the most important risk factors [125-128]. As mentioned, the ST131 clonal group has been isolated from both hospitals and LTCFs [120]; however, it is not known at present if this clonal group is disseminating predominantly within LTCFs or if colonisation in LTCFs is primarily due to acquisition during frequent

episodes of hospitalisation in the scenario which has been referred to as the 'revolving door' [119].

ST131 is most common among human isolates; however, it has also been reported among animals, food sources, and the environment [129-131]. Such environmental sources include the River Thames in the UK and river water in Barcelona [131, 132]. Pitout *et al.* (2009) and Kennedy *et al.* (2010) reported that foreign travel is one of the major risk factors for colonisation and infection with *E. coli* ST131 and other antimicrobial resistant organisms especially when travelling to high risk areas such as the Indian sub-continent, Europe, China, and the Middle East/Africa [133, 134]. Food and water from high risk areas are suggested as two possible reservoirs for the ST131 clonal group [133]. Consumption of antimicrobials while travelling has been linked to the acquisition of the antimicrobial resistant strains of bacteria [133]. ST131 has been detected in companion animals, seagulls, rats, poultry, and pigs [121]. Surface contamination with ST131 in the indoor environment of healthcare settings may play an important role in the dissemination of this clonal group; however, little evidence is present in the literature [135].

Other STs of *E. coli* such as ST95, ST73, ST127, ST69, and ST393 have been associated with community and healthcare acquired infections, with high prevalence among UTIs and bloodstream infections, in various geographical locations. However, ST131 has been reported as the most prevalent antimicrobial resistant ST of *E. coli*, in particular among children and the elderly, and is more frequently associated with healthcare associated infections [136, 137].

1.7.1.1 Antimicrobial resistance associated with *E. coli* ST131

ST131 is particularly associated with fluoroquinolone resistance, which usually occurs due to mutations within the quinolone resistance determining region

(QRDR) of *gyrA* and *parC*; the genes encoding fluoroquinolone targets DNA gyrase and topoisomerase IV respectively [138]. Subtypes of ST131 have been identified based on *fimH* alleles and *gyrA/parC* combinations [138].

FimH is a mannose-specific type-1 fimbrial adhesin common in most *Enterobacteriaceae* [139, 140]. FimH is a virulence factor in uropathogenic *E. coli* and FimH mediated binding to mannose receptors enhances colonisation, invasion of the bladder epithelium and intracellular bacteria, which are critical in causing UTIs [140]. The *fimH* gene is located in a recombinant fragment within the ST131 lineage [141, 142]. Allelic diversity of *fimH* can reflect functional differences in strains of the same ST [139, 142]. The sequence of the type-1 fimbrial adhesin gene *fimH* varies within ST131 and has been used to identify ST131 subclones [143, 144].

The ancestor allele 22 of *fimH* (*fimH22)* was historically isolated in fluoroquinolone susceptible isolates; however, since 2000 the allele 30 of *fimH* (*H30* ST131 subclone) has continued to increase in prevalence and is associated with multidrug-resistance, particularly fluoroquinolone resistance, and CTX-M-15 [138]. The *H30* ST131 subclone now predominates among ST131 isolates and is the single most common subclone of fluoroquinolone-resistant *E. coli* in the USA [138]. Unlike other lineages, the *H30* subclone is associated with a specific *gyrA/parC* allele combination represented by mutations in *gyrA* at position 87 (aspartic acid to asparagine) and mutations in *parC* at position 80 (serine to isoleucine) and position 84 (glutamic acid to valine*)* [116, 138]. This *gyrA/parC* allele combination confers fluoroquinolone resistance [116]. The *H30* subclone has been found in 88% of ST131 isolates; 99% of fluoroquinolone-resistant ST131 isolates and no fluoroquinolone susceptible ST131 isolates in healthcare settings in the USA [122]. The predominance of the *H30* subclone has been reported to be associated with healthcare settings and the elderly [122].

Using whole genome analysis Price *et al*. (2013) identified a further sub-lineage within the *H30* subclone, named *H30-Rx* because of its high antimicrobial resistance profile [143]. *H30-Rx* is associated with CTX-M-15 and differs from the other *H30* CTX-M-15 negative isolates by three core genome single-nucleotide polymorphisms (SNPs) [116, 143]. The whole genome analysis confirmed that the dissemination of CTX-M-15 in ST131 is due to global dissemination of the *H30-Rx* sub-lineage. The *H*30-Rx subclone has a reported association with sepsis, suggesting that virulence may be contributing to its epidemiologic success [143, 145].

The *aac(6')-Ib-cr* variant encodes an aminoglycoside acetlytransferase that confers reduced susceptibility to aminoglycosides (kanamycin and tobramycin) and certain fluoroquinolones such as ciprofloxacin by N-acetylation of its piperazinyl amine [146, 147]. The *aac(6')-Ib-cr* variant is often associated with ST131 conferring plasmid-mediated resistance to ciprofloxacin [72]. ST131 are also frequently reported as resistant to the expanded spectrum cephalosporins mediated by the production of an ESBL, most frequently a CTX-M variant particularly $bla\text{-}_{\text{CTX-M-15}}$ and to a lesser extent $bla_{\text{CTX-M-3,}}$ $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{CTX-M-32}}$ [68, 148-151]. Infrequent reports of ST131 producing SHV, TEM, OXA-1, and CMY β-lactamases have also emerged [121]. In addition to resistance to the expanded spectrum cephalosporins, carbapenem resistance has been reported in ST131 due to the acquisition of carbapenemase genes such as $bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$, $bla_{\text{VIM-1}}$, and $bla_{\text{OXA-48}}$ [152-155].

1.7.1.2 Laboratory detection and characterisation of *E. coli* ST131

There are two distinct MLST schemes available for *E. coli* and both schemes have been used to define ST131 based on the sequences of different housekeeping genes. The Achtman MLST scheme (http;//mlst.ucc.ie/mlst/dbs/Ecoli) is based on seven housekeeping genes: *adk* (adenylate kinase), *gryB* (DNA gyrase), *fumC* (fumarate hydratase), *icd* (isocitrate dehydorgenase), *mdh* (malate dehydrogenase),

purA (adenylosuccinate synthetase), and *recA* (ATP/GTP binding motif) [156]. The Institut Pasteur's MLST scheme (www.pasteur.fr/mlst) analyses 8 housekeeping genes: *dinB* (DNA polymerase), *icdA* (isocitrate dehydrogenase), *pabB* (p-aminobenzoate synthase), *polB* (polymerase PolII), *putP* (proline permease), *trpA* (tryptophan synthase subunit A), *trpB* (tryptophan synthase subunit B), and *uidA* (beta-glucuronidase). MLST is useful for the characterisation of bacteria and permits comparison of strains worldwide. MLST is portable, accurate and highly discriminatory for identifying ST131.

PCR assays have been developed for characterisation of ST131 by specific SNPs in *pabB* based on the Institut Pasteur's MLST scheme and SNPs in *mdh* and *gyrB* based on the Achtman multilocus sequence typing [157-159]. In general, both PCRs can detect ST131, however the dual SNP ST131 assay based on *mdh* and *gyrB* detects the classical O25b:H4 ST131 and the less common O16:H4 ST131 (associated with susceptibility to fluoroquinolones and extended-spectrum cephalosporins) [159]. The *pabB* based PCR detects O25b ST131 but misses O16 ST131 [159]. PCR amplification and DNA sequencing of *gyrA*, *parC, and fimH* have been performed to further identify sub-clones of the ST131 lineage [117, 138, 143, 144]. The major sub-clones of ST131 identified are the *H30* clade defined by carriage allele 30 of *fimH* (type 1 fimbrial adhesin gene) and the *H30*-Rx which is a subset within *H30* accounting for the majority of the CTX-M-15- producing isolates [143, 144]. The *H30* subclone can be detected by PCR using primers specific for allele 30 of *fimH* and the *H30*-Rx subclone can be detected by a specific SNP in the allantoin-encoding gene, *ybbW* [144, 145]. Whole genome sequencing can be performed to identify ST131, determine in-depth variation, the epidemiology, and evolutionary patterns in ST131 [141, 160].

PFGE was first described in 1984 by Schwarz and Cantor [161] and is a highly discriminatory molecular tool used to determine the epidemiological relatedness of bacteria. The advantage of PFGE in comparison to standard gel electrophoresis is its

ability to separate and permit visualisation of large fragments of DNA ≥ 40kb. Cell suspensions of bacteria are embedded in agar. Subsequently, bacterial cells are lysed and restriction enzymes (*Xba*I) are used to cut bacteria into large DNA restriction fragments which are separated based on size during electrophoresis in alternating electrical fields. The banding patterns can be visualised under UV light and a pulsed field profile (PFP) for each isolate can be determined. Comparison of PFP's can be performed visually and using specific computer software programmes such as BioNumerics (Applied Maths, Kortrijk, Belgium). Various cluster analysis methods can be applied. The cluster algorithm can be used to produce a phylogenetic tree known as a dendrogram. Data suggest that members of the ST131 clonal group cluster as ≥ 68% similar by PFGE [61].

1.8 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a ubiquitous microorganism which has been isolated from humans, animals, and the environment [162, 163]. *S. aureus* is carried on the skin and in the nasal area of about 20-30% of healthy people; however, *S. aureus* has the potential to cause various local or systemic infections including pneumonia, blood stream infections, and skin and soft tissue infections [162, 164-166]. Penicillin G was the antimicrobial prescribed for the treatment for *S. aureus* infections in the 1940s. In 1942, penicillin-resistant *S. aureus* emerged [167].

1.8.1 Meticillin-resistant *Staphylococcus aureus*

Meticillin was introduced into clinical practice in 1960 for the treatment of *S. aureus* infections; however, in 1961 MRSA was observed [168, 169]. MRSA is defined by the Centers for Disease Control and Prevention (CDC) as a *S. aureus* strain that is resistant to oxacillin, cefoxitin, or meticillin by standardised

antimicrobial susceptibility testing or by a laboratory test approved by Food and Drug Administration for MRSA detection [170].

Meticillin is no longer used and has been replaced by isoxazolyl penicillins such as flucloxacillin [171]. Vancomycin can be used to treat MRSA infections; however, vancomycin-intermediate *S. aureus* (VISA) strains and vancomycin-resistant *S. aureus* (VRSA) have been reported [172, 173]. In response, various new antimicrobials such as linezolid, daptomycin, tigecycline, and ceftobiprole may be required to treat MRSA infections [174].

Residence in a long-term care facility has been suggested as an important risk factor for MRSA acquisition [175]. MRSA carriage has been reported to persist for up to 8.5 months after hospital discharge [176]. Studies have shown that the environment may play a role in the dissemination of MRSA [177, 178]. Faires *et al.* (2013) identified MRSA on $\geq$ 1 surface in 16/39 (41%) patient's rooms in a hospital [177]. Creamer *et al.* (2014) identified MRSA environmental contamination on 3% of sites in eight acute hospital wards in Ireland, with highest recovery from 07:30 and 09:00 in the morning [179]. MRSA contaminated sites included mattresses, pillows, bed railings and bedside lockers [179]. Sexton *et al.* (2006) recovered MRSA from 54% of sites in isolation rooms in an Irish hospital; in particular MRSA was most frequently detected on mattresses and beds [180]. MRSA strains have disseminated worldwide causing outbreaks in various settings and a report from Köck *et al.* (2010) estimated extra costs of €380 million annually to EU hospitals as a result of MRSA infections [181].

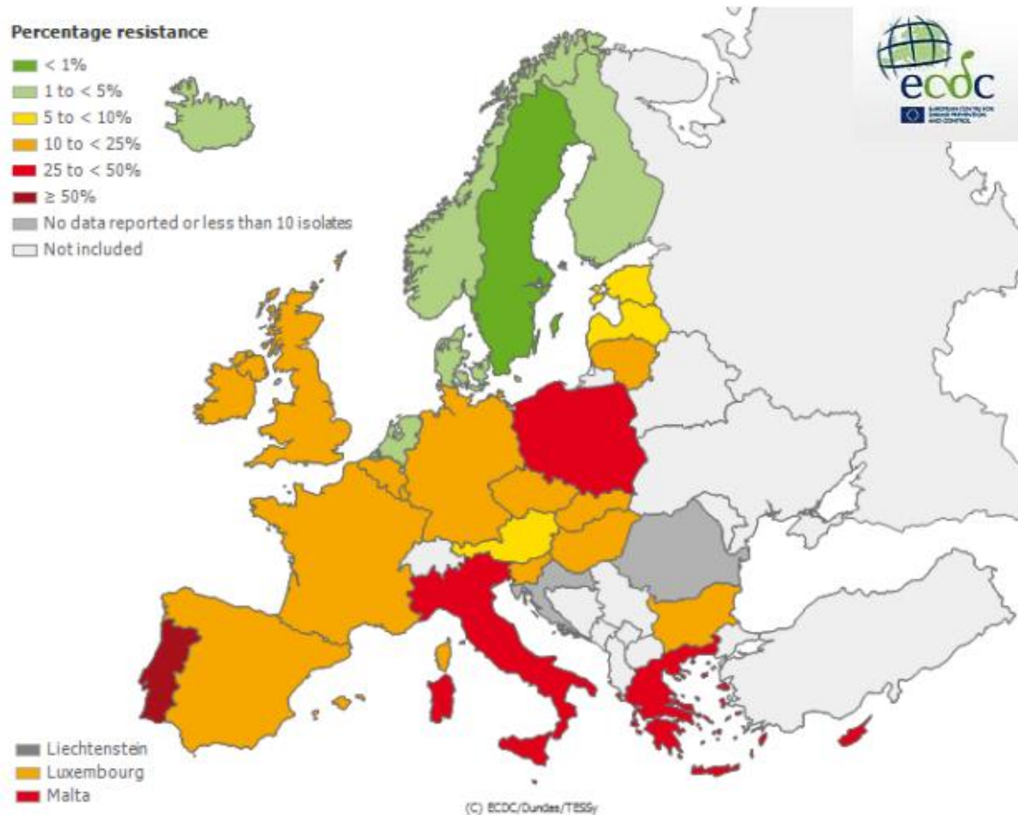
The *mecA* gene is a 2130bp segment of DNA, which encodes a low-affinity penicillin binding protein (PBP2a) [182]. The additional PBP (PBP2a) permits cell wall synthesis and reduced susceptibility/ resistance to β-lactams [182, 183]. PBP2a is also encoded by the recently described *mecC* gene (formerly known as $mecA_{LGA251}$) which is found mainly in animals such as cattle. The *mec* gene is not native to *S.*

aureus, therefore is not found in meticillin-susceptible *S. aureus*. The *Staphylococcus* cassette chromosome *mec* (SCC*mec*) is the only vector for the *mecA* gene and is the source of horizontal transmission of the *mecA* gene. SCC*mec* elements also encode additional antimicrobial resistant genes and recombinase genes [165]. MRSA strains carrying SCC*mec* elements I, II or III are most commonly reported in healthcare-associated MRSA (HA-MRSA) worldwide while SCC*mec* IV, V, VI are more frequently associated with community associated (CA-MRSA) [165, 184]. In contrast to CA-MRSA, HA-MRSA is usually associated with healthcare facilities, invasive procedures, higher levels of antimicrobial resistance and, unlike CA-MRSA, lack the genes encoding PVL [162, 184]. In Ireland the most prevalent strain circulating in Irish hospitals is ST22-MRSA-IV and accounts for 70 to 80% of MRSA recovered from bloodstream infections [184, 185].

In Ireland, the number of MRSA bloodstream isolates have decreased from 592/1424 (41.9%) *S. aureus* infections in 2006 to approximately 180/846 (21.3%) in 2013 (Figure 1.5 illustrates 2012 EARS-Net data) [186]. In 1999 to 2000, epidemic strains EMRSA-15 (ST22-MRSA-IV) and EMRSA-16 (ST36- MRSA-II) were widespread across the UK and commonly reported as co-resistant to erythromycin, ciprofloxacin, and the beta-lactams [171]. However, EMRSA-16 has since decreased from accounting for 35.4% of MRSA bloodstream isolates from 1998-2000 to accounting for 21.4% and 9% of MRSA bloodstream isolates in 2001 and 2007, respectively [171, 187]. The proportion of EMRSA-15 continues to rise, accounting for 85% of MRSA bloodstream isolates in the UK in 2007 according to Alan Johnson (2011), and as mentioned above EMRSA-15 accounts for approximately 80% of all MRSA from bloodstream infections in Ireland [165, 171]. The spread of the community associated clones such as ST8:USA300 are particularly problematic in the community in the USA and are responsible for over 80% of MRSA infections [188]. ST8:USA300 is easily spread via hands and can result in severe infections such as sepsis [162]. Risk of infection is higher among residents colonised with USA300 in

comparison to residents negative for MRSA [189]. USA300 has been detected in European counties; however, to a much lesser extent than in the USA [190].

Figure 1.5: Percentage of invasive isolates with MRSA in EU/EEA countries in 2012 (EARS-Net database 2012).



1.8.2 Laboratory detection and characterisation of meticillin-resistant *Staphylococcus aureus*

- **Phenotypic detection methods**

Chromogenic agar can be used for screening clinical samples for MRSA. Screening for MRSA can be performed on chromogenic agar such as MRSA*Select* (Bio-Rad), CHROMagar (CHROMagar Microbiology), BBL-CHROMagar (BD Diagnostics), ChromID™ MRSA (BioMérieux), or Brilliance™ MRSA (Oxoid). Chromogenic agars vary in chromogenic substrates, added antimicrobials, and concentrations of agents.

Chromogenic agars inhibit most bacteria and yeasts; however, coagulase-negative *Staphylococcus* can still grow on some agars listed but do not produce the typical MRSA coloured colonies. Antimicrobial susceptibility testing by broth microdilution or disk diffusion is performed to assess cefoxitin susceptibility. Cefoxitin is the recommended antimicrobial to be used for the detection of MRSA as resistance to cefoxitin is a sensitive and specific marker for *mecA/mecC* encoded *S. aureus* [75].

- **Molecular detection and characterisation methods**

The *mecA* gene can be detected using a PCR specific assay or by DNA microarray assays (StaphyType kit (Alere Technologies GmbH), which also detect *S. aureus* resistance genes and pathogenicity markers [191].

Epidemiological investigations into the dissemination of MRSA both locally and internationally require efficient typing methods and assays to characterise clonal complexes, identify corresponding virulence, and antimicrobial resistant genes. PFGE is used for inter-strain comparison of *Staphylococcus species* by digesting chromosomal DNA. PFGE is generally performed in conjunction with an additional typing method such as Staphylococcal Protein A (*spa)* typing or MLST. *spa* typing is based on sequencing the polymorphic region of the *spa* gene which is present in all *S. aureus* isolates, therefore is not an MRSA specific method; however, is useful for the characterisation of *S. aureus* clonal complexes [192]. *spa* sequences are analysed using Ridom StaphType software (Ridom, GmbH, Würzburg, Germany) and subsequently can be clustered into *spa* clonal complexes using Based upon Repeating Patterns (BURP) analysis. This method is rapid, reproducible, portable, and accurate for identifying variations in strains of MRSA in an outbreak. MLST for *S. aureus* was developed by Mark Enright and the scheme is based on variations in the DNA sequences of seven housekeeping genes, *arcC* (Carbamate kinase), *aroE* (Shikimate dehydrogenase), *glpF* (Glycerol kinase), *gmk* (Guanylate kinase),

pta (Phosphate acetyltransferase), *tpi* (Triosephosphate isomerase), and *yqiL* (Acetyle coenzyme A acetyltransferase) [193].

1.9 *Enterococcus*

Enterococcus commonly colonise the gut and are associated with a number of infections such as urinary tract infections, wound and pelvic infections, bacteraemia, and endocarditis. The majority of enterococcal infections are due to *E. faecium* and *E. faecalis*, in particular *E. faecium. Enterococci* are intrinsically resistant to aminoglycosides (e.g. kanamycin) and lincosamides (e.g. clindamycin) and have low-affinity PBPs with reduced susceptibility to beta-lactams [194]. Intrinsic resistance to antimicrobials commonly prescribed in the healthcare setting has resulted in the prescribing of other antimicrobials such as vancomycin to treat enterococci infections.

1.9.1 Vancomycin-resistant *Enterococcus*

The first vancomycin-resistant *E. faecalis* and *E. faecium* isolates were identified in England in 1988 shortly after the introduction of vancomycin as a treatment option for *Clostridium difficile,* MRSA, and coagulase-negative staphylococci infections [195]. Normal peptidoglycan precursors have a D-alanyl-D-alanine (D-Ala-D-Ala) dipeptide termini; however, in vancomycin-resistant enterococci, there is a substitution of D-Ala-D-Ala with D-alanyl-D-lactate (D-Ala-D-Lac) resulting in a reduction of binding of vancomycin to the target. There are 6 main VRE phenotypes: Van A, Van B, Van C, Van D, Van E, and Van G; however, Van A and Van B are most prevalent [174]. Both Van A and Van B are transferred mainly on mobile elements such as transposons and plasmids [174]. Van A is the most common phenotype of VRE and demonstrates higher level of resistance to vancomycin and teicoplanin. The spread of the *vanA* gene from enterococci to *S. aureus* in the same patient demonstrates the mobility of these resistance genes [196, 197]. The Van B

phenotype demonstrates a lower level of vancomycin resistance and is teicoplanin susceptible; however, teicoplanin resistance has occurred during treatment of an infection caused by Van B [198]. The *vanC* gene is only present on the chromosome of *E. gallinarum*, *E. casseliflavus* and is not monitored for public health surveillance.

Factors associated with VRE colonisation include the use of glycopeptides, length of hospital stay, and presence of a VRE positive patient in the same care area [174]. *E. faecalis* can survive for up to 5 days and *E. faecium* up to 7 days on environmental surfaces therefore strict cleaning procedures are required to minimise spread [174]. VRE infections are associated with high mortality, increased hospital stay, and prolonged treatment. Antimicrobials prescribed to treat VRE infections include linezolid, quinupristin/dalfopristin, daptomycin, and tigecycline. Resistance to these antimicrobials can occur resulting in fewer treatment options.

Ireland has the highest proportion of vancomycin-resistant *Enterococcus faecium* (VREfm) bacteraemia in Europe with trends increasing from 37.1% in 2006 to 45.4% in 2012 and decreased to 43.1% in 2013 [87]. According to EARS-NET Data, 44% of *E. faceium* BSI isolates in Ireland were resistant to vancomycin in 2012 [88]. In this same year, the prevalence of VREfm ranged from 0% to 23.3% in the 29 other European countries reporting to EARS-NET, with only 6 countries (Portugal, Greece, Germany, United Kingdom, Czech Republic, and Cyprus) exceeding a resistance rate higher than 10% [88]. Data regarding VRE colonisation within health-care settings is limited; however, a study from 2010 identified VRE carriage rate in 40% of 200 hospitalised patients in a particular hospital in Ireland [199].

1.9.2 Laboratory detection of vancomycin-resistant *Enterococcus*

- **Phenotypic Methods**

Chromogenic agars specific for VRE are convenient for initial screening of clinical samples for VRE. *E. faecalis* and *E. faecium* can be differentiated on chromogenic agar (e.g. Chrom ID VRE or Brilliance VRE). *E. faecalis* and *E. faecium* susceptibility to vancomycin can be identified by MIC determination using agar/broth dilution or Etests strips, by disk diffusion using a 5µg vancomycin disc, and by breakpoint agars with Brain Heart Infusion agar [75].

- **Molecular Methods**

Real- time PCR is a rapid and sensitive method for detecting *van A* and *vanB* genes. DNA micro-arrays can also be used to identify enterococci and resistance determinants [200]. Molecular methods can be performed directly from clinical specimens; however, the Van B operon is naturally occurring in obligate anaerobes highlighting the importance of organism identification.

1.10 Long-term care facilities

The term long-term care facilities (LTCF) encompasses a greater diversity of care settings than the term nursing home, for example, facilities for intellectual disability, psychiatric care, palliative care, rehabilitation, and for the physically disabled [201]. However, in numerical terms, most residents in LTCF are in nursing homes and therefore for many purposes the terms are largely interchangeable. For the purpose of this introduction, the term LTCF will be used as a general term to provide consistency.

The Population Division at the United Nations have estimated that by 2050 there will be a greater proportion of the world's population aged > 60 years than persons aged < 15 years. [202]. The population of Ireland in 2011 was 4,588,252 according to the national census [203]. The CSO estimate that by 2046 approximately 1.4 million people will be over the age of 65 years in Ireland and the population over 80 will increase from 128,000 in 2011 to between 470,000 and 484,000 in 2046 [204]. A report published by the Economic and Social Research Institute (ESRI) in 2009 outlined that the demand for LTCF is influenced by population growth, increases in life expectancy, disability trends, and trends in household composition [205]. Therefore with an increase in the population aged over 65 years this is likely to increase the demand for accommodation in LTCFs [206]. ECDC reported the population of people in LTCFs in Europe was 3.7 million in 2010 [207] and when focussing particularly on Ireland, the Information Unit, Department of Health Ireland reported that on 31 December 2012 19,752 people were long stay residents at LTCFs in Ireland [208]. Research performed by the Centre for Ageing Research and Development in Ireland (CARDI) has found that the number of people aged over 65 years using residential long term care in Ireland will increase by 59% from 2006 to 2021 and there will be a 57% increase in this older population using formal home care in Ireland during this same time period [206]. There are over 400 public and private registered LTCFs in Ireland (http://nursinghomes.ie).

1.10.1 The governance of long-term care facilities in Ireland

Long-term residential care in Ireland can be divided into three categories: public, private, and voluntary. The public health system in Ireland is based on the Health Act 2004 which established the Governing body in 2005 known as Health Service Executive (HSE). The HSE is responsible for the organisation and allocation of funding for long-term care in Ireland. The number of public beds provided by the HSE is declining and in response the number of private LTCFs are increasing [206]. In 2009, the HSE administered the Nursing Home Support Scheme (NHSS) to provide financial support for long-term residential care [206, 209]. Under the NHSS,

the individuals receiving the care pay a contribution towards the cost based on their financial situation and the Government pay the remainder [209]. The individual must choose from a list of nursing homes that are enrolled in the scheme which includes private, public, and voluntary institutions. A care needs assessment is performed for each NHSS applicant to ensure long-term residential care is needed. According to the Department of Health, the cost of the "care for older people" rose from €1 billion in 2006 to €1.6 billion in 2011 and these costs are predicted to continue to rise [206]. Currently the focus of care of the dependent elderly is mainly based on residential care in LTCFs; however, more support for independent living at home is recommended [206]. Until 30th June 2009, the HSE was responsible for performing inspection of private LTCFs; however, since the 1st June 2009 the inspections and registrations are performed by the Chief Inspector of Social Services, part of the Health Information and Quality Authority (HIQA), of all private, public and voluntary centres. The inspections are performed to determine if the quality of care in the LTCFs is in compliance with the Health Act 2007 (Care and Welfare of Residents in Designated Centres for Older People), Regulations 2009, and in accordance with the National Quality Standards for Residential Care Settings for Older People in Ireland (developed by the Health Information and Quality Authority) also approved by the Minister in 2009.

1.10.2 Antimicrobial prescribing and healthcare-associated infections in long-term care facilities

A study performed in Cork and Northern Ireland has shown that that people aged 65 years and older receive 2.3 times more medication than the younger population [210]. The Healthcare-Associated infections in Long-Term care facilities (HALT) project is a funded by ECDC and has been carried out EU wide in 2010 and 2013. The HALT study included collation of data on antimicrobial prescribing in LTCFs. The results of the 2013 HALT survey in Ireland showed that of 906/9269 (9.8%) people in LTCFs were receiving antimicrobials on the day of the survey [201]. Antimicrobials were prescribed mainly for the treatment of UTI and respiratory

tract infections and antimicrobial prophylaxis was most common for UTI prevention [201]. Co-amoxiclav was the most commonly prescribed antimicrobial; however, nitrofurantoin, trimethoprim, flucloxacillin, ciprofloxacin, amoxicillin, clarithromycin and cephalexin were also frequently prescribed [201]. Only 51% of residents who were prescribed an antimicrobial for a suspected urinary tract infection had a specimen collected and sent to the regional microbiology laboratory for analysis [201]. A study evaluating antimicrobial prescribing in LTCFs in Cork, Ireland reported 73% of 315 residents from 14 Cork LTCFs had at least 1 inappropriately prescribed medication [210]. This inappropriate prescribing can have detrimental effects on the patient but can also have implications for the healthcare system.

The EU-funded ESAC project demonstrated great variation in antimicrobial use in LTCFs across Europe reporting a mean prevalence of 6.5% and 5.0% for antimicrobial use in European LTCFs in April and November 2009, respectively [211]. In April of the point prevalence study, methenamine (17.5%), trimethoprim (11.4%), co-amoxiclav (11.1%), nitrofurantoin (8.9%), ciprofloxacin (6.8%), and pivmecillinam (6.1%) were the most commonly prescribed antimicrobials in descending order [211]. In November the most common antimicrobials prescribed were co-amoxiclav (12.2%), nitrofurantoin (12.2%), methenamine (11.5%), trimethoprim (7.3%), amoxicillin (7.1%), and ciprofloxacin (5.8%); however, there was large differences in antimicrobial prescribing between LTCFs [211]. Mc Clean *et al.* (2011) looked specifically at the ESAC results from Northern Ireland LTCFs and observed that antimicrobial prescribing was much higher in Northern Ireland than reported in other countries with 13.2% and 10.7% being reported in April and November 2009, respectively [212]. Trimethoprim was the most commonly prescribed antimicrobial in Northern Ireland LTCFs [212].

European point prevalence studies were performed as part of the HALT project and results from a 2013 survey showed that 4.2% of LTCF residents in Ireland had at least one HAI on the day of the study in comparison to 3.4% as a European mean prevalence [83, 201]. Presence of urinary catheters, pressure sores, and wounds are

the most common HAI risk factors in Irish LTCFs according to the 2013 HALT study [201]. The prevalence of multidrug-resistant organisms in LTCF has been reported in Ireland and worldwide [118, 122, 213-215]. A study by Rooney *et al.* (2009) reported 119/294 (40.5%) of residents in Northern Ireland LTCFs as carriers of multidrug-resistant *E. coli* [118]. Antimicrobial resistance, persistence, and dissemination of a pathogen may depend on the strain type. For example, ST131 accounts for a large proportion of antimicrobial resistant *E. coli* worldwide and carriage can persist for 12 months after infection [119, 126, 216]. There is great variation in the data available on MRSA colonisation in LTCFs. MRSA prevalence was 23.3%, 22%, 19.5%, 9.3%, 7.8%, 4.9%, 3%, and 1.1-3% among residents of LTCFs in Northern Ireland (2005-2006), UK (2005), Belgium (2005), Slovenia (2005), Italy 2009), Belgium (1997), and Germany (2001), respectively [217]. MRSA isolated from older people residing in LTCFs is often a HA-MRSA which tend to cause pneumonia, bacteraemia, in comparison to CA-MRSA found among previously healthy younger patients which predominately cause skin and soft tissue infections [162]. A study from Cheng *et al.* (2013) reported that residents in a LTCF are 3.4 times more likely to be MRSA positive in comparison to those not residing in a LTCF and that 19% of MRSA positive residents acquired MRSA during their stay at the LTCF [175]. Great diversity of MRSA strain types from residents of LTCFs has been also reported in the USA and it suggested that this may be due to the importation of different strains from the hospital setting to the LTCF and subsequent dissemination [218]. VRE have been reported to rarely spread or cause infections in LTCFs and VRE colonisation is commonly associated with hospitalisation and patients who have an underlying illness such as cancer or who have a low functional ability [219-221]. No cases of CPE were reported for the 2013 Ireland HALT survey in LTCFs [201]. There is little information reported on CPE prevalence in LTCFs in Europe; however, carbapenemase-producing *Klebsiella pneumoniae* have been reported in LTCFs in Italy, Israel and five LTCFs in Chicago and Rhode Island, USA [222-225].

Infection prevention and control procedures applied in the hospital setting are not always appropriate in the LTCFs. Applying source isolation procedures and barrier nursing could adversely affect the quality of life and wellbeing of the individual

[226]. Residents of LTCFs are frequently transferred between the LTCF and hospitals, therefore a high prevalence of antimicrobial resistant organisms in LTCFs may result in an increase in the prevalence of antimicrobial resistant organisms in hospitals in the same region [119, 227]. Appropriate infection prevention and control measure are essential to prevent the spread of antimicrobial resistant bacteria; however, when LTCFs were surveyed as part of the European HALT study it was reported that 62% of LTCFs overall and 10% of private LTCFs had access to a staff member trained in infection prevention and control (IPC) [201]. In most instances this staff trained staff member was not in the LTCF on a daily basis [201]. Sixty four percent of the participating LTCFs had no surveillance programme for HAIs [201].

1.11 Aim of thesis

The aim of this thesis was to improve understanding of the role LTCFs play in the dissemination of antimicrobial resistance and to determine the factors associated with antimicrobial resistance in LTCFs.

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Chapter 2

Comparison of extended-spectrum beta-lactamase-producing *Escherichia coli* associated with nursing homes with other ESBL isolates.

2.1 Abstract

Objectives: *E. coli* O25b-ST131 has disseminated worldwide in hospitals and the community. The objective of this study was to determine the extent to which *E. coli* O25b-ST131 accounts for extended-spectrum beta-lactamase (ESBL)-producing *E. coli* from clinical samples from all sources in this region.

Methods: Between January and June 2010 ESBL *E. coli* were collected from 94 routine samples including 47 from residents of 25 nursing homes, 15 categorized as hospital acquired and 32 others. PCR was performed for detection of bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, bla_{SHV} and for the identification of members of the *E. coli* O25b:ST131 clonal group. PFGE was carried out using *Xba*I in accordance with PulseNet protocols.

Results: The majority (97%) of isolates harbored a bla_{CTX-M} gene. *E. coli* O25b-ST131 accounted for 87% of all ESBL *E. coli* and for 96% of isolates from nursing home residents.

Conclusion: The *E. coli* O25b-ST131 clonal group predominated in the collection of ESBL *E. coli*, particularly in nursing home isolates.

2.2 Introduction

The *E. coli* O25b:ST131 clonal group was first reported in 2008 as a major carrier of CTX-M-15 however subsequent studies have identified the presence of this clonal group in earlier isolates dating back to 1967. [1] Since then it has been reported globally in both the hospital and community setting and is associated primarily with urinary tract infections and bacteraemia. Reported risk factors for *E. coli* O25b-ST131 include foreign travel, antimicrobial usage, catheterisation and admission to a nursing home. [2-4] *E. coli* O25b-ST131 are often resistant to fluoroquinolones and cephalosporins such as ceftazidime. [1] The dissemination of $bla_{CTX-M-15}$ is closely associated with *E. coli* O25b-ST131 however $bla_{CTX-M-9}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-32}$ have also been observed. In addition, a broad range of other antimicrobial resistance genes (bla_{OXA-1}, bla_{TEM}, *tetA*, *aac(6')-Ib-cr* and *aac(3)-II*) primarily carried on transferable IncF plasmids are also associated with the group. [5]

An increasing population of older people reside in nursing homes for extended periods. Nursing homes are associated with a significant number of antimicrobial-resistant (AMR) bacteria related to frequent transfer of patients from hospitals and also to factors within the nursing homes including the vulnerability of residents and antimicrobial consumption. [6] The relative importance of acquisition of AMR bacteria during hospital stay compared with dissemination within nursing homes is not well understood. We have examined the diversity of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolates from all clinical samples submitted to one laboratory over 6 consecutive months to compare isolates from nursing home residents with other isolates.

2.3 Methods

Galway University Hospitals [University Hospital Galway (UHG) and Merlin Park University Hospital (MPUH)] and Roscommon County Hospital (RCH) account for an approximate combined total of 1 052 inpatient beds and about 44 000 admissions per year. The Clinical Microbiology Laboratory service at GUH serves these hospitals and the nursing homes and General Practitioners (GP) throughout the region serving a population of approximately 445 356 (http://www.cso.ie).

Between January 1st and June 30th 2010, approximately 3 599 clinical isolates of *E. coli* were reported by the laboratory with urine, blood, wound and respiratory samples accounting for approximately 3337, 82, 74 and 66 isolates, respectively. All *E. coli* isolates are tested for susceptibility to cefpodoxime (Oxoid Ltd, UK) and cefpodoxime non-susceptible isolates were initially assessed for ESBL production using cefpodoxime/clavulanic acid discs in accordance with Clinical and Laboratory Science Institute (CLSI) methods and criteria. Ninety four ESBL *E. coli* were collected from urine (*n*=80), blood (*n*=3), sputum (*n*=9), drainage fluid (*n*=1), and tissue (*n*=1).

Forty seven ESBL *E. coli* isolates were from residents of 25 nursing homes (21 private and 4 public; NH=47), and 47 were non-nursing home isolates. Of the 25 nursing homes, 18 were represented by a single isolate, 2 by 2 isolates and the remaining 5 nursing homes by 3 or more isolates. The number of beds in each

nursing home varied from 28-62. The nursing home samples were submitted in the course of routine investigation of clinical episodes of suspected infection and the interval between admission to the nursing home and date of sample collection was not available. Fifteen ESBL-producing *E. coli* were categorized as likely hospital associated (HA) on the basis that the clinical samples were collected more than 48 hours after admission (range 3 – 79 days). Of the remaining 32 isolates of ESBL-*E. coli,* 4 were from General Practitioners and 28 were from hospitalized patients but with samples collected (a) at or within 48 hours of admission (n= 16) or (b) submitted from a hospital but with interval from admission to sampling not accessible (n= 12). For purposes of this study these 32 isolates are classified as “others”. Thirty of the 32 “others” had previous hospital contact (out-patient and in-patient). There was no record of nursing home admission for those patients hospitalized at time of sampling.

The *E. coli* ST131 clonal group was identified by PCR specific to the detection of the *pabB* gene (specific marker the *E. coli* O25b-ST131 clonal group) and the *trpA* gene (to act as a control and to ensure quality of DNA). [7] All ESBL isolates were also tested for bla_{TEM}, bla_{SHV}, bla_{OXA-1} and bla_{CTX-M} as previously described and representative *bla* genes were sequenced using primers specific to the entire coding region of $bla_{CTX-M-group\ 1}$, bla_{TEM} and bla_{SHV} (Sequiserve, Vaterstetten, Germany). [8,9] Pulsed field gel electrophoresis (PFGE) using *Xba*I was performed by the Pulse-Net protocol with analysis of profiles (PFPs) using the Dice coefficient with clustering by the unweighted pair group method with arithmetic averaging (UPGMA). [10] Three major UK ST131 variants (Strains A, C and D) were included in the PFGE analysis for comparison. [5] Chi Square tests were applied to compare demographic and clinical data from the hospital and nursing homes collections.

2.4 Results

Table 1 shows results for ST131 status, bla_{CTX-M}, bla_{OXA-1}, bla_{TEM} and bla_{SHV} genes detected in the 3 categories of isolates. All isolates were confirmed as carrying one or more ESBL encoding genes. The ST131 clonal group accounted for 82 of the 94 isolates with bla_{CTX-M} in 81 of 82 ($bla_{CTX-M-group1}$ *n*=73; $bla_{CTX-M-group9}$ *n*=8) and bla_{TEM-10}

in the remaining isolate. Sequencing of a *bla*$_{CTX\text{-}M\text{-}group1}$ gene from an ST131 isolate showed *bla*$_{CTX\text{-}M\text{–}15}$ while the *bla*$_{CTX\text{-}M\text{-}group1}$ gene in a non ST131 isolate was *bla*$_{CTX\text{-}M\text{–}61}$. One NH *E. coli* harbored *bla*$_{SHV\text{-}2}$, *bla*$_{CTX\text{-}M\text{–}group\ 1}$, *bla*$_{OXA\text{-}1}$ and *bla*$_{TEM.}$ A higher percentage of nursing home isolates were ST131 (96%) compared with hospitals associated (87%) and "other" ESBL *E. coli* (75%) although the differences did not reach statistical significance (Table 1). Among the 12 non-ST131, *bla*$_{CTX\text{-}M}$ was also predominant (n=10) with the remaining isolates being *bla*$_{SHV\text{-}2}$ (*n*=1) in one case and both *bla*$_{SHV\text{-}12}$ and *bla*$_{TEM}$ in the other.

PFGE analysis identified 65 pulsed field profiles (PFPs) within the 94 isolates and the reference Strain A. PFP's of the *E. coli* ST131 were $\geq$ 78% similar. The ST131 isolates formed 3 clusters X to Z, based on a similarity of $\geq$ 85%. Cluster X included the pandemic UK Strain A and 45 local isolates (NH *n*=19; OT=17; HA *n*=9; UK Strain A control *n*=1). Cluster Y comprised mainly nursing home isolates (NH *n*=9, HP *n*=3; OT=1). Most cluster Z isolates (NH *n*=10; OT=2; HP *n*=1) were from nursing homes and in particular one nursing home provided 7 (54%) of all cluster Z isolates (Table 2). The two non-ST131 nursing home isolates were from residents of the same nursing home and were indistinguishable by PFGE, and one of these was confirmed by sequencing to harbor *bla*$_{CTX\text{-}M\text{-}61}$. The 10 non-ST131 isolates from 2 hospital patients and 8 from the OT category were all quite distinct on PFGE.

2.5 Discussion

The association of AMR with nursing homes is well described however the relative importance of dissemination within nursing homes compared with acquisition during episodes of care for nursing home residents at acute hospitals is not well understood. Rooney et al (2009) reported that CTX-M –producing *E. coli* O25b-ST131 accounted for 49% of ESBL *E. coli* from nursing homes in Northern Ireland. [3] Burke et al (2012) reported *E. coli* O25b-ST131 represented 85% and 51% respectively of nursing home and hospital inpatient ESBL *E. coli* isolated from Dublin, Ireland. [6] *E. coli* O25b-ST131 was reported to account for 21%, 43% and 41% of ESBL *E. coli* isolates from Japan, South Africa and Canada, respectively. [4] In addition, *E. coli* O25b-ST131 was identified in 27% of isolates in the United States;

however, *E. coli* O25b-ST131 was detected in 76% of isolates from long-term care facilities in this region of the United States. [2] Our data confirm other reports of global dissemination of $bla_{CTX-M-15}$ carrying *E. coli* O25b-ST131. Our study also demonstrates the striking predominance of this clonal group in this region of Ireland. Even by comparison with Burke and colleagues the predominance we observe (87% of all ESBL *E. coli*) in this collection of consecutive clinical isolates from all sources is exceptionally high. In 2012, ESBL *E. coli* accounted for 8.8% of all *E. coli* blood stream infections in Ireland as a whole. In comparison, ESBL *E. coli* accounted for approximately 21.5% of *E. coli* (20-23%) blood stream infections for most hospitals in the West of Ireland where this study was performed (S. Murchan, Health Protection Surveillance Centre, personal communication, January 8, 2013). It is interesting to speculate if this may be related to the prevalence of this clonal group in this region.

In conclusion this study demonstrates that a single clonal group, *E. coli* O25b-ST131 accounts for the vast majority of ESBL *E. coli* in the West of Ireland, with a particular predominance (96%) in nursing home isolates. The ST131 variant designated as UK strain A represents almost half of all ESBL *E. coli* from all sources. A unique aspect of this study is the comparison of the homogeneity of ESBL *E. coli* in consecutive isolates from nursing home and other settings in the same region during a defined time period. Although this study is not sufficient to form firm conclusions we suggest that there is a trend towards greater homogeneity of ESBL *E. coli* within the nursing home isolates. Given the growing nursing home population in developed countries and the practical challenges of implementing effective measures to control dissemination of AMR bacteria in nursing homes more comprehensive studies comparing nursing home isolates with non-nursing home isolates are required.

2.6 Acknowledgments

The authors thank Dr Neil Woodford (Health Protection Agency, Colindale, UK) for provision of reference strains [5].

2.7 References

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Table 1: Characteristics of nursing home, hospital acquired and other ESBL *E. coli* isolates detected from all sample types submitted over a 6 month period.

Isolate Characteristics	**Source of Isolates (*n*=94)**			**Total Isolates (n=94)**
	Nursing Home Isolates (*n*=47)	**Hospital Isolates (*n*=15)**	**Other (*n*=32)**	
ST131	45 (96%)	13 (87%)	24 (75%)	82 (87%)
bla$_{CTX-M}$	47 (100%)	15 (100%)	29 (91%)	91 (97%)
bla$_{CTX-M}$ Group 1	42 (89%)	12 (80%)	26 (81%)	80 (85%)
bla$_{CTX-M}$ Group 9	5 (11%)	3 (20%)	3 (9%)	11 (12%)
bla$_{OXA-1}$	39 (83%)	12 (80%)	20 (63%)	71 (76%)
bla$_{TEM}$	20 (43%)	4 (27%)	8 (25%)	32 (34%)
bla$_{SHV}$	1 (2%)	0 (0%)	2 (6%)	3 (3%)

ST, sequence type; *bla*, beta-lactamase

Table 2: Molecular typing of ESBL *E. coli* and source of isolates.

MLST Type [a]	PFGE Grouping [b]	Nursing home isolates (n=47)	Hospital isolates (*n*=15)	Other (*n*=32)	Total isolates (n=94)
ST131	X [c]	19 (41%)	9 (60%)	17 (53%)	45 (47%)
ST131	Y [c]	9 (19%)	3 (20%)	1 (3%)	13 (14%)
ST131	Z [c]	10 (21%)	1 (7%)	2 (6%)	13 (14%)
ST131	Diverse [d]	7 (15%)	0 (0%)	4 (13%)	11 (12%)
Non ST131	Diverse [d]	2 (4%)	2 (13%)	8 (25%)	12 (13%)

[a] MLST= Multilocus sequence typing, inferred from PCR specific assay

[b] PFGE= Pulsed Field Gel Electrophoresis

[c] X, Y, Z= Represent randomly assigned Pulsed Field Gel Electrophoresis clusters based on based on a similarity of $\geq$ 85%

[d] Diverse= Represents isolates not in clusters X, Y and Z based on < 85%

Chapter 3

Environmental contamination of a nursing home with antimicrobial-resistant organisms occurs rapidly from start of commissioning, preceding occupation by residents

3.1 Abstract

Nursing homes are reservoirs for antimicrobial-resistant organisms (AROs). This study examined the time to environmental contamination with AROs in a new-build nursing home. Environmental sites in an occupied nursing home (*N* = 18) and a newly built replacement nursing home (*N* = 21) were monitored during an 11 week period before and after residents transferred between buildings. Meticillin-resistant *Staphylococcus aureus* was detected during commissioning in the new building and was a frequent finding throughout the building after residents had moved in. Extended-spectrum beta-lactamase-producing *E. coli* O25b:ST131 was detected once.

3.2 Introduction

The environment plays an important role in the dissemination of antimicrobial-resistant organisms (AROs) such as meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE). [1, 2] Infection with AROs is associated with increased health care costs, length of hospitalization, morbidity and mortality. [3] We studied environmental contamination with MRSA, VRE and ESBL in an old and a new nursing home. The replacement building was commissioned by staff while also working in the old building, occupied by residents later transferred. Although some personal items were transferred to the new building following cleaning, most furniture and equipment was new.

3.3 Methods

A number of environmental sites in an occupied nursing home (*N* = 18) and an adjacent new replacement building (*N* = 19) were sampled on three occasions prior to commissioning of the new building (Table I). Sites sampled in the old building included seven sites within a 10-bed dormitory, four sites in a sitting room area and seven sites in an area used exclusively for day care (Table II). Selected sites in the new nursing home included five sites within each of two single rooms (assigned to

residents transferring from the dormitory), four sites in sitting room area and five sites in an area used exclusively for day care (Table II). Sampling of furniture took place before and after commissioning with the exception of the tables and chairs in the day-care area of the new building which were not in place until after the commissioning stage (Table I). When commissioning was complete substantially all of the rooms in the new building were occupied by transfer of residents from the old building.

Sites (10cm × 10 cm or entire surface of door handles) were swabbed using Copan ESwabs (BS ISO 18593:2004). Swabs were inoculated into peptone water overnight. Ten microlitres of peptone water were plated on to chromID^{TM} MRSA, chromID^{TM}ESBL agar and chromID^{TM}VRE (bioMerieux) for detection of MRSA, ESBL and VRE respectively.

Identification of suspect isolates of MRSA, VRE and ESBL *E. coli* were by standard methods and susceptibility testing and ESBL confirmation was performed and interpreted by Clinical and Laboratory Standards Institute (CLSI) methods and criteria. [4] Control strains included *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923 *E. coli* UK strain A, *K. pneumoniae* 700603, *E. coli* ATCC 25922, *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212. Isolates confirmed as ESBL were tested by polymerase chain reaction for $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and $bla_{\text{OXA-1}}$ [5, 6] and for the *pab*B region (specific to the O25b:ST131 clone) and *trp*A genes as previously described. [7] Amplicons of the $bla_{\text{CTX-M}}$ gene were sequenced.

3.4 Results

Environmental contamination with MRSA was detected in the old building on 17 out of 54 (31%) samples from chairs, bedside lockers, bed frames, tables, floors and doors (Table 1). VRE and ESBL were not detected. MRSA was first detected in the new building in a common-room area on the third sampling occasion after commissioning had begun. After the transfer of residents MRSA was detected on 63 out of 147 samples (43%) from all areas. CTX-M-15-producing *E. coli* O25b:ST131 was detected from a toilet seat in the ensuite of a single room on one occasion. This

isolate was also positive for *bla*$_{TEM}$ and *bla*$_{OXA-1.}$ VRE was not detected.

3.5 Discussion

Our study indicates a much higher frequency in detection of environmental contamination with MRSA. Floors, bed frames and bed side lockers were sites most frequently contaminated (> 50% of samples test positive). The finding of MRSA contamination before occupation by residents is new. As MRSA was not detected until commissioning by the staff of the new building commenced, it is likely that healthcare workers and/or transfer of various items contributed to the introduction of MRSA. This highlights the difficulties of avoiding the introduction of contamination when transferring care services from old to new buildings on adjacent sites.

Comparisons of our findings with previous studies should take account of differences in methodology. We performed environmental sampling with nylon flocked Copan ESwabs placed in Amies media (Copan, Brescia, Italy) and enriched in peptone water overnight. Previous studies indicated that the Copan ESwab method allows greater recovery of MRSA from environmental surfaces than saline-moistened cotton swabs or rayon swabs. (8)

There are several limitations to the study. Firstly, sampling times were not related to cleaning schedules, although the cleaning products and schedules were comparable in the old and new nursing homes. Each room was cleaned daily using kitchen sanitizer. A deep clean was performed monthly. Floors were cleaned using neutral floor cleaner and were wiped using a non-dip system. Infection prevention practice in both the old and new nursing homes is based on the Department of Health guidelines 2011. (9)

Limited recovery of ESBL-PE and the failure to detect VRE may be related to limitations of the detection method used in this study, rather than to the absence of these organisms. As prevalence of carriage of AROs in residents and staff was not established it is not clear to what extent differences in the frequency of detection of the three AROs is determined by difference in levels of colonization,

shedding and persistence in the environment or by differences in sensitivity of detection. However, hospital admission screening and other data indicate that both MRSA and ESBL colonization are both frequent occurrences in nursing home residents in this geographical area. [10] The sites selected for monitoring may not have given a comprehensive reflection of the level of environmental contamination of gastrointestinal tract colonizing organisms such as ESBL-PE and VRE, as we included limited sites in the toilet facility.

Environmental contamination of the new nursing home with MRSA commenced before residents moved in and it rapidly became very widely disseminated. Environmental contamination is likely to play a significant part in persistence and transmission of MRSA within nursing homes.

3.6 References

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Table I: Dates of swabs and significant events

Activity	Dates
Swabbing of old nursing	22/08/2011-29/08/2011
Swabbing of new nursing home before commissioning	22/08/2011-24/08/2011
Swabbing of new nursing after staff had entered the new building for training	29/08/2011
Tables and chairs put in day-care area[1]	29/08/2011-05/09/2011
Nursing home residents move to new building	05/09/2011
Swabbing of new nursing after residents had moved in	12/09/2011-21/11/2011

[1] All furniture except the tables and chairs were placed in the new nursing home prior to 22/08/2011

Table II: Detection of MRSA in old and new nursing homes

Environmental sites	Old occupied Nursing Home No of tests	Number With MRSA	New unoccupied Nursing Home No of tests	Number With MRSA	New occupied Nursing Home No of tests	Number With MRSA
Door Handles (*N*=92)	18	1	18	0	56	13
Floor Surfaces (*N* = 26)	6	4	6	1	14	11
Tables (*N*=23)	6	2	3	1	14	5
Bed Side Lockers (*N*=26)	6	4	6	0	14	10
Bed Frame (*N*=26)	6	2	6	0	14	11
Toilet Seat (*N*=36)	6	1	9	0	21	7
Arm Chair (*N*=23)	6	3	3	0	14	6

Chapter 4

Characterization of meticillin-resistant *Staphylococcus aureus* from residents and environment in a long-term care facility

4.1 Abstract

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern associated with residence in a long-term care facility (LTCF). The aim of this study was to characterize MRSA recovered from residents and their physical environment. During a one year prospective study MRSA was recovered from 17/64 residents (R) of a LTCF and from multiple environmental (E) sites. Isolates underwent antibiogram-resistogram typing, were screened for the presence of *mecA, mecC* and the genes encoding for Panton-Valentine Leukocidin (PVL), and were typed by Staphylococcal Protein A (*spa)* typing. All isolates carried the *mecA* gene and lacked the *mec*C and PVL genes. The *spa* types were as follows: 15 t032 (8 R, 7 E), 4 t727 (E), 3 t022 (R), 2 t002 (R), 2 t8783 (E), and 1 each of t1372 (E), t020 (E), t611 (R), t4623 (R), t379 (R), t045 (R). Twenty four isolates were of s*pa* types associated with the multi locus sequence type ST22. The t032 *spa* type (47%) and *spa*-CC 22 (75%) predominated. This reflects the most common types recovered in Irish hospitals where ST22 predominates. The uncommon *spa* type t727 was present in the environment but not detected in residents and is infrequently observed in Ireland.

4.2 Text

Staphylococcus aureus (*S. aureus*) is a ubiquitous microorganism which has been isolated from humans, animals, and the environment [1, 2]. *S. aureus* can colonize the skin and nasal passages of 20-30% of people without apparent adverse impact on health [3]. However in appropriate circumstances *S. aureus* can cause a wide variety of infections and can produce several toxins increasing the severity of some infections. Examples of *S. aureus* infections include pneumonia, blood stream infection, skin and soft tissue infection, and food-poisoning [3, 4].

Although the proportion of *S. aureus* blood stream infections attributed to MRSA has declined in recent years it remains a challenge with particular clonal lineages of health-care associated (HA) MRSA such as EMRSA-15 (ST22) and EMRSA-16 (ST36) and community associated strains of MRSA (CA-MRSA) such as USA300 (ST8) widely

disseminated [2, 3, 5]. In Ireland, multi locus sequence type (ST) 22 predominates accounting for 70-80% of invasive MRSA infections [5]. Residence in long-term care facility (LTCF) is a risk factor for MRSA acquisition and environmental contamination with MRSA in such facilities may play a role in dissemination of MRSA [1, 6, 7]. MRSA can be transmitted from person-person via MRSA colonized hands and by surface contamination from MRSA colonized objects [6]. MRSA has been reported to survive for months on contaminated inanimate surfaces [6]. The aim of this study was to determine the epidemiology of MRSA among residents and their corresponding environment.

In a one year study (July 2012-August 2013) of 64 residents of a LTCF, nasal colonization with MRSA was detected for 17 of the 64 residents [8]. In September 2011 the residents moved to a newly built LTCF. MRSA was recovered from environmental sites in both the original LTCF (Sample date: August 2011) prior to decommissioning (n = 17) and in the new LTCF (Sample date: August to November 2011 and August 2013) before (n = 2) and after (n = 75) occupation by residents. Environmental sites sampled included door handles, tables, floor surfaces, arm-chairs, bed-frames, bed-side lockers, on-call buttons, handles beside showers, toilet flushers, toilet seats, tap handles and railings beside common toilets. MRSA was recovered from all sites except the tap handles, railings beside common toilets, and on-call buttons. MRSA was recovered from 82/201 (40%) and from 12/69 (17%) environmental samples collected in 2011 and 2013 respectively. We suggest that the frequency of MRSA detection in 2013 may be lower due to better infrastructure in the new LTCF.

The first MRSA recovered from each resident (n = 17) together with 15 environmental isolates were selected. It was not possible within the scope of this project to perform typing on all environmental isolates therefore a subset was chosen to reflect the diversity of MRSA isolated over time. The subset included ten isolates from 2011 (n = 5) and 2013 (n = 5) recovered from the newly built LTCF and five isolates from the old facility (n = 5).

Isolates were stored on Protect beads (Technical Service Consultants Limited, Heywood, United Kingdom) at –70°C prior to subsequent detailed analysis prior to

analysis. All isolates underwent antibiogram- resistogram (AR) typing by testing susceptibility to 23 agents including amikacin (30µg), ampicillin (10µg), cadmium acetate (130µg), chloramphenicol (30µg), ciprofloxacin (5µg), erythromycin (15µg), ethidium bromide (60µg), fusidic acid (10µg), gentamicin (10µg), kanamycin (30µg), lincomycin (2µg), mercuric chloride (10µg), mupirocin (5µg), neomycin (30µg), phenyl mercuric acetate (10µg), rifampicin (5µg), spectinomycin (500µg), streptomycin (25µg), sulphonamide (300µg), tetracycline (30µg), tobramycin (10µg), trimethoprim (5µg) and vancomycin (30µg) [4].

Isolates were screened for the *mecA, mecC* and the genes encoding for Panton-Valentine Leukocidin (PVL) using real time PCR and were typed by Staphylococcal Protein A (*spa)* typing as previously described [9]. *spa* sequences were analysed using Ridom StaphType software (Ridom, GmbH, Würzburg, Germany). The *spa* types were clustered into *spa* clonal complexes using Based upon Repeating Patterns (BURP) analysis where isolates with <5 repeat successions were excluded and if the cost (number of genetic events) was <5. Where possible an MLST type was inferred from data available in an online database (Ridom *spa* server http://www.*spa*server.ridom.de/).

All isolates carried the *mecA* gene and lacked the *mec*C and PVL genes *lukF*-PV and *lukS*-PV. The resistance profile of isolates is shown in Table 1. In total, 11 *spa* types were recognized: t032 (15/32), t727 (4/32), t022 (3/32), t002 (2/32), and t8783 (2/32). There was a single isolate each of *spa* types t020, t379, t611, t4623, t045, and t1372. BURP analysis clustered 24 isolates from 14 residents and 10 environmental specimens in a single *spa* clonal complex (*spa*-CC22) (Table 1). *spa*-CC22 predominated in environmental isolates collected in 2011 and 2013. Type t002 and t045 were defined as singletons *spa* types while t727 and t1372 were excluded from clustering as they consisted of four and two repeat succession units respectively.

To our knowledge this is the first study to report the typing of isolates recovered from all colonized residents in a single facility along with isolates recovered from the associated environment over an extended period. A single *spa* type, t032 accounted for 45% of isolates. *spa* types associated with ST22, including t032,

accounted for 75% (24/32) of all isolates (82% (14/17) from residents and 67% (10/15) of environment isolates). In Ireland ST22 has been the predominant strain in hospitals since 2002 and accounts for 70-80% of MRSA isolates from blood stream infections [5]. Therefore the predominance was not unexpected. t727 is associated with ST45 (Ridom *spa* server http://www.*spa*server.ridom.de/) but no ST could be inferred for t1372 (Table 1).

ST5 [t002 (n = 2) and t045 (n = 1)] was recovered from three residents however was not found among the environmental specimens. These isolates were resistant to multiple antimicrobials (Table 1). *spa* type t002 (ST5) has previously been isolated from residents of LTCFs in California, where it accounted for 23% of isolates, although t008 type (ST8) predominated in hospitals [7]. A study in Israel identified ST5 as the predominant strain among residents of LTCFs [10]. In the current study, unfortunately, the environment of the rooms of residents carrying MRSA ST5 were not sampled and so this may explain why ST5 was not recovered from the environment.

spa type t727 (ST45) was recovered from the environment (4/15) in August 2011 from the old building and in August 2013 from the new building but it was not detected among residents. Based on the *spa* types submitted to the Ridom *spa* Server (http://spaserver.ridom.de/, 19 June 2014, date last accessed) *spa* type t727 has only been previously reported from Norway (n = 13) and Ireland.

The antimicrobial resistance profiles of the ST22 isolates reported here correlate with that of previously reported ST22 isolates. The isolates were non-multidrug resistant with ciprofloxacin resistance observed in 31 of 32 isolates, also typical of HA-MRSA in contrast to CA-MRSA [2, 4]. During the one year study, MRSA isolates from three of four new residents colonized when first tested were of three unique *spa* types (t002, t045 and t4623). Two of the *spa* types (t045, t4623) were not observed in any long-term residents and the other (t002) was not observed in any long-term resident until after the new admission. This suggests that these strains represent new variants which were introduced from outside the LTCF into the setting of a well established resident strain.

The finding of MRSA in a newly built facility is interesting. As MRSA was not detected until commissioning by the staff of the old building commenced it is possible that health care workers and or transfer of items may have contributed to the introduction of MRSA. As health care workers were not tested for MRSA colonization it is not possible to assess the extent to which this may have contributed to environmental contamination.

In summary the single clonal complex/sequence type (*spa*-CC 22) that predominates in both residents and the environment corresponds to that which causes most blood stream infection in Ireland. Two findings merit follow up. Three of four colonized new residents carry minority MRSA variants. It would be of interest to determine if such residents acquire the resident LTCF strain over time. Also it is of interest that *spa* type t727 was found in the environment over a two year period but was not identified in any resident. Further studies investigating the role of the LTCF admissions from hospitals and the impact of environment contamination are required to gain a broader understanding into the epidemiology of MRSA.

Declaration of interest: None

4.3 References

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Table 1: *spa* types, repeat succession, *spa* inferred MLST, clonal complexes, PCR results and antimicrobial susceptibility profiles for 32 environment and clinical MRSA.

spa type	N (% total MRSA)	Clinical (% total)	Environment (% total)	*spa* Repeat Succession	*spa* Clonal Complex	Inferred MLST	PVL[a]	*mecA*	Antibiogram [b]
t032	15 (47%)	8 (47%)	7 (46%)	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP,CIP, CDM (1), FUS (10), ERY (10), TET (1)
t022,	3 (10%)	3 (17%)	0 (0%)	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP, CIP, CDM, ERY
t020	1 (3%)	0 (0%)	1 (7%)	26-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP, CIP, ERY, FUS
t379	1 (3%)	1 (6%)	0 (0%)	26-23-23-13-23-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP, CIP, ERY, FUS
t611	1 (3%)	1 (6%)	0 (0%)	26-23-23-13-23-31-17-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP, CIP, FUS
t4623	1 (3%)	1 (6%)	0 (0%)	26-23-13-23-31-29-132-17-31-29-17-25-17-25-16-28	22	ST22	-	+	AMP, CDM
t8783	2 (6%)	0 (0%)	2 (13%)	26-23-13-23-31-29-17-31-29-17-31-17-25-16-28	22	ST22	-	+	AMP, CIP, ERY (1)
t002	2 (6%)	2 (12%)	0 (0%)	26-23-17-34-17-20-17-12-17-16	Singleton	ST5, ST231	-	+	AMP, CIP, CDM, ERY, EB, MC, PMA
t045	1 (3%)	1 (6%)	0 (0%)	26-17-20-17-12-17-16	Singleton	ST5, ST225	-	+	AMI, AMP, CIP, ERY, KAN, LIN, NEO, SPC, TOB,

Table 1 continued: *spa* types, repeat succession, *spa* inferred MLST, clonal complexes, PCR results and antimicrobial susceptibility profiles for 32 environment and clinical MRSA.

spa type	N (% total MRSA)	Clinical (% total)	Environment (% total)	*spa* Repeat Succession	*spa* Clonal Complex	Inferred MLST	PVL[a]	*mecA*	Antibiogram [b]
t727	4 (13%)	0 (0%)	4 (27%)	08-16-02-43	Singleton	ST45	-	+	Amp, Cip, CDM, Ery, Fus (2), MC (2), PmA (2)
t1372	1 (3%)	0 (0%)	1 (7%)	26-28	Singleton	None	-	+	Amp, Cip, Fus

[a]PVL, Panton-Valentine Leukocidin

[b]Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including amikacin (AMI), ampicillin (AMP), cadmium acetate (CDM), chloramphenicol, ciprofloxacin (CIP), erythromycin (ERY), ethidium bromide (EB), fusidic acid (FUS), gentamicin, kanamycin (KAN), lincomycin (LIN), mercuric chloride (MC), mupirocin, neomycin (NEO), phenyl mercuric acetate (PMA), rifampin, spectinomycin (SPC), streptomycin, sulfonamide, tetracycline (TET), tobramycin (TOB), trimethoprim and vancomycin.

Chapter 5

Colonization with ESBL-producing and carbapenemase-producing *Enterobacteriaceae,* vancomycin-resistant enterococci, and meticillin-resistant *Staphylococcus aureus* in a long-term care facility over one year.

5.1 Abstract

Objectives: Residence in a long-term care facility (LTCF) is a risk factor for colonization with antimicrobial resistant organisms (AMRO). This study examines colonization with and characteristics of AMRO in residents of a LTCF over a year.

Methods: Residents of a LTCF were recruited to the study. At baseline 51 of 88 residents agreed to participate and 13 subsequent admissions joined (n = 64). Data on clinical conditions, dependency levels, hospitalization in the previous year, and antimicrobial prescribing was collected from participants. Nasal and rectal swabs and catheter urine specimens were collected quarterly and examined for extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and meticillin-resistant *S. aureus* (MRSA) on chromogenic agars with confirmation by conventional means.

Results: In total, 243 rectal swabs, 243 nasal swabs, and 30 urine samples were collected from 64 residents. ESBL-producing *Enterobacteriaceae* and MRSA were detected in 36/64 (56%) and 17/64 (27%) residents, respectively. Sixteen residents who participated in the study for the entire year remained ESBL-EC free throughout, and 5/16 (31%) were colonized with non-ESBL-producing ciprofloxacin-resistant *E. coli.* ESBL-KP was detected in 5/64 (8%), VRE in 2/64 (3%), and CPE were not detected. ESBL and MRSA colonization was associated with location in the LTCF, and previous exposure to antimicrobials. Colonization was generally persistent.

Conclusion: Colonization with AMRO in residents of a LTCF is common and persistent. Location in the LTCF and antimicrobial use are associated with colonization. LTCFs are a critical element in control of antimicrobial resistance.

5.2 Introduction:

Antimicrobial resistance has been identified as a key public health challenge (1). Amongst the major acquired antimicrobial resistant organisms (AMRO) are extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*,

carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and meticillin-resistant *Staphylococcus aureus* (MRSA).

Both ESBL-producing *Enterobacteriaceae* and CPE are detected with increasing frequency in Ireland (2-4). The first *Klebsiella pneumoniae* carbapenemase (KPC)-producers were reported in Ireland in 2009 and the first *E. coli* with a KPC in 2011 (5, 6). VRE blood stream infection has also been increasing in Ireland (7). MRSA in Ireland is still predominantly hospital associated with strains that have been prevalent for decades and the incidence of blood stream infection has been in decline (571 cases in 2006 to 235 in 2012) (7, 8). The proportion of the population in the European Union aged 65 year and over is 16% and will increase to 29.3% (152.6 million) by 2060 (9). This is likely to result in further increase the population in long-term care facilities (LTCFs), currently at 19,800 (2012) in Ireland and approximately 3.7 million in the EU (2010) (9, 10). There is increasing evidence that LTCFs are important reservoirs for AMRO including reports of high levels of colonization and outbreaks (11-15).

The aims of this study were: (i) to determine the baseline prevalence of colonization; (ii) to monitor the colonization status at quarterly intervals over one year (iii) to assess risk factors associated with colonization; and (iv) to characterise antimicrobial susceptibility of the AMRO. Detailed molecular characterization of the isolates is described in an accompanying paper (16).

5.3 Materials and Methods:

5.3.1 Setting: A newly built 100 bed LTCF with 88 residents at the start of the study. There are four discrete care areas with residents grouped by dependency level. Each area has 21 single and two double en-suite rooms, dining area, and day room. There are two lifts and two sets of stairs.

5.3.2 Ethical Approval: Ethical approval was granted by the Galway University Hospital Ethics Committee. Written consent was obtained at the outset and residents could withdraw from the study at any time.

5.3.3 Design and data collection: The study period was July 2012-August 2013. For each participating resident data collected were gender, age on admission, date of admission, where the resident was admitted from, presence of an indwelling urinary catheter, systemic antimicrobial treatment, hospitalization in the previous 12 months, and previous ESBL, CPE, VRE, and MRSA result recorded. At each quarterly interval antimicrobial treatment, number of residents in the same room, hospitalization, presence of a break in the skin, Barthel Index score (dependency Index), and location within the LTCF (areas 1 to 4 scored on level of dependency) was recorded for each participant. Antimicrobials were classified as: (1) narrow spectrum beta-lactams, (2) broad spectrum beta-lactams: (3) quinolone/ fluoroquinolones, (4) nitromidazole, (5) nitrofurantoin, (6) others. An antimicrobial day was defined as any day a resident received an antimicrobial.

5.3.4 Clinical sample collection: At quarterly intervals nasal and rectal swabs were collected from each participating resident and urine samples were obtained from catheterized residents.

5.3.5 Laboratory detection: Rectal swabs were cultured on chromogenic agar for detection of ESBL-producing *Enterobacteriaceae* and vancomycin resistant enterococcci ((ID^{TM} ESBL agar and chrom ID^{TM} VRE agar (bioMérieux, Marcy l'Etoile, France)). The Centre of Disease Control and Prevention (CDC) method was applied to detect CPE (17, 18). Nasal swabs were cultured on chrom ID^{TM} MRSA agar (bioMérieux, Marcy l'Etoile, France). Retrospectively, all stored rectal swabs (n = 141) from January to August 2013 were screened for ciprofloxacin resistant *E. coli* by enrichment in 5ml typticase soy broth containing one ciprofloxacin (5µg) disc and one vancomycin (5µg) disc overnight followed by culture on CPS ID 3 agar (bioMérieux, Marcy l'Etoile, France). In each case suspect colonies were subcultured for identification by standard methods and susceptibility testing was performed in accordance with EUCAST disk diffusion methods (19).

5.3.6 Statistical analysis: The descriptive statistics relating to patients' characteristics are provided in absolute frequencies for categorical data, and medians and ranges for continuous data. Outcome variables were dichotomous, yes/no colonization with ESBL/CPE/VRE/MRSA/ respectively. As there were too few

occurrences of colonization with VRE and CPE, these were not further analysed. For ESBL and MRSA colonization univariate comparisons were made to identify associations between the colonization and patient characteristics. Generalised Estimating Equations (GEE) models with an exchangeable correlation structure were used to investigate the longitudinal effects taking dependency between repeated measurements in the same individual into account (repeated individual measures at 0, 3, 6, 9 and 12 months). A forward selection procedure was used to estimate the relation between ESBL/MRSA colonization and specific or general antimicrobial use. Potential confounding effects included in the models were previous colonization, care area, hospitalization, age, gender, and other patient characteristics. Age and gender were kept in the final model; however, other variables were only included if found to be a confounder. Interaction terms were checked but omitted if not significant. P-values <0.05 were considered statistically significant. Results are presented as adjusted odds ratios (OR) (adjusted for potential confounders) with corresponding 95% confidence intervals (CI).

There were four care areas; however, residents in care areas 1 and 2 were similar with respect to dependency levels as were residents in areas 3 and 4 therefore to simplify the models, these areas were subsequently grouped into two care areas.

Statistical analysis was performed using SPSS for windows version 20 and STATA/IC version 13.0 for the GEE estimates.

5.4 Results:

5.4.1 Participating residents

Of 88 residents present at the outset, 51 (58%) were recruited. Of these, 34 completed the study and 17 withdrew before completion. During the study period 13/29 (45%) new residents participated. Eleven were admitted from hospital, one

from home, and one from a LTCF. Key participant characteristics are summarised in Table 1.

Ten baseline and one new participant were hospitalized during the study (2 to 40 hospital days). Of the participants, 28 of 51 baseline and five of 13 new participants received antimicrobials (3 to 203 antimicrobial days).

5.4.2 Detection of Antimicrobial Resistant Organisms

Results are summarized in Table 2. ESBL-producing *E. coli* (ESBL-EC) were detected in 35 (55%) participants (Table 2). Of these, seven (20%) were previously identified as colonized from clinical urine samples. Just over half (26/51) baseline participants remained un-colonized. Most (71%) of the ESBL-EC colonized participants were from two care areas (Table 3). ESBL-EC were resistant to ciprofloxacin, and susceptible to cefoxitin, ertapenem, meropenem, and gentamicin. The retrospective analysis of stored samples taken at 6, 9, and 12 months detected only the ESBL-EC in those participants previously confirmed as ESBL positive but also detected non-ESBL-producing ciprofloxacin-resistant *E. coli* in 5/16 baseline and 2/2 new participants who were not ESBL colonized for the entire study.

At baseline none of the participants tested positive for ESBL-producing *K. pneumoniae* (ESBL-KP); however, five from two care areas subsequently tested positive. At 3, 6, 9, and 12 months 2, 0, 2, 2 residents respectively tested positive for ESBL-KP (Table 2). Four were also colonized with ESBL-EC. None of the five ESBL-KP positive participants were previously identified as ESBL positive. Four of the five ESBL-KP positive participants identified had not been hospitalized in the previous three months. The fifth ESBL-KP positive participant was admitted from hospital; however, ESBL-KP was detected in the LTCF prior to this admission. Four of the five ESBL-KP positive participants identified had not received antimicrobials in the previous three months. All ESBL-KP isolates were resistant to ciprofloxacin and gentamicin, and susceptible to piperacillin/tazobactam, cefoxitin, ertapenem, and meropenem.

CPE were not detected and vancomycin resistant *E. faecium* were detected in only two participants.

MRSA was detected in 8 of 51 participants on initial testing, and subsequently from 5 additional baseline participants and 4 new participants (Table 2 and Table 4). Of the seventeen participants colonized with MRSA at some point, six were positive for MRSA on two or more subsequent tests (Table 4). Five (29%) of MRSA positive participants were previously recognised from clinical samples from wound (n = 2) or nasal/body swabs (n = 3).

5.4.3 Longitudinal analysis of ESBL and MRSA colonization

A total of 241 observations from 64 patients were included in the GEE analysis. ESBL colonization was associated with overall antimicrobial use (odds ratio 2.4 (1.5-3.8)). A more detailed model including class of antimicrobials showed broad spectrum beta-lactam antimicrobials (OR 2.1 (1.2-3.4) and nitrofurantoin use in the previous three months (OR 3.5 (1.1-11.3) to be independently associated with ESBL colonization. Care area was also an independent risk factor for ESBL colonization with participants in area 1 and 2 nearly four times more likely to be colonized (OR 3.9 (1.6-9.7). Age, gender, and previous ESBL colonization were not independently associated with ESBL colonization.

For MRSA colonization, participants who received antimicrobials in the previous three months were 3.1 (1.1-9.1) times more likely to test positive for MRSA. Care area was also independently associated with MRSA colonization, with people in care area 1 and 2, 6.9 (1.6-29.3)) times more likely to test positive for MRSA. Previous MRSA positivity (OR 17.7 (3.8-83.1) and age ((1.12 (1.0-1.2)) were both independently associated with the outcome. No specific antimicrobial class could be identified which was independently associated with MRSA colonization.

5.5 Discussion

A number of studies have reported the prevalence of colonization with MRSA and ESBL-EC in residents of LTCFs. Our findings are broadly consistent with a previous study in Northern Ireland showing a high proportion of residents of LTCFs colonized. However, the level of ESBL-producing *Enterobacteriaceae* colonization at 56% (ESBL-EC = 55% and ESBL-KP = 9%) is higher than the Northern Ireland study (41%) (12).

This level of colonization contrasts with a 2008-2010 study in Sweden which reported *E. coli* resistant to third-generation cephalosporins in just one of 268 residents and with ESBL-*E. coli* in 12% of residents in a Melbourne study (20, 21).

In addition to ESBL-EC we also found colonization with ESBL-KP (9%) and VRE (3%), but not with CPE. Colonization with ESBL-producing *Enterobacteriaceae* and MRSA were associated with antimicrobial consumption and with residence in particular care areas within the LTCF. Fourteen of 36 (39%) ESBL-producing *Enterobacteriaceae* positive residents were also positive for MRSA compared with three of 28 non-ESBL colonized residents.

A study in France identified 6.6 months as the median time to ESBL-producing *Enterobacteriaceae* clearance after hospital discharge (22). A study from Thailand reported the median duration of outpatient colonization with ESBL-producing *Enterobacteriaceae* as 98 days after discharge (23). However, these studies did not address residents of LTCFs. In our study 22 of 25 (88%) colonized residents for whom there at least two follow up swabs remained positive, thus spontaneous clearance of colonization is uncommon in this setting. Furthermore, two ESBL colonized residents detected on initial testing were negative at 3 or 6 months but reverted to positive at nine and 12 months therefore one should be cautious in accepting failure to detect colonization as confirmation of clearance. Previous studies show that 15-25% of patients colonized with multidrug resistant Gram negative organisms will subsequently develop an infection from the strain they were colonized with originally (24, 25).

While the focus of discussion tends to be on those who are colonized, it is important to note the 16 participants co-resident with colonized residents in whom ESBL colonization was not detected at any time over one full year. Retrospective examination of stored samples of these residents indicated that five (33%) were colonized with non-ESBL-producing ciprofloxacin-resistant *E. coli*.

VRE colonization was uncommon in residents in this LTCF although VRE now accounts for 44% of *Enterococcus faecium* blood stream infection in Ireland (7). The two VRE colonized residents were also colonized with MRSA and ESBL-producing *Enterobacteriaceae*. One of the VRE colonized residents was a new admission from an acute hospital and the other had a history of previous hospitalization and 71 antimicrobial days in the previous 18 months. Both were highly dependent based on the Barthel Index. VRE colonization of LTCF residents was not detected in Belgium but has been reported in Australia (2%), Israel (10%), and in the USA (4 to 45%) (21, 26-29). Donskey and colleagues (2003) reported that VRE positive patients have a higher frequency of colonization with MRSA and ceftazidime-resistant Gram negative bacilli than patients not colonized with VRE (30).

MRSA colonization was detected at some time in 27% of residents which is similar to a previous study from Northern Ireland (23% of residents in 45 LTCFs but higher than an earlier study performed in six LTCFs in Ireland between 1995 and 1996 (9% and 10% respectively) (15, 31). It is possible that 27% represents an underestimate of colonization as our sampling was limited to nasal swabs because of practical constraints. Low levels of MRSA colonization in LTCF residents were reported from Sweden, the Netherlands and Denmark (32-34). However, these countries have a low incidence of MRSA blood stream infection compared with Ireland (7).

Of 17 MRSA colonized residents, from whom there were at least two subsequent samples 8/11 (73%) were positive on one subsequent sample and 5/11 (45%) remained positive on two or more subsequent samples. Thus, MRSA colonization was less consistently detected in those participants who were colonized at any time compared with ESBL-EC. There was no programme of decolonization.

Fifty two percent of the residents in our study received antimicrobials in the course of the one year study period. Broad spectrum beta-lactam antimicrobials (included amoxicillin, co-amoxiclav, and cefuroxime) were the most frequently consumed antimicrobials, with amoxicillin-clavulanic acid accounting for the majority. This is consistent with the European Surveillance of Antimicrobial Consumption point prevalence studies which reported the beta-lactam antimicrobials and penicillins as the most common antimicrobials prescribed in Irish LTCFs (35). The 2013 HALT survey for Ireland reported 9% and 11% respectively as the median overall antimicrobial use prevalence in general nursing homes (n = 103) and mixed care facilities (n = 26) both with length of stay > 12 months (36). Likewise in 722 European LTCFs the most commonly prescribed agents were amoxicillin-clavulanic acid (12.7%), nitrofurantoin (10.4%), trimethoprim (9.9%), amoxicillin (7.3%), and ciprofloxacin (6.9%) (9). Higher point prevalence of antimicrobial prescribing (16%) have been reported in LTCFs in other European studies (37, 38). Benoit *et al.* (2008) reported 42% of residents in 73 LTCFs as receiving antimicrobials in the previous six months in the USA (39). Given that third generation cephalosporins and fluoroquinolones were infrequently used in this facility it appears that selective pressure from these agents is not likely to be a major factor in the high level of colonization with ESBL-producing *Enterobacteriaceae* or MRSA in this facility.

Participants were 2.4 times more likely to be ESBL colonized and 3.1 times more likely to be MRSA colonized if they received antimicrobials in the three months before sampling. It was surprising to note that nitrofurantoin was independently associated with ESBL colonization (3.4 (1.1-11.3)) as it is generally considered to have limited selective impact on gut flora (40). However, record review indicated that 8/9 participants who received nitrofurantoin prior to sampling had already been identified as ESBL-positive before the nitrofurantoin was prescribed, based on clinically-submitted urine samples. Since nitrofurantoin use in these patients was most likely guided by prior susceptibility test reports, and since nitrofurantoin is one of few oral antimicrobials active against ESBL-EC, this association can be explained on that basis.

Location within the LTCF was also an important risk factor in ESBL and MRSA colonization with residents in area 1 and 2, 3.9 times more likely to be ESBL colonized, and 6.9 times more likely to be MRSA colonized.

This is one of a very limited number of longitudinal studies of colonization with antimicrobial resistant organisms in a LTCF. There are significant challenges in conducting such a study resulting in some practical limitations. Although initial participation in the study and retention was good it was incomplete. Antimicrobial exposure may have been underestimated as antimicrobial agents prescribed during hospital admission were not captured.

With those limitations however, there are a number of novel findings. We have demonstrated that long term persistence of ESBL-EC colonization is the norm in this setting. We report the first emergence of ESBL-KP in LTCFs in Ireland. We note that although present in five residents over an extended time period it did not appear to disseminate to the extent that ESBL-EC has. It is of interest to consider why a large number of residents in daily contact with multiple residents with ESBL-EC and in a setting in which it seems likely that person to person spread can occur appear to be resistant to colonization. While care area and lack of antimicrobial exposure are important factors associated with non- colonization, we note that nine of the non colonized participants had received antimicrobials during the year of the study and some were in care areas with high levels of colonization. Further studies are needed to establish if there are other characteristics of such individuals e.g. gut microbiota that may contribute to such colonization resistance. While such possibilities are under investigation; however, the key message of this study is that antimicrobial prescribing in LTCFs is an immediately modifiable risk factor for colonization with antimicrobial resistant organisms in LTCFs.

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Table 1: Demographics and clinical details of all participating residents (n = 64)

Factor	Description	N	%	Min-max days
Gender	Male	34	53	N/A
	Female	30	47	N/A
Admission group	Hospital	6	9	N/A
	Long-term care facility	19	30	N/A
	Home	39	61	N/A
Diabetic	Yes	16	25	N/A

Table 1 continued: Demographics and clinical details of all participating residents (n = 64)

Factor	Description	N	%	Min-max days
Care area	1&2	30	47	N/A
	3&4	34	53	N/A
Urinary catheter	Yes	9	14	N/A
Break in the skin	Yes	8	13	N/A
Antimicrobials [a]	In year previous	31	48	0-66
Antimicrobials [b]	During study	33	52	0-203
Hospitalization [c]	In year previous	11	17	0-163
Hospitalization [d]	During study	37	58	0-40
	Mean (SD)	**Min-max**	**Median**	
Age	80 (10.52)	37-98	80	
Initial Barthel Index	33 (33.17)	0-100	17.5	

[a-d]; Details were only accessible for residents in the long-term care facility during the time period therefore no information on prior hospitalization and antimicrobial usage was included for new admissions

Table 2: Results for all residents tested from 0 to 12 months for colonization with targeted antimicrobial resistant organisms.

Resistance phenotype	July 2012[7]	October 2012[7]	January 2013[7]	May 2013[7]	August 2013[7]	Any time point
ESBL-EC [1]	20/51 (39%)	21/51 (41%)	14/50 (28%)	21/49 (43%)	19/45 (42%)	35/64 (55%)
ESBL-KP[2]	0/51 (0%)	2/51 (4%)	0/50 (0%)	2/49 (4%)	2/45 (4%)	5/64 (8%)
CPE [3]	0/51 (0%)	0/51 (0%)	0/50 (0%)	0/49 (0%)	0/45 (0%)	0/64 0%)
VRE [4]	0/51 (0%)	0/51 (0%)	1/50 (2%)	1/49 (2%)	1/45 (2%)	2/64 (3%)
MRSA [5]	8/51 (16%)	9/51 (18%)	7/50 (14%)	7/49 (14%)	4/45 (9%)	17/64 (27%)
Any AMRO [6]	24/51 (47%)	25/51 (49%)	17/50 (34%)	23/49 (47%)	20/45 (44%)	39/64 (61%)

[1] ESBL-EC; Extended-spectrum beta-lactamase producing-*Escherichia coli*

[2]ESBL-KP; Extended-spectrum beta-lactamase producing-*Klebsiella pneumoniae*

[3] CPE; Carbapenemase producing *Enterobacteriaceae*

[4] VRE; Vancomycin resistant enterococci

[5] MRSA; Meticillin resistant *Staphylococcus aureus*

[6] AMRO; Antimicrobial resistant organisms

[7] Number of residents tested at each interval varied accounting for residents who died, withdrew from the study or new admissions who joined the study.

Table 3: Details of care areas corresponding to all extended-spectrum beta-lactamase-producing *Escherichia coli* positive residents including new admissions (n = 35)

Care area	Total residents at baseline	Total participants at baseline	Total new admissions during the study	Total new admissions participating	Total residents participating (n = 64)	No of all residents colonized at initial test	No of residents colonized first detected on second or subsequent test	No of residents never colonized
1	21	15/21	8	7/8	22	13/22	3/22	6
2	23	7/23	5	1/5	8	7/8	0/8	1
3	20	12/20	10	4/10	16	5/16	4/16	7
4	24	17/24	6	1/6	18	3/18	0/18	15

Table 4: Details of care areas corresponding to all meticillin resistant *Staphylococcus aureus* positive residents including new admissions (n = 17)

Care area	Total residents at baseline	Total participants at baseline	Total new admissions during the study	Total new admissions participating	Total residents participating (n = 64)	No of all residents colonized at initial test	No of residents colonized detected on second or subsequent test	No of residents never colonized
1	21	15/21	8	7/8	22	7/22	1/22	14/22
2	23	7/23	5	1/5	8	2/8	2/8	4/8
3	20	12/20	10	4/10	16	1/16	2/16	13/16
4	24	17/24	6	1/6	18	1/18	1/18	16/18

Chapter 6

Molecular characterization of ciprofloxacin-resistant *E. coli* and extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from long-term care facility residents in Ireland

6.1 Abstract

Introduction: *Escherichia coli* sequence type 131 (ST131) is associated with antimicrobial resistance, virulence, and dissemination. Long-term care facilities (LTCFs) are recognised as a reservoir for ST131 *E. coli* and other antimicrobial-resistant pathogens. We studied molecular diversity and strain persistence over one year among surveillance isolates of extended-spectrum β-lactamase (ESBL)-producing *E. coli* (ESBL-EC), ESBL-producing *Klebsiella pneumoniae* (ESBL-KP), and non-ESBL-producing, ciprofloxacin-resistant *E. coli* (non-ESBL-FQREC) from LTCF residents.

Methods: ESBL-EC (n = 98), ESBL-KP (n = 6), and non-ESBL-FQREC (n = 20) were collected prospectively from residents of one LTCF in Ireland (July 2012 to August 2013). Isolates underwent *Xba*I pulsed-field gel electrophoresis (PFGE) and PCR screening for $bla_{\text{CTX-M,}}$ $bla_{\text{TEM,}}$ $bla_{\text{SHV,}}$ $bla_{\text{OXA-1}}$, ST131, and the resistance-associated *H*30 and *H*30-Rx ST131 subclones.

Results: All 118 ESBL-EC and non-ESBL-FQREC isolates were ST131. The 98 ESBL-EC isolates, from 35 residents, represented the *H*30-Rx ST131 subclone, contained $bla_{\text{CTX-M-group 1}}$, and were split by PFGE into two clusters (≥ 85% profile similarity). The major cluster (90 isolates, from 33 residents) clustered with (ST131) UK epidemic strain A and a representative of international pulsotype PFGE812. Sequential isolates from a given resident were closely related. All 20 non-ESBL-FQREC, from 13 residents, were also *H*30 ST131, but only five represented the *H*30-Rx subclone. The six ESBL-KP isolates, from five residents, contained $bla_{\text{CTX-M-group 1}}$ and were closely related.

Conclusion: ESBL-EC, ESBL-KP, and non-ESBL-FQREC in this LTCF are highly clonal. All *E. coli* represented the *H*30 ST131 subclone. Residents generally remained colonized with the same strain. ESBL-KP remained restricted to a few residents.

6.2 Introduction

Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* were first reported in the early 1980s and have become increasingly common throughout the world. In Ireland, ESBL-producing *E. coli* (ESBL-EC) represented 10% of all *E. coli* bloodstream infections in 2013, compared with 3% in 2006 (1).

TEM and SHV enzymes dominated reports of ESBLs until the late 1990s, when the CTX-M beta-lactamases emerged (2). Although CTX-M-15 is common throughout the world, the relative importance of other CTX-M variants differs by region, with CTX-M-2 predominating in South America, CTX-M-3 in Poland, and CTX-M-14 in Spain and throughout Asia (3). Spread of CTX-M enzymes is linked to association with specific epidemic clonal groups such as *E. coli* sequence type ST131 (typically of serotype O25b:H4) and specific epidemic plasmids, e.g. IncFII (3). *E. coli* ST131 producing CTX-M-15 was first reported in 2008. Subsequent reports have documented ST131 isolates containing other CTX-M variants, e.g. $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-10}}$, and $bla_{\text{CTX-M-14}}$ (3-5). Although ST131 has been identified from as early as 1967, ST131 isolates containing ESBLs, predominantly CTX-M-15, emerged mainly in the 2000s (6, 7). ST131 isolates collectively exhibit over 170 distinct PFGE patterns/pulsotypes (> 94% similar *Xba*I PFGE profiles), some of which are associated with particular sources and antimicrobial resistance patterns (6).

Fluoroquinolones are also important therapeutic agents for treatment of bloodstream infection; however, resistance to fluoroquinolones has become a major problem. In Ireland in 2013, 25% of all *E. coli* bloodstream isolates were ciprofloxacin-resistant. Most ESBL-EC are co-resistant to fluoroquinolones; however, non-ESBL-producing, ciprofloxacin-resistant *E. coli* (i.e., non-ESBL-FQREC) account for the majority of ciprofloxacin-resistant *E. coli* infections (1).

The *H*30 ST131 subclone, so named for its carriage of allele 30 of *fimH* (type 1 fimbrial adhesin gene), reportedly expanded and disseminated rapidly after 2000 to become the most common subclone among clinical *E. coli* isolates globally (8, 9). *H*30 is closely associated with fluoroquinolone resistance (8, 9). ESBL-EC, most of

which carry *bla*$_{CTX\text{-}M\text{-}15}$, belong mainly to the *H*30-Rx subset within *H*30, which is strongly associated with sepsis (8, 10).

Analogous to ST131 among ESBL-EC, several widely disseminated clonal groups of ESBL-producing *K. pneumoniae* (ESBL-KP) have also been identified, including *K. pneumoniae* ST11, ST15, ST16, ST23, and ST48 (11-15). Recently, emerging carbapenem resistance has been reported in both *E. coli* and *K. pneumoniae,* further limiting options for antimicrobial therapy for these organisms (16, 17).

Risk factors for acquisition of ESBL-producing organisms and urinary tract infection such as old age, urinary catheterization, antimicrobial consumption, and hospitalization are common among residents of long-term care facilities (LTCFs) (18-20). Multiple point-prevalence studies have found a high prevalence of ESBL-producing organisms colonization among LTCF residents, with a predominance of *E. coli* ST131 (21, 22) and a lower frequency of ESBL-KP (23). The temporal dynamics of molecular correlates of colonization with ESBL-producing *Enterobacteriaceae* among residents in LTCFs have not been well described and represent an important area for research (24). Here we report the molecular characterization of all ESBL-producing *Enterobacteriaceae* isolates and non-ESBL-FQREC from a one-year study of colonization in a LTCF.

6.3 Materials and Methods

As described elsewhere, between July 2012 and August 2013 residents of a LTCF in western Ireland underwent longitudinal surveillance for antimicrobial-resistant organisms (25). At intervals of three months for one year rectal swabs were collected from each participating resident and urine samples were obtained from catheterized residents. Samples were cultured on selective media (chromID ESBL agar, bioMérieux, Marcy l'Etoile, France). Retrospectively, all stored rectal swabs (n = 141) from January to August 2013 were screened for non-ESBL-FQREC by overnight enrichment in 5ml typticase soy broth containing one ciprofloxacin (5µg) disc and one vancomycin (5µg) disc, followed by culture on CPS ID 3 agar

(bioMérieux, Marcy l'Etoile, France). All susceptibility testing was performed in accordance with EUCAST disk diffusion methods, and antimicrobial-resistant organisms were identified as described in the accompanying paper (25, 26).

As described in the accompanying paper, 104 total ESBL-producing *Enterobacteriaceae* were identified, including 98 ESBL-EC isolates from 35 residents and six ESBL-KP isolates from five residents, four of whom also had ESBL-EC. Additionally, 20 non-ESBL-FQREC were recovered from 13 residents (25). All ESBL-EC, ESBL-KP, and non-ESBL-FQREC isolates were tested by PCR for $bla_{\text{CTX-M,}}$ $bla_{\text{TEM,}}$ $bla_{\text{SHV, and}}$ $bla_{\text{OXA-1}}$ as described previously (27, 28). Additionally, the *E. coli* isolates were screened for O25b:ST131 status by PCR-based detection of ST131-specific single-nucleotide polymorphisms (SNPs) in *pabB* (29), and the ST131 isolates so identified were additionally screened for *H*30 and *H*30-Rx subclone status by using established SNP-based PCR assays (8, 30).

*Xba*I pulsed-field gel electrophoresis (PFGE) analysis was done according to the Pulse-Net protocol (31). PFGE profiles were analysed using the Dice coefficient with clustering by the unweighted pair group method with arithmetic averaging (UPGMA). For reference, *E. coli* ST131 isolates representing UK Strains A, C, and D, and international pulsotypes PFGE788, PFGE797, PFGE800, PFGE806, PFGE812, PFGE837, PFGE842, PFGE903, PFGE905, PFGE906, PFGE945, PFGE968, PFGE987, and PFGE1140 (as defined within a large private PFGE library: JRJ) were included in the *E. coli* PFGE analysis (4, 6). Similarly, representative *K. pneumoniae* isolates for STs ST14, ST15, ST16, ST23, ST35, ST37, ST45, ST48, ST101, ST147, ST161, ST258, ST280, ST307, ST392, ST429, and ST1236 were included in the *K. pneumoniae* PFGE analysis.

Plasmid analysis was performed by S1 nuclease-PFGE, as described previously (32), on all six ESBL-KP isolates and the four ESBL-EC isolates from the four residents with both ESBL-EC and ESBL-KP. Plasmid analysis was also performed on two additional ESBL-EC strains from different PFGE clusters (32). Strains with plasmids of known molecular weight, including PB1 (108kb), PB2 (93kb), PB3 (189kb), PB5 (39kb), NCTC

50192 (7kb, 38kb, 66kb, 154kb), were used as size standards. *E. coli* J53 was used as a negative control (33).

6.4 Results

During the one-year surveillance period of LTCF residents, 98 ESBL-EC isolates were recovered from 35 residents, 20 non-ESBL-FQREC from 13 residents, and six ESBL-KP from five residents (25). All 98 ESBL-EC represented the *H*30-Rx ST131 subclone and contained $bla_{\text{CTX-M group 1}}$. Eighty-five (87%) also contained $bla_{\text{OXA-1}}$, whereas none contained bla_{TEM} or bla_{SHV} (Table 1). All 85 $bla_{\text{OXA-1}}$-positive isolates were co-amoxiclav-resistant, whereas all $bla_{\text{OXA-1}}$ negative isolates were co-amoxiclav-susceptible.

Like the ESBL-EC, all 20 non-ESBL-FQREC were ST131. However, only five were *H*30-Rx; the other 15 were *H*30 (non-Rx). Fifteen (75%) contained bla_{TEM}; none contained $bla_{\text{CTX-M}}$ or bla_{SHV} (Table 1).

In a PFGE dendrogram that included each colonized resident's first ESBL-EC (n = 35) or non-ESBL-FQREC (n = 13) isolate, the study isolates clustered with the eight UK and international reference ST131 strains at 73% profile similarity (Figure 1). The *H*30-Rx ST131 isolates were divided into two clusters (clusters A and B), each with ≥ 85% profile similarity, whereas the *H*30 (non-Rx) ST131 isolates formed a third cluster (cluster C) at 94% similarity. Cluster A comprised 95 isolates from 37 residents and included reference isolates for UK strain A and international PFGE812. Cluster B comprised six isolates from two residents. Cluster C comprised 15 isolates from nine residents and the reference isolate for international pulsotype PFGE945. Plasmid analysis identified plasmids ranging from 3 kb to 186 kb in the seven ESBL-EC isolates examined. Plasmids of 3 kb and 5 kb predominated.

The six ESBL-KP isolates were 91% similar by PFGE (Figure 2), but were variously positive for $bla_{\text{CTX-M group 1}}$, bla_{SHV}, and $bla_{\text{OXA-1}}$ (Table 1). All six carried two plasmids

of 4.1 kb and 6.3 kb, respectively (not shown). Two isolates, collected from two residents in October 2012, were indistinguishable by PFGE and had a 208 kb plasmid (not shown). The remaining four isolates, collected from three residents in May and August 2013 (one patient was positive in both May and August), likewise were indistinguishable by PFGE, but differed from the October isolates by three PFGE profile bands (Figure 2) and presence of an 8.4 kb plasmid instead of a 208 kb plasmid.

6.5 Discussion

We describe here what to our knowledge is the first longitudinal study of carriage of ESBL-EC, non-ESBL-FQREC, and ESBL-KP among LTCF residents. The need to address this issue was recognised as urgent in a recent editorial on *E. coli* ST131 (24). Our study yielded five key findings. First, all ESBL-EC isolates represented the *H*30-Rx ST131 subclone. Second, PFGE divided the *H*30-Rx subclone into two clusters, one of which included UK Strain A and a representative of the international PFGE812 group and accounted for 92% of *H*30-Rx isolates. Third, the non-ESBL-FQREC isolates, although also *H*30 ST131, were mostly (75%) not *H*30-Rx, and clustered separately from *H*30-Rx isolates in the PFGE dendrogram. Fourth, once subjects acquired ESBL-EC they tended to remain colonized with the same strain. Fifth, all ESBL-EC and ESBL-KP contained $bla_{\text{CTX-M group 1}}$.

The rapid dissemination of ESBL-EC has been identified as a global public health problem in LTCFs, accounted for in part by the dissemination of the pandemic clonal group *E. coli* ST131*.* Banerjee *et al.* (2013) reported that, among consecutive *E. coli* clinical isolates from Rochester, Minnesota, ST131 accounted for 80/299 (27%) of isolates from healthcare or community-associated infections, but for fully 28/37 (76%) of isolates from LTCFs (22). A surveillance study of feces by Rooney *et al.* (2009) identified ESBL-EC in the feces of 118/294 (40%) of residents of 25 LTCFs in Northern Ireland, with 99% of these isolates representing ST131 (21, 34). The proportion of ST131 we encountered among fecal ESBL-EC and non-ESBL-FQREC

(100%) was similar to that reported by Rooney *et al.* (2009) and Dhanji *et al*. (2011), and higher than in other international studies (21, 22, 34).

The predominance of ST131 in this LTCF may indicate that this facility contributes importantly to the dissemination of ST131 to other local healthcare institutions and the wider community, a principle that likely applies to LTCFs generally, regardless of locale. This problem is likely to be exacerbated by growing number of LTCF residents, which currently is approximately 19,800 (2012) in Ireland, 3.7 million in the EU (2010), and 1.5 million in the United States (2009) (24, 35, 36).

Genomic diversification within the ST131 clonal group has been described previously based on sequencing the *fimH*, *gyrA,* and *parC* genes, PFGE, and whole genome analysis (6, 9, 10). The *H*30 subclone and its *H*30-Rx subset have been identified as the major lineages of *E. coli* associated with antimicrobial-resistant infections. Colpan *et al.* (2013) found that the *H*30 subclone as a whole accounted for ≥ 95% of fluoroquinolone-resistant and ESBL-producing ST131 isolates from US. Similarly, in the present study all ESBL-EC and non-ESBL-FQREC represented the *H*30 ST131 subclone, with all of the ESBL-EC belonging to the *H*30-Rx subclone. The *H*30-Rx subclone has a reported association with sepsis, suggesting that virulence may be contributing to its epidemiologic success (8, 10).

Johnson *et al.* (2012) and Colpan *et al.* (2013) identified various pulsotypes within ST131, with PFGE968, PFGE800, and PFGE812 being the most prevalent (6, 30). Here, 95/103 (92%) of the *H*30-Rx isolates formed a single PFGE cluster, at the ≥ 85% similarity level, which included the ST131 UK epidemic strain A and a representative of international PFGE812 (Figure 1). The latter two reference strains represent, respectively, the most prevalent ST131 variant in the UK (4, 37) and the most prevalent European-associated ST131 pulsotype in a large private PFGE library (JRJ). Thus, the predominance of this clonal group within the study LTCF is part of the international dissemination of this clonal group. Six of the remaining eight *H*30-Rx isolates from two residents formed a distinct PFGE cluster, Cluster B (Figure 1). It is of interest that this variant was not displaced by the predominant variant; thus, two variants of the *H*30-Rx subclone were circulating in the LTCF.

Although the non-ESBL-FQREC also were from the *H*30 ST131 subclone, most (15/20, 75%) were not from the *H30*-Rx subclone, and this subset formed a third PFGE cluster (Cluster C) at the 94% similarity level that included the reference strain for international PFGE945. This pulsotype, the 5th most common in a large private ST131 PFGE library (n = 14), includes fluoroquinolone-resistant human clinical and avian isolates from multiple international locales, with variable presence of ESBL production (unpublished data, JRJ).

We found that, despite the presence within this LTCF of two different PFGE variants of ESBL-EC (both, ST131), the variant identified in a given resident was stable over time. Thus, residents tend to remain colonized with the same ESBL-EC strain for an extended period.

Banjeree *et al.* (2013) and Price *et al.* (2013) demonstrated a high prevalence (91%) of $bla_{\text{CTX-M-15}}$ within the *H*30-Rx subclone (8, 10). Our findings corresponded with these, and additionally showed that most isolates (87%) also carried $bla_{\text{OXA-1,}}$ which was associated with co-amoxiclav resistance, reflecting inefficient inhibition of OXA enzymes by clavulanic acid (38).

Although limited in numbers, the ESBL-KP in our study were 91% similar by PFGE (Figure 2). All carried $bla_{\text{CTX-M group 1}}$ and $bla_{\text{OXA-1}}$ and were resistant to co-amoxiclav. Comparison with a collection of ESBL-KP isolates from other regions of Ireland showed that the present isolates were 86% similar to other Irish isolates belonging to *K. pneumoniae* clonal complex (CC) CC43 (i.e., ST48 and ST123) (unpublished data). This is consistent with clonal dissemination of ESBL-KP in Ireland, similar to that described elsewhere (39). Various clonal groups of *K. pneumoniae* have been reported worldwide, such as CC43, which is commonly associated with resistance enzymes such as TEM-3, SHV-12, and CTX-M-15 (13, 15). We detected ESBL-KP for the first time in this LTCF three months after commencing the study. Given the propensity for dissemination of such strains of *K. pneumoniae* it seems surprising that after nine months of follow-up only five residents had evidence of colonization.

Although we regard the observed clonality of the ESBL-EC and ESBL-KP study isolates as supporting dissemination within this LTCF, it is impossible to differentiate definitively between acquisition of ESBL-producing organisms within the LTCF vs. during episodes of hospitalization or via other exposures. Emerging higher-resolution typing methods such as whole-genome sequencing could be used in the future to clarify transmission pathways (40).

In summary, we demonstrate the clonality and temporal stability of ESBL-producing *Enterobacteriaceae* within a single LTCF. Although multiple ST131 variants were detected, all represented the *H*30 subclone. Colonized residents tended to retain the same pulsotype over an extended period. Further studies are essential to clarify the acquisition, retention, and dissemination of antimicrobial-resistant clones within LTCFs and to define modifiable risk factors for these critical processes.

6.6 Acknowledgements

The authors thank Prof. Neil Woodford (Health Protection Agency, Colindale, UK) for providing UK reference strains and Brian Johnston and Connie Clabots (Veterans Affairs Medical Center, Minneapolis, Minnesota) for providing international reference strains

6.7 References

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Table 1: Correlation of pulsed-field gel electrophoresis (PFGE) clusters with *bla* type, sequence type, and subclone among 124 antimicrobial-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from long-term care facility residents.

Organism	PFGE cluster	No. of Isolates	*bla* genes, no. of isolates				ST[a] and subclone (no. of isolates)
			$bla_{CTX\text{-}M\text{-}group\text{-}1}$[b]	bla_{TEM}	bla_{SHV}	$bla_{OXA\text{-}1}$	
E. coli	EcA[c]	95	90	1	0	79	ST131 *H*30-Rx(95)
E. coli	EcA1[d]	2	2	0	0	0	ST131 *H*30-Rx (2)
E. coli	EcB[e]	6	6	0	0	6	ST131 *H*30-Rx (6)
E. coli	EcC[f]	15	0	14	0	0	ST131 *H*30 (non-Rx) (15)
K. pneumoniae	KpA[g]	6	6	0	6	6	ST1236/ST48 (6)[h]

[a] ST = sequence type (from multilocus sequence typing). Inferred from PCR-based assays for *E. coli* and by PFGE similarity to reference strains of known sequence type (ST) for *K. pneumoniae* strains

[b] Although other $bla_{CTX\text{-}M}$ genes were tested for including $bla_{CTX\text{-}M\text{-}group\text{-}2}$, $bla_{CTX\text{-}M\text{-}group\text{-}8}$, $bla_{CTX\text{-}M\text{-}group\text{-}9}$, and $bla_{CTX\text{-}M\text{-}group\text{-}25}$, none were detected other than $bla_{CTX\text{-}M\text{-}group\text{-}1}$

[c] EcA = ESBL-EC (n = 90) and non-ESBL-FQREC (n = 5) represented in PFGE Cluster A and similar to representative of UK Strain A and international PFGE812.

[d] EcA1 = ESBL-EC (n = 2) demonstrating 83% similarity to strains in PFGE Cluster A.

[e] EcB = ESBL-EC (n = 6) represented in PFGE Cluster B.

[f] EcC = non-ESBL-FQREC (n = 15) represented in PFGE Cluster C A and similar to representative international PFGE945.

[g] KpA = ESBL-KP PFGE cluster similar to ST1236

[h] ST1236 is a single-locus variant of ST48; both are part of clonal complex 43.

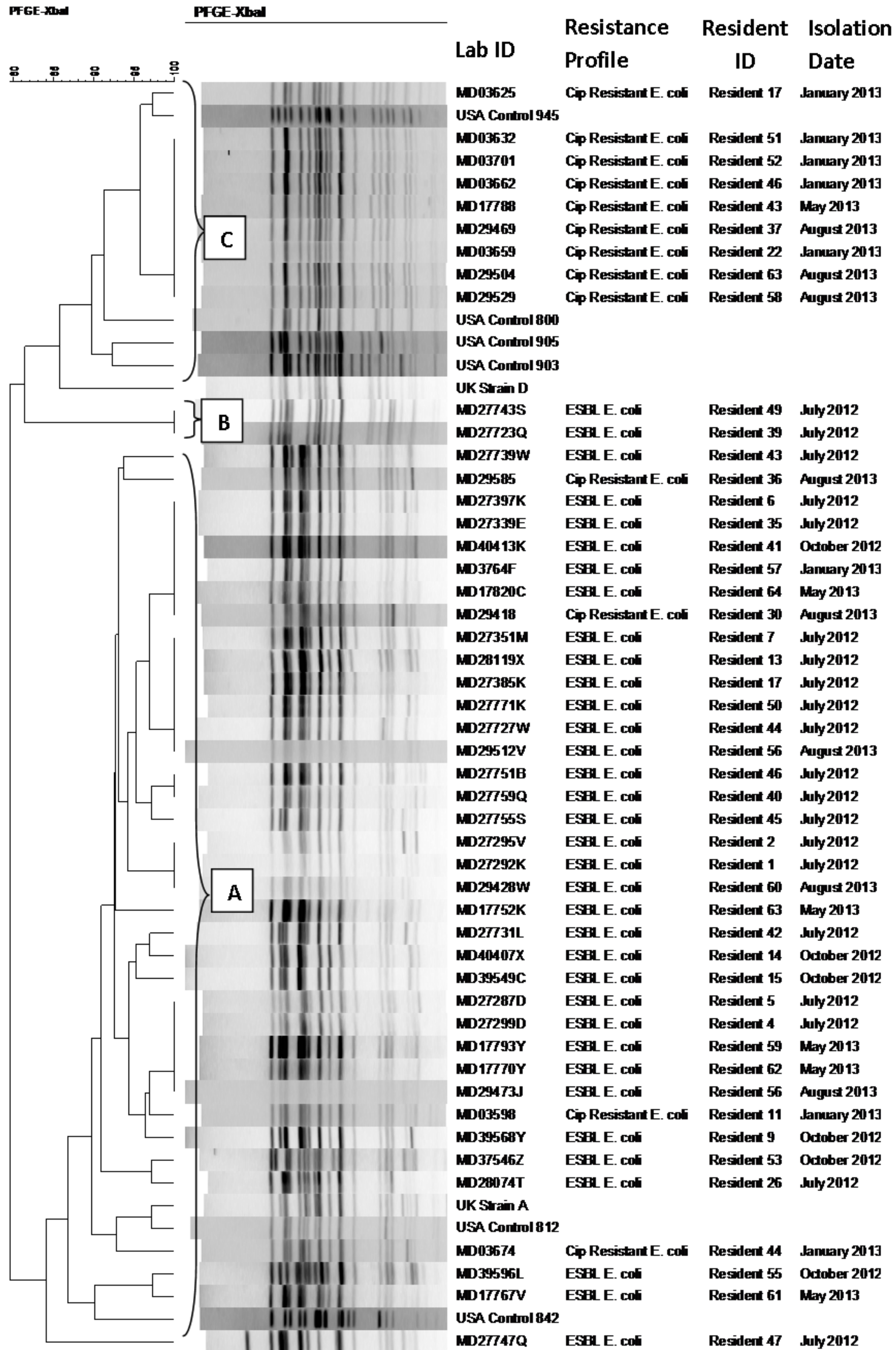
PFGE-XbaI
PFGE-XbaI
Lab ID
Resistance Profile
Resident ID
Isolation Date
C
B
A
MD03625 Cip Resistant E. coli Resident 17 January 2013
USA Control 945
MD03632 Cip Resistant E. coli Resident 51 January 2013
MD03701 Cip Resistant E. coli Resident 52 January 2013
MD03662 Cip Resistant E. coli Resident 46 January 2013
MD17788 Cip Resistant E. coli Resident 43 May 2013
MD29469 Cip Resistant E. coli Resident 37 August 2013
MD03659 Cip Resistant E. coli Resident 22 January 2013
MD29504 Cip Resistant E. coli Resident 63 August 2013
MD29529 Cip Resistant E. coli Resident 58 August 2013
USA Control 800
USA Control 905
USA Control 903
UK Strain D
MD27743S ESBL E. coli Resident 49 July 2012
MD27723Q ESBL E. coli Resident 39 July 2012
MD27739W ESBL E. coli Resident 43 July 2012
MD29585 Cip Resistant E. coli Resident 36 August 2013
MD27397K ESBL E. coli Resident 6 July 2012
MD27339E ESBL E. coli Resident 35 July 2012
MD40413K ESBL E. coli Resident 41 October 2012
MD3764F ESBL E. coli Resident 57 January 2013
MD17820C ESBL E. coli Resident 64 May 2013
MD29418 Cip Resistant E. coli Resident 30 August 2013
MD27351M ESBL E. coli Resident 7 July 2012
MD28119X ESBL E. coli Resident 13 July 2012
MD27385K ESBL E. coli Resident 17 July 2012
MD27771K ESBL E. coli Resident 50 July 2012
MD27727W ESBL E. coli Resident 44 July 2012
MD29512V ESBL E. coli Resident 56 August 2013
MD27751B ESBL E. coli Resident 46 July 2012
MD27759Q ESBL E. coli Resident 40 July 2012
MD27755S ESBL E. coli Resident 45 July 2012
MD27295V ESBL E. coli Resident 2 July 2012
MD27292K ESBL E. coli Resident 1 July 2012
MD29428W ESBL E. coli Resident 60 August 2013
MD17752K ESBL E. coli Resident 63 May 2013
MD27731L ESBL E. coli Resident 42 July 2012
MD40407X ESBL E. coli Resident 14 October 2012
MD39549C ESBL E. coli Resident 15 October 2012
MD27287D ESBL E. coli Resident 5 July 2012
MD27299D ESBL E. coli Resident 4 July 2012
MD17793Y ESBL E. coli Resident 59 May 2013
MD17770Y ESBL E. coli Resident 62 May 2013
MD29473J ESBL E. coli Resident 56 August 2013
MD03598 Cip Resistant E. coli Resident 11 January 2013
MD39568Y ESBL E. coli Resident 9 October 2012
MD37546Z ESBL E. coli Resident 53 October 2012
MD28074T ESBL E. coli Resident 26 July 2012
UK Strain A
USA Control 812
MD03674 Cip Resistant E. coli Resident 44 January 2013
MD39596L ESBL E. coli Resident 55 October 2012
MD17767V ESBL E. coli Resident 61 May 2013
USA Control 842
MD27747Q ESBL E. coli Resident 47 July 2012

Figure 1: **Dendrogram of *Xba*I pulsed-field gel electrophoresis (PFGE) profiles for *Escherichia coli* isolates.**

One representative of each PFGE type per subject was included in the dendrogram, which includes both extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC) (n = 35) and non-ESBL-producing, ciprofloxacin-resistant *E. coli* (non-ESBL-FQREC) (n = 13) isolates, plus reference strains representing UK Strains A, C, and D, and international pulsotypes labelled USA Control 800, 812, 842, 903, 905, and 945. Reference strains with ≥ 85% profile similarity to study isolates are shown. The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on Dice similarity coefficients. Three PFGE clusters (A, B, C) were defined based on ≥ 85% profile similarity. Isolates labelled "Cip Resistant E. coli" represent non-ESBL-FQREC. Isolates labelled "ESBL E. coli” represent ESBL-EC.

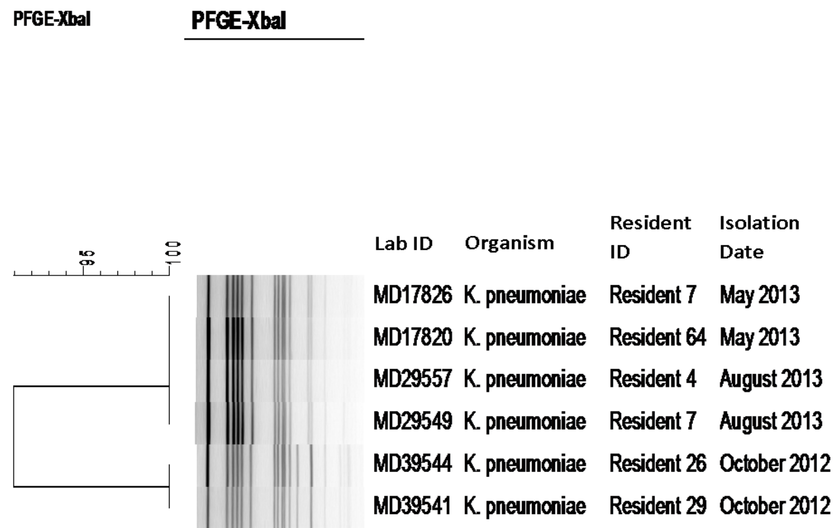

Figure 2: Dendrogram of *Xba*I pulsed-field gel electrophoresis (PFGE) profiles for *Klebsiella pneumoniae* isolates.

PFGE dendrogram of the six ESBL-producing *Klebsiella pneumoniae* isolates, from 5 residents from October 2012 – August 2013. The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on Dice similarity coefficients. All profiles are ≥ 91% similar.

Chapter 7

Discussion

7.1 Discussion

The objective of this thesis was to improve the understanding of the role long-term care facilities (LTCFs) play in the dissemination of antimicrobial resistance.

This work represents the first longitudinal study monitoring colonisation with extended-spectrum β-lactamase (ESBL)-producing and carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and meticillin-resistant *Staphylococcus aureus* (MRSA) in a LTCF and correlating microbiological findings with clinical data. Results of this study indicate high prevalence of colonisation, long-term persistence of rectal colonisation with ESBL-producing *E. coli* (ESBL-EC) (Paper IV), and that in general, residents remained colonised with the same strain over extended periods (Paper V). Second, clonal dissemination of ST131 in LTCFs was identified (Paper I and Paper V). All non ESBL-producing ciprofloxacin-resistant *E. coli* detected represented the ST131 *H30* subclone and ESBL-EC represented ST131 *H30-Rx* (Paper V). Third, to the best of our knowledge this thesis reports the first emergence of ESBL-producing *K. pneumoniae* (ESBL-KP) in a LTCF in Ireland (Paper IV). Fourth, just under half of the residents in a LTCF did not become colonised with antimicrobial resistant organisms and over 70% did not become colonised with MRSA, although regular contact with both organisms was presumably unavoidable given the high prevalence of colonisation overall (Paper IV). Fifth, environmental contamination is likely to play a significant part in persistence and transmission of MRSA within the LTCF, but is of less apparent significance with respect to other antimicrobial resistant organisms (Paper II). Sixth, a single MRSA *spa* clonal complex (*spa*-CC 22) predominated in the LTCF, although diversity in *spa* types was observed in the environment and resident isolates (Paper III).

This thesis demonstrates that ESBL-EC are more widespread than CPE, VRE, and MRSA in one LTCF (Paper IV). Various point prevalence studies have reported high numbers of LTCF residents as colonised with ESBL-EC [1-4]; however, due to the

lack of longitudinal studies little is known as to whether or not LTCF residents can eliminate ESBL-EC from their gut and if so how long does this take. This is important to know as it may be relevant to selection of empiric antimicrobial therapy and in guiding effective infection prevention and control (IPC) measures. This research shows that clearance of ESBL-EC generally does not occur. This suggests that caution is required in considering failure to detect ESBLs in such patients on one or more occasions as evidence of clearance. This may be due to limitations in methodologies and/or of masking by other gut flora in the absence of antimicrobial selection pressure. Similar findings were reported by Birgand *et al.* (2013) who reported alternating ESBL positive and negative results in consecutive samples [5]. The majority of studies looking for ESBL clearance are performed in the hospital setting and monitoring of colonisation is based on samples collected on re-admission to hospitals. The median time to reported clearance varies greatly depending on the study e.g. from 98 days up to 58 months; however, it is important to note that these studies include large ranges for time of colonisation [5-7]. Alsterlund *et al*. (2012) monitored long-term carriage of ESBL-EC after an outbreak of ESBL-EC in a town named Kristianstad in Sweden. By following up the subjects involved in the outbreak (n = 27) and additional ESBL-EC positive individuals detected at the same time but not part of the outbreak (n = 15), it was recorded that 5/42 individuals remained colonised after 41-59 months. In contrast to the data presented in this thesis, the criterion for clearance was 3 consecutive ESBL negative swabs and it was not performed in a LTCF [6]. Shorter time from discharge to re-admission and antimicrobial use are also recognised as risk factors for persistent carriage of ESBL- producing *Enterobacteriaceae* [7, 8].

In addition, ESBL-EC and MRSA co-colonisation was common in the studied LTCF (Paper IV), illustrating carriage of various multidrug-resistant organisms in residents, which would significantly limit treatment options in the case of an infection occurring. Due to the nature of LTCFs, shared facilities are the norm to encourage social interactions between individuals. These shared facilities create opportunities for direct and indirect transmission of ESBL-EC and MRSA within the LTCF. In this

thesis ESBL-EC environmental contamination was detected to a much lesser extent than MRSA contamination.

This thesis provides evidence that the *E. coli* ST131 is widely disseminated in LTCFs in one region of Ireland and accounts for the all CTX-M *E. coli* isolates in this LTCF over a one year period (Paper I and Paper V). These findings raise concern that LTCFs may be playing a central role in the spread of ST131. All non-ESBL ciprofloxacin resistant *E. coli* colonising the LTCF residents were representatives of the *H*30 ST131 subclone and all ESBL ciprofloxacin resistant *E. coli* were representatives of the virulent *H30-Rx* subclone. It was noted that all residents colonised with ESBL-EC remained colonised with the same *H30-Rx* strain for an extended period of time. It was interesting that in any individual resident only non-ESBL ciprofloxacin resistant-EC (*H30*) or the ESBL-EC (*H30-Rx*) were detected at one time and not both.

This work represents the first report in the literature of ESBL-KP in a LTCF in Ireland (Paper IV). Even though colonisation with ESBL-KP was detected less often than colonisation with ESBL-EC in this LTCF, it is worrying that the same clonal complex (CC) of ESBL-KP circulating in the LTCF is also disseminating nationally in hospitals (Paper V). *K. pneumoniae* are one of the important pathogens causing bloodstream infection in Ireland and the proportion of ESBL-KP infections are dramatically increasing [9]. If this CC (ST48 and ST123) of ESBL-KP achieves the same success as other multidrug-resistant globally disseminated clonal complexes such as *K. pneumoniae* CC 258 (ST258 and its single locus variants ST11 and ST437) or *E. coli* ST131 it may have serious healthcare consequences. Similar to the ESBL-EC, all ESBL-KP were positive for CTX-M group 1 enzymes.

A major benefit of performing the longitudinal study was the ability to monitor colonisation status at 3 month intervals over one year (Paper IV). Interestingly, just

under half of the residents were not detected as colonised with ESBL-producing *Enterobacteriacea*e or MRSA over the extended period of time, although living in close proximity to colonised residents. Previous studies have not discussed this phenomenon and it is interesting to speculate that their gut microbiota is playing a vital role in preventing acquisition of antimicrobial resistant organisms (AROs). Residents who did not acquire AROs tend to have less frequent use of antimicrobials and required less help with daily activities such a bathing, dressing, toilet use etc, all of which may impact on the gut microbiota.

The data presented in this thesis indicates that environmental contamination appears to be a more important reservoir for transmission for MRSA than for ESBL-producing *Enterobacteriaceae.* A similar pattern was also observed in the LTCF prior to occupation by residents (Paper II). With the demand for LTCFs increasing due to the ageing population, it is important to consider how the movement of healthcare staff or equipment to new facilities may support dissemination of MRSA. Also, by monitoring environmental contamination it will provide guidance to IPC teams as to what sites are most commonly contaminated. This may encourage additional cleaning for these areas. The lack of detection of ESBL (with the exception of one occasion) and VRE environmental contamination, suggest that indirect transmission through the environment may not be as significant in their transmission as person to person contact. Studies from France and New Zealand have also reported infrequent detection of ESBL-producing *Enterobacteriaceae* contamination (3.4-4%) in rooms or bathrooms of colonised or infected hospital inpatients [10, 11]. There is a general consensus from results of research studies that ESBL-KP environmental contamination is significantly more common than ESBL-EC contamination [10-12]. Freeman *et al.* (2014) also reported that the presence of indwelling urinary catheters increases the likelihood of environmental contamination with ESBL-producing *Enterobacteriaceae*, while patients receiving a carbapenem is inversely associated with environmental contamination suggesting reduction in bacterial load [11]. Guet-Revillet *et al.* (2012) and Gbaguidi-Haore *et al.* (2013) also reported environmental contamination with ESBL-producing *Enterobacteriaceae* being more

frequent in a room of a patient colonised with ESBL-KP than a patient with ESBL-EC [10, 12]. The infrequent detection of ESBL environmental contamination detected in this thesis may be due to the limited number of ESBL-KP colonised residents and a small number of residents with indwelling urinary catheters in the LTCF (9/64, 14%).

Paper III identified a single MRSA *spa* clonal complex (*spa*-CC 22) predominating in the LTCF; however, variations in *spa* types were observed within the LTCF. *spa* type t727 was only found in the environment and t727 strains were resistant to numerous antimicrobials including ampicillin, ciprofloxacin, cadmium acetate, erythromycin, fusidic acid, mercuric chloride, and phenyl mercuric acetate. The predominant ST identified in this series of studies was ST22 and ST22 has also predominated in Irish hospitals for over 10 years [13]. It has previously been reported that MRSA strain replacement occurs approximately every 10 years. It is imperative that we notice any new strains emerging, particularly strains resistant to a large panel of antimicrobials, before they become endemic in the healthcare setting [13]. Monitoring the epidemiology of MRSA in Ireland will provide recognition of new mechanisms of resistance which may influence antimicrobial prescribing. The frequent movement of residents from LTCFs to hospitals, previously been referred to as 'the revolving door', could facilitate the introduction of new resistant strains to other healthcare institutions [14]. This may exacerbate the problem of antimicrobial resistance in healthcare institutions.

Monitoring antimicrobial prescribing and improvements in IPC practices in LTCFs may provide effective steps to minimise the transmission of AROs. Even with the most effective practical measures, preventing spread of AROs in a LTCF is likely to be challenging, therefore supporting ageing in the community should be considered a central part of the global effort to contain the spread of acquired antimicrobial resistance.

7.2 Limitations

There were a number of limitations to this thesis. We do not know the prevalence of ESBL-PE, CPE, VRE and MRSA colonisation among all of the LTCF residents due to an overall participation rate of 55%. One care area in particular had a low participation rate in part related to a very high proportion of residents who were unable to consent due to impaired executive function. Seven of the eight residents who participated from this particular care area were colonised with AROs, therefore it would have been interesting to know the true prevalence in this care area. In addition, healthcare workers were not screened therefore we also do not know the prevalence of AROs among this key population. Healthcare workers were not included as this is generally not appropriate according to local guidelines [15, 16].

Due to practical clinical constraints only nasal swabs were used for screening for MRSA and this may result in under-estimation of the number of residents colonised with MRSA. Other sites that could be also sampled include the axilla and groin area; however, highest MRSA detection rates have been found for nasal swabs [17, 18].

At baseline, screening was performed for ESBL-PE, CPE, VRE, and MRSA; however, it was decided at the end of the study to retrospectively look for non-ESBL ciprofloxacin-resistant *E. coli.* Ideally this would have been performed prospectively from the start of the study.

As hospital charts were not accessible antimicrobials prescribed during hospital admissions in the course of the study were not captured. Details regarding hospitalisations or antimicrobial prescriptions prior to admission to the LTCF could not be recorded for new study participants due to practical and time constraints.

The study was focussed on gathering qualitative data from a single LTCF therefore the sample size was limited. The LTCF studied was a public LTCF and it is uncertain to what extent the findings are generalisable to other LTCFs in Ireland or elsewhere.

Due to practical limitations environmental and clinical samples chosen for molecular characterisation were representatives of the total collection. It may have been of interest to perform molecular characterisation on all isolates and a larger numbers of environmental isolates to see if this would reveal more diversity of the MRSA strains. Also, *spa* types detected only in the environmental collection e.g. t727 and in the clinical collection e.g. t002 could possibly be present in the opposite collection but were not detected as were not in the subset of isolates analysed.

7.3 Future Work

If time permitted it would have be of value to perform whole genome sequencing to determine

- The basis of the differences in PFGE patterns within ST131
- How homogeneous are ST131 *E. coli* within multiple LTCFs
- The route of transmission of ESBL and MRSA within the LTCFs and to determine if new admissions from hospitals play a role in introducing new strains to a LTCF
- The characteristics of persistence associated the *H30-Rx* strains such as virulence factors and/or biofilm formation. In addition, it would be interesting to know how the *H30-Rx* strains colonising the LTCF residents in this study compare to *H30-Rx* strains causing sepsis.

It would also be of interest to perform metagenomics studies on all the rectal swabs collected to monitor changes in the gut microbiota, particular when exposed to

antimicrobials, and also to compare the gut microbiota of the residents colonised with ESBL-EC and the residents that never acquired ESBL-EC.

7.4 References

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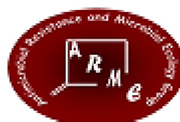
Appendix 1: Longitudinal study information leaflet for residents (Paper IV and V)

Antimicrobial Resistance and Microbial Ecology Group
NATIONAL UNIVERSITY OF IRELAND GALWAY

Antibiotic Resistance Bacteria in Nursing Homes

Information Leaflet on Antibiotic Resistance Bacteria in Nursing Homes



Information Leaflet on Antibiotic Resistance Bacteria in Nursing Homes

What is the purpose of this study?

We want to find out if you are carrying antibiotic resistant bacteria in your gut. If you are willing to take part in this study, one of the nurses in the nursing home will take the swab from your back passage and it will be tested in the laboratory. The result of the test will be given to your GP and nursing home staff. The staff or GP will inform you of your results.

What type of sample will be taken?

A swab from your back passage and nose will be taken by a nurse in the nursing home. A urine sample may also be taken.

Will the samples be used in another study at

The bacteria that we grow may be used in a future project.

Will anyone look at my medical records?

Yes information about recent antibiotic use and general information such as age will be recorded.

What will you benefit from this study?

If you get an infection caused by one of these antibiotic resistant bacteria it can sometimes be difficult for the doctor to find the right antibiotic to treat the infection. Knowing before you get an infection, that you are carrying antibiotic resistant bacteria in your gut or nose could help the doctor with the choice of antibiotic used to treat your infection. This could mean that the infection clears up more quickly.

Appendix 2: Longitudinal study information leaflet for residents regarding results (Paper IV and V)

Antimicrobial Resistance and Microbial Ecology Group
NATIONAL UNIVERSITY OF IRELAND GALWAY

Antibiotic Resistance Bacteria in Nursing Homes

Information leaflet regarding results of study

Information leaflet regarding results of study

What if the test is positive?

The test results will not make any difference to your care in the nursing home. There is no need for any antibiotic treatment no matter what the test result is. If you have a positive test and you get an infection sometime in the months or years ahead it could be useful for the doctor to know you had a positive MRSA, ESBL, VRE or CPE test because there it could help the doctor pick the correct antibiotic right away.

Will you be told your rest results and how?

GPs and nursing staff at St. Brendans will be notified of the test results. The GP for the nursing home will inform you of your test result.

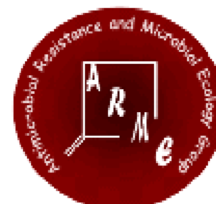
Can infection with antibiotic resistant bacteria treated?

Yes there are specific antibiotics to help treat these infections.

If colonised or infected with antibiotic resistant bacteria is it still safe to have visitors?

Yes. There is no need to worry about visitors regard-less of their age.

Appendix 3: Consent form for residents participating in the longitudinal study (Paper IV and Paper V)



Antimicrobial Resistance and Microbial Ecology Group

National University of Ireland Galway

SAMPLE CONSENT FORM FOR RESEARCH STUDY

Working Title of Project: Antibiotic Resistant Bacteria in Nursing Homes

- I understand that this project involves testing a sample from my back passage, nose and urine for antibiotic resistant bacteria.
- I agree to take part in the above research study and consent to give to a rectal swab and a swab from my nose. I also agree to give a urine sample.
- I give permission for my medical records to be looked at for this study.
- I allow for the bacteria obtained from the swabs in this study to be used in future studies.

________________ ________________ ____________________________

Name of Patient   Date   Signature

If you require further information after reading this leaflet, please contact:

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Appendix 4: Awards and honours received during this Ph.D. thesis

2014- Travel grant for attendance at the Infectious Diseases Society of Ireland Annual Society Meeting, Cork. Value: €100

2013- Best Oral Presentation-The Environmental Science Association of Ireland.

2013- Runner Up Oral Presentation at the National University of Ireland Galway College of Medicine, Nursing and Health Sciences Postgraduate Research Day.

2013- Travel grant for attendance at the Infectious Diseases Society of Ireland Annual Society Meeting, Cork. Value: €100.

2013- Travel grant from the British Society for Antimicrobial Chemotherapy (BSAC) for attendance at the Antimicrobial Resistance Mechanisms (ARM) Workshop for Researchers, Birmingham.

2012- Travel grant from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for attendance at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), London. Value: €500.

2012- Travel grant from the American Society for Microbiology (ASM) for attendance at the interscience conference on antimicrobial agents and chemotherapy (ICAAC), San Francisco. Value: $750.

2012- Travel grant from the British Society for Antimicrobial Chemotherapy (BSAC) for attendance at the interscience conference on antimicrobial agents and chemotherapy (ICAAC), San Francisco. Value: £1,500.

Appendix 5: Dissemination of Research

- **Peer reviewed publications**

Ludden, C., M. Cormican, A. Vellinga, B. Austin, and D. Morris, Colonization with ESBL-producing and carbapenemase-producing *Enterobacteriaceae*, vancomycin resistant enterococci and meticillin-resistant *Staphylococcus aureus* in a long-term care facility over one year. In Submission.

Ludden, C., M. Cormican, J. R. Johnson, B. Austin, and D. Morris, Molecular characterization of ciprofloxacin-resistant *E. coli* and extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from long-term care facility residents in Ireland. In Submission.

Ludden, C., G. Brennan G, D. Morris, **B. Austin, B. O'Connell B, M. Cormican,** Characterization of meticillin-resistant *Staphylococcus aureus* from residents and environment in a nursing home. In Submission.

Ludden, C., B. Hanahoe, F. Boyle, B. Kanagaratnam, M. Cormican, and D. Morris, Comparison of extended-spectrum beta-lactamase-producing *Escherichia coli* associated with nursing homes with other ESBL isolates. 2014. *J Microb Infect Dis.* 4 (3): 92-96

Ludden, C., M. Cormican, B. Austin, and D. Morris, Rapid environmental contamination of a new nursing home with antimicrobial-resistant organisms preceding occupation by residents. 2013. *J Hosp Infect*. 83(4): 327-329.

Morris D, O'Connor M, Izdebski R, Corcoran M, **Ludden C**, McGrath E, Buckley V, Cryan B, Gniadkowski M, Cormican M. Dissemination of clonally related ESBL-producing *Klebsiella pneumoniae* in Ireland. 2014. Under Review, *J Hosp Infect*.

Morris D, Boyle F, **Ludden C,** Condon I, Hale J, O'Connell N, Power L, Boo TW, Dhanji H, Lavallee C, Woodford N, Cormican M. Production of KPC-2 Carbapenemase by an *Escherichia coli* Clinical Isolate Belonging to the International ST131 Clone. 2010. *Antimicrob Agents Chemother.* 55(10):4935-4936.

Morris D, McGarry E, Cotter M, Passet V, Lynch M, **Ludden C,** Hannan MM, Brisse S, Cormican M. Detection of OXA-48 Carbapenemase in the Pandemic Clone *Escherichia coli* O25b:H4-ST131 in the Course of Investigation of an Outbreak of OXA-48-Producing *Klebsiella pneumoniae*. 2012. *Antimicrob Agents Chemother.* 56(7):4030-4031.

- **Lead Authorship - Conference Proceedings**

Oral Presentations

Characterisation of meticillin-resistant *Staphylococcus aureus* strains isolated from residents and the environment of a nursing home. Infectious Diseases Society of Ireland (IDSI) Annual Society Meeting, Cork, April 23rd- 25th, 2014.

Epidemiological investigation into the spread of antimicrobial resistant organisms in nursing homes in Ireland. Antimicrobial Resistance Mechanisms (ARM) Workshop for Researchers, Birmingham, November 28th-29th, 2013.

Whole genomic sequencing evidence for replacement of an established ESBL *E. coli* ST131 strain by an introduced strain in a nursing home. Infectious Disease Genomics and Global Health Conference, Wellcome Trust, Cambridge, October 16th-18th, 2013.

The Investigation of Colonization and Risk Factors Associated with Meticillin-Resistant *Staphylococcus aureus,* Extended Spectrum Beta Lactamase-Producing *Enterobacteriaceae*, Vancomycin-Resistant Enterococci and Carbapenemase-Producing *Enterobacteriaceae* Among Nursing Home Residents Over a 3 Month

Period. National University of Ireland Galway Postgraduate Research Day, Galway, May 27th, 2013.

The Investigation of Colonization and Risk Factors Associated with Meticillin-Resistant *Staphylococcus aureus,* Extended Spectrum Beta Lactamase-Producing *Enterobacteriaceae*, Vancomycin-Resistant Enterococci and Carbapenemase-Producing *Enterobacteriaceae* Among Nursing Home Residents Over a 3 Month Period. Infectious Diseases Society of Ireland (IDSI) Annual Society Meeting, Cork, May 23rd- 25th, 2013.

Longitudinal study of environmental contamination with antimicrobial resistant organisms in a newly built nursing home. Environ Conference, Galway, January 30th- February 1st, 2013.

Environmental contamination with antimicrobial resistant organisms in a newly built nursing home. Proceedings of the National University of Ireland Galway College Postgraduate Research Day, Galway, May 28th May, 2012.

Longitudinal study of environmental contamination with antimicrobial resistant organisms in a newly built nursing home. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), London, March 31st- April 3rd, 2012.

The introduction of antimicrobial resistant organisms to a newly built nursing home during commissioning. Society of General Microbiology (SGM), Dublin, March 26th-29th, 2012.

Clonal group O25b-ST131 accounts for more than 90% of clinical isolates of CTX-M producing *E. coli* from residents of 26 nursing homes in one region. Infectious Diseases Society of Ireland (IDSI) Dublin, June 11th- 12th, 2011.

Is containment of antimicrobial resistance a hidden benefit of supporting ageing in the community? Centre for Ageing Research and Development in Ireland (CARDI), Dublin, November 2nd- 3rd, 2011.

Clonal group O25b-ST131 accounts for more than 90% of clinical isolates of CTX-M producing *E. coli* from residents of 26 nursing homes in one region. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Milan, May 7th-10th, 2011

Poster Presentations:

Meticillin-resistant *Staphylococcus aureus* from residents in one nursing home are more closely related than environmental isolates. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, May 10th- 13th, 2014

Whole genomic sequencing evidence for replacement of an established ESBL *E. coli* ST131 strain by an introduced strain in a nursing home. Federation of Infection Societies Scientific Conference (FIS), Birmingham, November 11th- 13th, 2013

Emergence of ESBL *K. pneumoniae* during a longitudinal study of colonisation with antimicrobial-resistant microorganisms in a nursing home. Federation of Infection Societies Scientific Conference (FIS), Birmingham, November 11th- 13th, 2013.

Longitudinal study of role of nursing homes in the dissemination of meticillin-resistant *Staphylococcus aureus* (MRSA), ESBL-producing *Enterobacteriaceae* (ESBL-PE), Vancomycin-Resistant Enterococci (VRE) and Carbapenemase Producing *Enterobacteriaceae* (CPE). Five Nations Health Protection Conference, Dublin, May 14th- 15th, 2013.

Emergence of ESBL *K. pneumoniae* during a longitudinal study of colonisation with antimicrobial-resistant microorganisms in a nursing home. European Congress of

Clinical Microbiology and Infectious Diseases (ECCMID), London, April 27th- 30th, 2013.

Changes in dominant extended spectrum β-lactamase producing *E. coli* in a nursing home over a seven year period. Antimicrobial Resistance Mechanisms (ARM) Workshop for Researchers, Birmingham, November 28th-29th, 2012.

E. coli O25b:ST131 accounts for a significantly higher proportion of nursing home compared with hospital isolates of CTX-M producing *E. coli.* Interscience *Conference* on *Antimicrobial* Agents and Chemotherapy (ICAAC), San Francisco, September 9th-12th, 2012.

CTX-M *E. coli* is common in retail chicken in Ireland but is not closely linked to human isolates. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, September 9th- 12th, 2012.

Changes in dominant extended spectrum β-lactamase producing *E. coli* in a nursing home over a seven year period. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, September 9th- 12th, 2012.

Rapid dissemination of antimicrobial resistant organisms in the environment of a newly built nursing home. Royal Academy of Medicine in Ireland Biomedical Sciences Conference, Galway, June 14th, 2012.

Most extended-spectrum beta-lactamase producers in nursing homes and hospitals in Ireland belong to a single clonal group. Royal Academy of Medicine in Ireland Biomedical Sciences Conference, Galway, June 14th, 2012.

Clonal dissemination of *Escherichia coli* O25b:ST131 in hospitals, nursing homes and the community in one region of Ireland. Postgraduate Research Day, Galway, May 28th 2012.

Clonal group O25b-ST131 accounts more than 88% of clinical isolates of ESBL producing *E. coli* from hospital and nursing homes in one region of Ireland. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), London, March 31st- April 3rd, 2012

E. coli clonal group O25b-ST131 producing CTX-M-type extended spectrum beta-lactamase producers in nursing homes in Ireland. Society of General Microbiology (SGM), Dublin, March 26th- 29th, 2012.

Environmental contamination of nursing homes with antimicrobial resistant organisms may precede occupation by residents. Federation of Infection Societies Scientific Conference (FIS), Manchester, November 16th- 18th, 2011.

Emergence of VIM-producing *Enterobacter cloacae* in a university hospital in the western region of Ireland. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Milan, May 7th- 10th, 2011

Appendix 6: Rapid environmental contamination of a new nursing home with antimicrobial-resistant organisms preceding occupation by residents

Journal of Hospital Infection 83 (2013) 327–329

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Short report

Rapid environmental contamination of a new nursing home with antimicrobial-resistant organisms preceding occupation by residents

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ARTICLE INFO

Article history:
Received 20 September 2012
Accepted 26 November 2012
Available online 29 January 2013

Keywords:
Antimicrobial
Meticillin-resistant
Staphylococcus aureus
Residential care
Resistance

SUMMARY

Nursing homes are reservoirs for antimicrobial-resistant organisms (AROs). This study examined the time to environmental contamination with AROs in a new-build nursing home. Environmental sites in an occupied nursing home ($N = 18$) and a newly built replacement nursing home ($N = 21$) were monitored during an 11-week period before and after residents transferred between buildings. Meticillin-resistant *Staphylococcus aureus* was detected during commissioning in the new building and was a frequent finding throughout the building after residents had moved in. Extended-spectrum beta-lactamase-producing *E. coli* O25b:ST131 was detected once.
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Introduction

The environment plays an important role in the dissemination of antimicrobial-resistant organisms (AROs) such as meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE).[1,2] Infection with AROs is associated with increased healthcare costs, length of hospitalization, morbidity and mortality.[3] We studied environmental contamination with MRSA, VRE and ESBL in an old and a new nursing home. The replacement building was commissioned by staff while also working in the old building, occupied by residents later transferred. Although some personal items were transferred to the new building following cleaning, most furniture and equipment was new.

Methods

A number of environmental sites in an occupied nursing home ($N = 18$) and an adjacent new replacement building ($N = 19$) were sampled on three occasions prior to commissioning of the new building (Table I). Sites sampled in the old building included seven sites within a 10-bed dormitory, four sites in a sitting room area and seven sites in an area used exclusively for day care (Table II). Selected sites in the new nursing home included five sites within each of two single rooms (assigned to residents transferring from the dormitory), four sites in the sitting room area and five sites in an area used exclusively for day care (Table II). Sampling of furniture took place before and after

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http://dx.doi.org/10.1016/j.jhin.2012.11.023

Table I

Dates of swabs and significant events

Activity	Dates
Swabbing of old nursing home	22 Aug 2011 to 29 Aug 2011
Swabbing of new nursing home before commissioning	22 Aug 2011 to 24 Aug 2011
Swabbing of new nursing home after staff had entered the new building for training	29 Aug 2011
Tables and chairs put in day-care area[a]	29 Aug 2011 to 05 Sep 2011
Nursing home residents move to new building	5 Sep 2011
Swabbing of new nursing home after residents had moved in	12 Sep 2011 to 21 Nov 2011

[a] All furniture except the tables and chairs were placed in the new nursing home before 22 August 2011.

commissioning with the exception of the tables and chairs in the day-care area of the new building which were not in place until after the commissioning stage (Table I). When commissioning was complete, substantially all of the rooms in the new building were occupied by transfer of residents from the old building.

Sites (10 cm × 10 cm or entire surface of door handles) were swabbed using Copan ESwabs (Copan, Brescia, Italy) (BS ISO 18593:2004). Swabs were inoculated into peptone water overnight. Ten microlitres of peptone water were plated on to chromID™ MRSA, chromID™ ESBL agar and chromID™ VRE (bioMérieux, Marcy-l'Etoile, France) for detection of MRSA, ESBL and VRE respectively.

Identification of suspect isolates of MRSA, VRE and ESBL *E. coli* were by standard methods and susceptibility testing and ESBL confirmation was performed and interpreted by Clinical and Laboratory Standards Institute (CLSI) methods and criteria.[4] Control strains included *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *E. coli* UK strain A, *K. pneumoniae* 700603, *E. coli* ATCC 25922, *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212. Isolates confirmed as ESBL were tested by polymerase chain reaction for $bla_{CTx\text{-}M}$, bla_{TEM}, bla_{SHV} and $bla_{OXA\text{-}1}$ and for the *pab*B region (specific to the O25b:ST131 clone) and *trp*A genes as previously described.[5–7] Amplicons of the $bla_{CTx\text{-}M}$ gene were sequenced.

Results

Environmental contamination with MRSA was detected in the old building in 17 out of 54 (31%) samples from chairs, bedside lockers, bed frames, tables, floors and doors (Table I). VRE and ESBL were not detected. MRSA was first detected in the new building in a common-room area on the third sampling occasion after commissioning had begun. After the transfer of residents, MRSA was detected in 63 out of 147 samples (43%) from all areas. CTX-M-15-producing *E. coli* O25b:ST131 was detected from a toilet seat in the ensuite section of a single room on one occasion. This isolate was also positive for bla_{TEM} and $bla_{OXA\text{-}1}$. VRE was not detected.

Discussion

This study indicates a high frequency in detection of environmental contamination with MRSA. Floors, bed frames and bedside lockers were sites most frequently contaminated (>50% of samples test positive). The finding of MRSA contamination before occupation by residents is new. As MRSA was not detected until commissioning by staff of the new building had commenced, it is likely that healthcare workers and/or transfer of various items contributed to the introduction of MRSA. This highlights the difficulties of avoiding the introduction of contamination when transferring care services from old to new buildings on adjacent sites.

Comparison of our findings with previous studies should take account of differences in methodology. We performed environmental sampling with nylon flocked Copan ESwabs placed in Amies media (Copan) and enriched in peptone water overnight. Previous studies indicated that the Copan ESwab method allows greater recovery of MRSA from environmental surfaces than saline-moistened cotton swabs or rayon swabs.[8]

There are several limitations to the study. First, sampling times were not related to cleaning schedules, although the cleaning products and schedules were comparable in the old and new nursing homes. Each room was cleaned daily using a kitchen sanitizer. A deep clean was performed monthly. Floors were cleaned using a neutral floor cleaner and were wiped using a non-dip system. Infection prevention practice in both the old and new nursing homes is based on the Department of Health guidelines 2011.[9]

Limited recovery of ESBL-PE and the failure to detect VRE may be related to limitations of the detection method used in this study, rather than to the absence of these organisms. As prevalence of carriage of AROs in residents and staff was not established, it is not clear to what extent differences in the frequency of detection of the three AROs is determined by difference in levels of colonization, shedding and persistence in the environment or by differences in sensitivity of detection. However, hospital admission screening and other data indicate

Table II

Detection of meticillin-resistant *Staphylococcus aureus* (MRSA) in old and new nursing homes

Environmental sites	Old occupied nursing home		New unoccupied nursing home		New occupied nursing home	
	No. of tests	No. with MRSA	No. of tests	No. with MRSA	No. of tests	No. with MRSA
Door handles ($N = 92$)	18	1	18	0	56	13
Floor surfaces ($N = 26$)	6	4	6	1	14	11
Tables ($N = 23$)	6	2	3	1	14	5
Bedside lockers ($N = 26$)	6	4	6	0	14	10
Bed frames ($N = 26$)	6	2	6	0	14	11
Toilet seats ($N = 36$)	6	1	9	0	21	7
Arm chairs ($N = 23$)	6	3	3	0	14	6

that both MRSA and ESBL colonization are both frequent occurrences in nursing home residents in this geographical area.[10] The sites selected for monitoring may not have given a comprehensive reflection of the level of environmental contamination of gastrointestinal tract colonizing organisms, such as ESBL-PE and VRE, as we included limited sites in the toilet facility.

Environmental contamination of the new nursing home with MRSA commenced before residents moved in and it rapidly became very widely disseminated. Environmental contamination is likely to play a significant part in persistence and transmission of MRSA within nursing homes.

Conflict of interest statement

None declared.

Funding sources

None.

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Appendix 7: Production of KPC-2 carbapenemase by an *Escherichia coli* clinical Isolate belonging to the international ST131 clone

Antimicrobial Agents and Chemotherapy

Production of KPC-2 Carbapenemase by an Escherichia coli Clinical Isolate Belonging to the International ST131 Clone

Dearbháile Morris, Fiona Boyle, Catherine Ludden, Iris Condon, James Hale, Nuala O'Connell, Lorraine Power, Teck Wee Boo, Hiran Dhanji, Christian Lavallee, Neil Woodford and Martin Cormican

Antimicrob. Agents Chemother. 2011, 55(10):4935. DOI: 10.1128/AAC.05127-11.
Published Ahead of Print 18 July 2011.

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2011, p. 4935–4936 Vol. 55, No. 10
0066-4804/11/$12.00 doi:10.1128/AAC.05127-11
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Production of KPC-2 Carbapenemase by an *Escherichia coli* Clinical Isolate Belonging to the International ST131 Clone[∇]

The rapid international dissemination of *Klebsiella pneumoniae* carbapenemase (KPC)-producing organisms is of major concern. Of the 13 variants of KPC described to date, KPC-2 is the most widely reported and disseminated. KPC-producing isolates of *K. pneumoniae* have reached epidemic proportions in the United States, Israel, and Greece, with increasing reports from other European countries (1, 2, 7, 8).

Escherichia coli O25b:H4-ST131 is a very successful pandemic uropathogenic clone associated predominantly with community-acquired antimicrobial-resistant infection. The close association between the extended-spectrum β-lactamase (ESBL) CTX-M-15 and *E. coli* ST131 has been implicated in the international dissemination of this enzyme (2, 12). We report the KPC-2 carbapenemase in an isolate belonging to the ST131 clone; this has not been reported previously.

E. coli 490995 and *K. pneumoniae* 490995.1 were isolated in April 2010 from the urine of an 84-year-old woman. She was an independently mobile long-term resident of an elderly care facility in the Mid-Western region of Ireland, with no history of foreign travel or admission to hospital in the 6 months prior to isolation of these organisms. She had received four courses of amoxicillin-clavulanic acid, two courses of quinolones (ofloxacin and ciprofloxacin), and two courses of cephalosporins (cefuroxime and cefixime) in the previous 6 months for treatment of lower respiratory tract and urinary tract infections. Screening using rectal swabs at the time of isolation of these organisms and 6 months later did not identify carriage of a carbapenemase producer among the seven other residents sharing her room.

The meropenem, ertapenem, and imipenem MICs for *E. coli* 490995 were 8 µg/ml, >16 µg/ml, and 16 µg/ml, respectively, as determined by agar dilution, and the isolate was susceptible to chloramphenicol, minocycline, amikacin, and kanamycin and resistant to ampicillin, ceftazidime, cefotaxime, cefpodoxime, cefoxitin, aztreonam, amoxicillin-clavulanic acid, piperacillin-tazobactam, tetracycline, sulfonamides, streptomycin, gentamicin, ciprofloxacin, nalidixic acid, and trimethoprim by the CLSI disk diffusion method (4). The meropenem, ertapenem, and imipenem MICs for *K. pneumoniae* 490995.1 were >32 µg/ml, >16 µg/ml, and 64 µg/ml, respectively, and the isolate was susceptible to only 3 of the 19 antimicrobial agents screened: streptomycin, gentamicin, and tetracycline. Both isolates were confirmed as carbapenemase producers by the modified Hodge method of the CLSI, and the KPC enzyme was indicated by a commercial synergy test (Rosco Diagnostica, Taastrup, Denmark). PCR and sequencing confirmed that both isolates harbored bla_{KPC-2}, bla_{TEM-1}, and $bla_{CTX-M-15}$ (6, 13, 14); *K. pneumoniae* 490995.1 additionally harbored bla_{SHV-12}. *E. coli* 490995 was confirmed to belong to the sequence type 131 (ST131) clonal group by PCR (3) and multilocus sequence typing (MLST) (http://mlst.ucc.ie/mlst/dbs/Ecoli). Both isolates harbored four plasmids ranging in size from 7 kb to 190 kb. A 120-kb IncFIIK2 plasmid was common to both isolates and carried bla_{KPC-2} in an isoform "a" Tn*4401* element (5).

The occurrence of an isolate belonging to the pandemic *E. coli* clonal group O25b:H4-ST131 that produces a KPC-type carbapenemase has not been reported previously, although NDM-1 and VIM-1 carbapenemases have recently been reported in this clone (9, 10, 11). Given that carbapenems are vital therapeutic agents for treatment of severe infection and the success with which CTX-M ESBL-producing *E. coli* O25b:H4-ST131 has disseminated throughout the world, the spread of carbapenemases into this clonal group is a cause of serious concern.

(This work was presented in part at the 21st European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Milan, Italy, 7 to 10 May 2011.)

The MLST group at UCC is funded by Science Foundation Ireland under SFI grant code 05/FE1/B882.

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[▽] Published ahead of print on 18 July 2011.

Appendix 8: Detection of OXA-48 Carbapenemase in the pandemic clone *Escherichia coli* O25b:H4-ST131 in the course of investigation of an outbreak of OXA-48-producing *Klebsiella pneumoniae*.

Antimicrobial Agents and Chemotherapy

Detection of OXA-48 Carbapenemase in the Pandemic Clone Escherichia coli O25b:H4-ST131 in the Course of Investigation of an Outbreak of OXA-48-Producing Klebsiella pneumoniae

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Antimicrob. Agents Chemother. 2012, 56(7):4030. DOI: 10.1128/AAC.00638-12.
Published Ahead of Print 7 May 2012.

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LETTER TO THE EDITOR

Detection of OXA-48 Carbapenemase in the Pandemic Clone *Escherichia coli* O25b:H4-ST131 in the Course of Investigation of an Outbreak of OXA-48-Producing *Klebsiella pneumoniae*

Reports of carbapenemase-producing *Enterobacteriaceae* (CPE) have increased dramatically in the past decade. OXA-48 was first described in Turkey in 2008, and outbreaks of OXA-48-producing *Enterobacteriaceae* have since been reported worldwide, including Ireland (3, 12, 16). *Escherichia coli* O25b:H4-ST131 is a very successful uropathogenic clonal group, and its close association with the extended-spectrum β-lactamase (ESBL) CTX-M-15 has been implicated in the dissemination of this enzyme (2, 14). We report, for the first time, OXA-48 carbapenemase in a member of the sequence type 131 (ST131) clonal lineage.

An 81-year-old male patient (patient 1) (Table 1) was admitted to the medical ward in October 2011 with a diagnosis of lower respiratory tract infection. He had multiple comorbidities and had been treated initially with co-amoxiclav and subsequently with piperacillin-tazobactam. A *Klebsiella pneumoniae* isolate (isolate number 31799) resistant to amoxicillin, co-amoxiclav, piperacillin-tazobactam, and ertapenem was isolated from a midstream urine and sputum specimen cultured on day eight after admission. On the basis of a preliminary identification as a probable carbapenem-resistant *K. pneumoniae* isolate, rectal screening of all patients cared for on the same ward was initiated together with measures to control transmission of infection in accordance with draft national guidance (http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCRE/). In the course of investigation, carbapenem-resistant *K. pneumoniae* isolates were identified from six other patients, including patient 2 (Table 1).

Patient 2 was an 82-year-old man admitted in late October 2011 with a diagnosis of health care-associated pneumonia. He was treated empirically with piperacillin-tazobactam for 7 days, as he had recently been discharged from a health care facility (9). He had a history of peripheral vascular disease with gangrene of his right foot and multiple comorbidities. He had a recent lengthy hospital stay (August to October 2011) for management of lower limb soft tissue infection and ischemia. On day 10 of his October admission, he developed a progressive soft tissue infection of his right foot. He was treated with broad-spectrum antibiotics for 10 days and required subsequent forefoot amputation. On day 22, in response to the progressive soft tissue infection, piperacillin-tazobactam treatment was commenced and further debridement of his foot was performed. The rectal swab was taken 5 weeks after admission, and in addition to *K. pneumoniae* (isolate number 110833-3), an *Escherichia coli* (isolate number 110833-1) isolate was found.

Isolate identification was confirmed by Vitek2 (bioMérieux, Hampshire, United Kingdom). Isolates were identified as carbapenemase producers by the modified Hodge method of the Clinical and Laboratory Standards Institute. Results of a commercial synergy test (Rosco Diagnostica, Taastrup, Denmark) were not consistent with a KPC enzyme or metallo-β-lactamase. PCR and sequencing confirmed that *E. coli* 110833-1 harbored bla_{OXA-48}, bla_{TEM-1}, and bla_{OXA-1} (7, 17, 18) and belonged to the ST131 clonal group (4). *E. coli* 110833-1 harbored 2 plasmids of 61 kb and 4 kb (1). All 7 *K. pneumoniae* isolates were indistinguishable by pulsed-field gel electrophoresis (PFGE), and a 61-kb plasmid was detected in all cases (15). Multilocus sequence typing (MLST) was performed according to the method of Diancourt et al. (6) and indicated that all *K. pneumoniae* isolates belonged to ST913. Meropenem and ertapenem MICs for *E. coli* 110833-1 were 0.25 μg/ml and 1.0 μg/ml, respectively, as determined by Etest (AB Biodisk, Solna, Sweden), and the isolate was susceptible to ceftazidime, cefotaxime, cefpodoxime, aztreonam, cefoxitin, amikacin, kana-

Published ahead of print 7 May 2012
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doi:10.1128/AAC.00638-12

TABLE 1 Microbiological and molecular analyses of all OXA-48-producing *Enterobacteriaceae*

Isolate no.	Patient no.	Species	Isolate source	MIC (μg/ml)		Antibiogram[a]	Genes harbored[b]	PFP[c]	MLST result	Plasmid size(s) (kb)
				Meropenem	Ertapenem					
31779	1	*K. pneumoniae*	Urine	0.5	1	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
110833-1	2	*E. coli*	Rectal swab	0.25	1	AAugPtzTeSuCGnNaCipTm	bla_{OXA-48}, bla_{TEM-1}, bla_{OXA-1}	EcI	ST131	61, 4
110833-3	2	*K. pneumoniae*	Rectal swab	1.5	6	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
110758	3	*K. pneumoniae*	Rectal swab	1.5	4	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
110856	4	*K. pneumoniae*	Rectal swab	1.5	24	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
111385	5	*K. pneumoniae*	Rectal swab	1	24	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
111518	6	*K. pneumoniae*	Rectal swab	12	>32	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61
101101	7	*K. pneumoniae*	Rectal swab	4	>32	AAugPtz	bla_{OXA-48}, bla_{SHV}	KpN	ST913	61

[a] A, ampicillin; Cpd, cefpodoxime; Ctx, cefotaxime; Caz, ceftazidime; Fox, cefoxitin; Azt, aztreonam; Aug, amoxicillin-clavulanic acid; Ptz, piperacillin-tazobactam; Na, nalidixic acid; Cip, ciprofloxacin; Gn, gentamicin; K, kanamycin; Amk, amikacin; C, chloramphenicol; S, streptomycin; Su, sulfonamides; T, tetracycline; Tm, trimethoprim; Mn, minocycline.
[b] Based on PCR results.
[c] PFP, pulsed-field profile.

mycin, and streptomycin by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (Table 1) (5).

This is the first report of an isolate belonging to the pandemic *E. coli* clonal group O25b:H4-ST131 that produces an OXA-48 carbapenemase, although KPC-2, NDM-1, and VIM-1 carbapenemases have recently been reported in this group (8, 10, 11, 13). In general, OXA-48 does not confer frank resistance to the carbapenems as defined by current interpretive standards. It is likely that many OXA-48-producing *Enterobacteriaceae* may go unrecognized given the low MIC compounded by the lack of an enzyme inhibitor to facilitate phenotypic detection. The worldwide dissemination of CTX-M-15 is attributed in part to its association with *E. coli* O25b:H4-ST131. As carbapenems are vital therapeutic agents for treatment of severe infection, the introduction of OXA-48 and other carbapenemases into this clonal group is of major concern.

ACKNOWLEDGMENTS

We are grateful to all the staff of the diagnostic laboratories and the clinical areas of the hospital.

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Appendix 9: ‘Wordle’ of Ph.D. thesis