The Spread and Detection of Carbapenemase-Encoding Bacteria

Human-Microbiome-like Correlations and Opportunities for Precision Medicine

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List of abbreviations

AmpC	“AmpC”-gene encoded Beta-lactamases
ATCC	American Type Culture Collection
BLRG	Beta-lactam resistance-genes
C	Cytosine
CEB	Carbapenemase-encoding bacteria
CGE	Center for Genomic Epidemiology
CPM	Carbapenemase
CTX-M	Cefotaxime-resistant beta-lactamase
DALYs	Disability-adjusted life years
DNA	Deoxyribonucleic acid
EEA	European Economic Area
ESBL	Extended-spectrum beta-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
G	Guanine
GES	“Guiana-extended spectrum” beta-lactamase
ICU	Intensive-care unit
IMP	“Active on imipenem” beta-lactamase
KPC	*K. pneumoniae* carbapenemase
LOS	Length of stay in the hospital
MALDI-TOF	Matrix-assisted laser desorption/ionization time of flight
MBL	Metallo-beta-lactamases
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRGN	Multidrug-resistant gram-negtive
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
NDM	“New Delhi” metallo-beta-lactamase
OXA	“Active on oxacillin” beta-lactamase
PBP	Penicillin-binding protein

PCR	Polymerase chain reaction
SHV	“blaSHV”-gene encoded beta-lactamase
TEM	Carbapenemase named after the patient Temoneira
UHB	University Hospital Bonn
VIM	“Verona” integron-encoded metallo-beta-lactamase
VRE	Vancomycin-resistant *Enterococcus*
WGS	Whole-genome sequencing

Units Used:

l	Liter
mg	Milligram
µl	Microliter
µm	Micrometer

1. Introduction

1.1 The Relationship between Bacteria and Humans

Bacteria are ubiquitous single-celled organisms that in the great majority of cases range from 0.2-2 µm in size. They are the most abundant form of life on the planet and thrive in the most diverse environments. Among these we count the inside and the surface of many multicellular organisms such as for example the human body. Bacterial cells are estimated to outnumber the eukaryotic cells of the human body (Sender et al. 2016). However due to their much smaller size they make up less than 1 % of the human body weight. More bacteria live in a human's mouth than there are human beings in the world.

The vast majority of bacteria do not hurt us. Instead many of these organisms are indispensable for our well-being. Among the systems of the human body, the gastrointestinal one hosts the largest amount of bacteria being a comfortable setting with plenty of nutrients available for their sustenance. The relationship is in fact not merely commensal but mutualistic. They help the immune system to correctly develop and function, they break down certain carbohydrates and toxins and aid in the uptake of certain fatty acids (Bry et al. 1996). Other important sites of bacterial colonization are the skin, genital areas and upper respiratory tract with each displaying its unique and variable bacterial micro-environmental flora. The human microbiome encompasses the collective genomes of the microorganisms that reside in and on the human body as well as the microorganisms themselves. Climate, habitat, ethnicity, genetics, nutrition and activity cause a person's microbiome to fluctuate in its diversity and can alter the susceptibility of the host to opportunistic pathogens (Gilbert et al. 2018; Penders 2006).

A small minority of bacteria has the potential to be pathogenic and among these an even smaller amount are obligate pathogens. These damage host cells by interfering with their functions or provoke immune responses that damage host cells. Pathogenic bacteria have been responsible for a tremendous amount of deaths throughout our history on this planet and continue to do so today in many underdeveloped regions of the world. And while the history of all former societies is said to be the history of class struggles (Marx & Engels 1848), even before the emergence of societies the history of mankind has definitely been one of struggle against infectious disease.

That is the reason for which the discovery of antibacterial substances in the early 20th century revolutionized medicine. With the discovery of penicillin early in the twentieth century, treatments of infectious diseases entered the modern era in which antibiotics control and eliminate infections that would otherwise be intractable. To this date many antibiotics have been developed and have been classified into several categories based on their mechanism of action, chemical structure and spectrum of activity. Regrettably, the use of these substances has soon been accompanied by the appearance of resistances against them.

1.2 Resistance to Antibiotics

Bacteria appeared on this planet billions of years ago, so have their skills sharpened due to genomic flexibility at shielding themselves from toxic chemicals. The possibility of recombining genes from different bacterial populations is huge, and it seems that bacteria do not need much time to acquire the genetic resources to thrive in an environment that would otherwise have hindered their growth. Resistance, which is becoming increasingly common, limits therapeutic options, with the result that certain human infections cannot be treated (Sultan et al. 2018).

Antibiotic resistance is ancient and the expected result of the interaction of many organisms with their environment. Many antibiotic compounds are molecules found in nature, produced by certain microorganisms to suppress others and, as such, co-resident bacteria have evolved mechanisms to overcome their action in order to survive. Some organisms are often considered to be intrinsically resistant to one or more antibiotics. However, when discussing the issue of antibiotic resistance, the main focus of the problem is acquired resistance in a bacterial population that was originally susceptible to the antibiotic compound (Munita & Arias 2016). Even the most resistant bacteria can be inhibited or killed by a sufficiently high concentration of an antibiotic, but patients would not be able to tolerate the high concentration required in some cases.

The bacterial species differ greatly in their susceptibility to an antibiotic. For example, most strains of the bacterium *Streptococcus pneumoniae* are inhibited by benzyl penicillin in a concentration of 0.01 mg/l, while the bacterium *Escherichia coli* requires a

concentration of 32-64 mg/l to be inhibited in its growth, a level that cannot be achieved in the human body. This introduces the concept of the minimum inhibitory concentration (MIC) and clinical resistance. Clinical resistance is a complex concept in which the nature of the infecting bacterium, its location in the body, the patient's immune status and the distribution of the antibiotic in the body and its concentration at the site of infection, all interact (Hawkey 1998).

1.2.1 The Global Issue of Growing Antibiotic Resistance

Over several decades, after the introduction of antibiotics into medical practice, to varying degrees, bacteria causing common or severe infections have developed resistance to each new antibiotic coming to market. The advent of multidrug resistance among pathogenic bacteria is imperiling the worth of antibiotics, which have previously transformed medical sciences. Growing antibiotic resistance poses a serious global threat of growing concern to human health and a substantial economic burden to the whole world. WHO considers the growing antimicrobial resistance issue one of the three major public health challenges of the 21st century, leading to rising healthcare costs, extended hospital stays, treatment failures and often death (Dadgostar 2019; Naylor et al. 2019). Firstly in 2013, the World Economic Forum devoted a chapter in its *Global Risk Report* to the growing public health challenge of antimicrobial resistance. In the 2018 report, the forum reported that the issue continued to intensify and during the annual meeting in January 2020 antimicrobial resistance was finally predicted to become the worldwide leading cause of death by 2050 if no action is taken.

Several areas of modern medicine are dependent on the availability of effective antibiotics; chemotherapy for cancer treatment, organ transplantation, hip replacement surgery, intensive care of premature babies and many other activities could not be carried out without effective antibiotics. Indeed, infections caused by multi-resistant bacterial strains are among the main factors influencing morbidity and mortality in patients undergoing these procedures (Prestinaci et al. 2015).

The most significant contribution to genetic selection pressure leading to the emergence of multidrug-resistant bacteria in the environment and of multidrug-resistant bacterial infections in the community have been the excessive use of antibiotics in animal

farming, pets and humans, antibiotics sold over-the-counter, increased international travel, poor sanitation and release of non-metabolized antibiotics or their residues into the environment through manure (Aslam et al. 2018; Michael et al. 2014).

Every year in the European Union (EU) and the European Economic Area (EAA) alone, an estimated 33 000 patients die because of a serious resistant bacterial infection. However only a minority of EU and EEA countries have identified specific funding sources to implement national action plans to tackle the problem and today investments in public health actions are still insufficient.

All age groups are affected by infections with antibiotic-resistant bacteria, although their burden is significantly higher among infants than in any other age group (see Fig. 1). Among adults, the burden increases with age. About a third of the deaths due to infections with antibiotic-resistant bacteria in the EU and EEA were in Italy (Cassini et al. 2019).

Since the late 1990s there has been systematic effort to educate and persuade prescribers of antibiotics to follow evidence-based prescribing, in order to halt antibiotic overuse, and thus the development of antibiotic resistance. This effort is known as antibiotic stewardship and it has continuously evolved since its implementation. However despite robust evidence supporting optimal practice, antibiotic decision-making remains sub-optimal in many settings.

Efforts to develop new antimicrobials have over the past two decades been woefully behind the rapid evolution of resistance genes developing among pathogens. The development of new antibacterial agents has declined significantly in recent decades despite the current demand for new antimicrobial drugs. The costs for research and development of antimicrobial agents have risen to a level that allows low and thus only unattractive profitability of new drug development in the pharmaceutical industry (Cheesman et al. 2017).

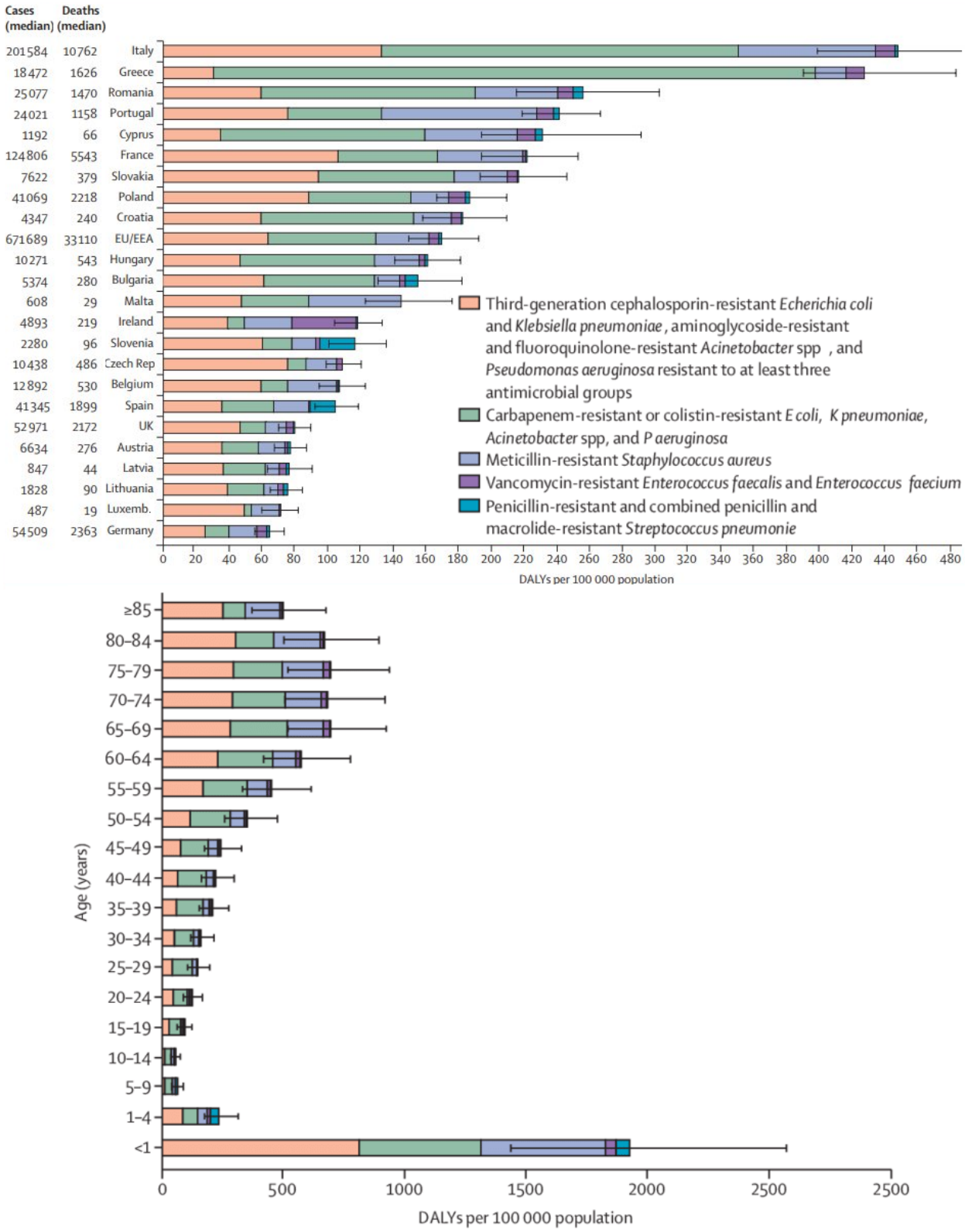


Fig. 1: Model estimates of the burden of infections with antibiotic-resistant bacteria of public health importance in Disability-adjusted life years (DALYs), by country (above) and age group (below), EU and European Economic Area, 2015 (adapted from: Cassini et al. 2019).

1.2.2 The Mechanisms underlying Antibiotic Resistance

The many mechanisms that bacteria use to protect themselves against antibiotics can be divided into a few basic types (see Fig. 2). Some antibiotic resistant bacteria protect themselves by preventing the antibiotic from entering the cell or by pumping it out faster than it can flow in. Other resistant bacteria might instead for example enzymatically degrade or modify the antibacterial substance rendering it inactive. Another mechanism is to stop displaying or altering the structure the antibiotic substance targets (Hawkey 1998).

It is also possible that two or more mechanisms of antibiotic resistance coexist in the same bacteria at a given time producing an additive effect and, often, increasing the levels of resistance. Generally however bacterial species seem to have evolved a preference for some mechanisms of resistance over others. For example the predominant mechanism of resistance to β-lactam antibiotics in gram-positive bacteria is by modifications of their target site, the penicillin-binding proteins, whereas resistance to these compounds in gram-negative organisms is mostly achieved by the production of β-lactamases, enzymes that inactivate these substances. In fact one of the most successful bacterial strategies to cope with the presence of antibiotics is to produce enzymes that inactivate the drug by adding specific chemical moieties to the compound or that destroy the molecule itself, rendering the antibiotic unable to interact with its target. Regardless of the biochemical reaction, the resulting effect is often related to steric hindrance that decreases the avidity of the drug for its target (Munita & Arias 2016).

1.2.3 The Acquisition of Antibiotic Resistance

Acquired resistance occurs when a bacterium that is sensitive to an antibiotic develops resistance. This can happen through mutation or through the acquisition of new DNA. Mutation is a spontaneous event that occurs independently of the presence of antibiotics. In the presence of an antibiotic a bacterium that carries such a mutation conferring resistance has a great advantage because the antibiotic kills the susceptible cells quickly, leaving behind only the resistant subpopulation. Transmissible resistance was first identified in 1959 and occurs mainly by means of plasmids.

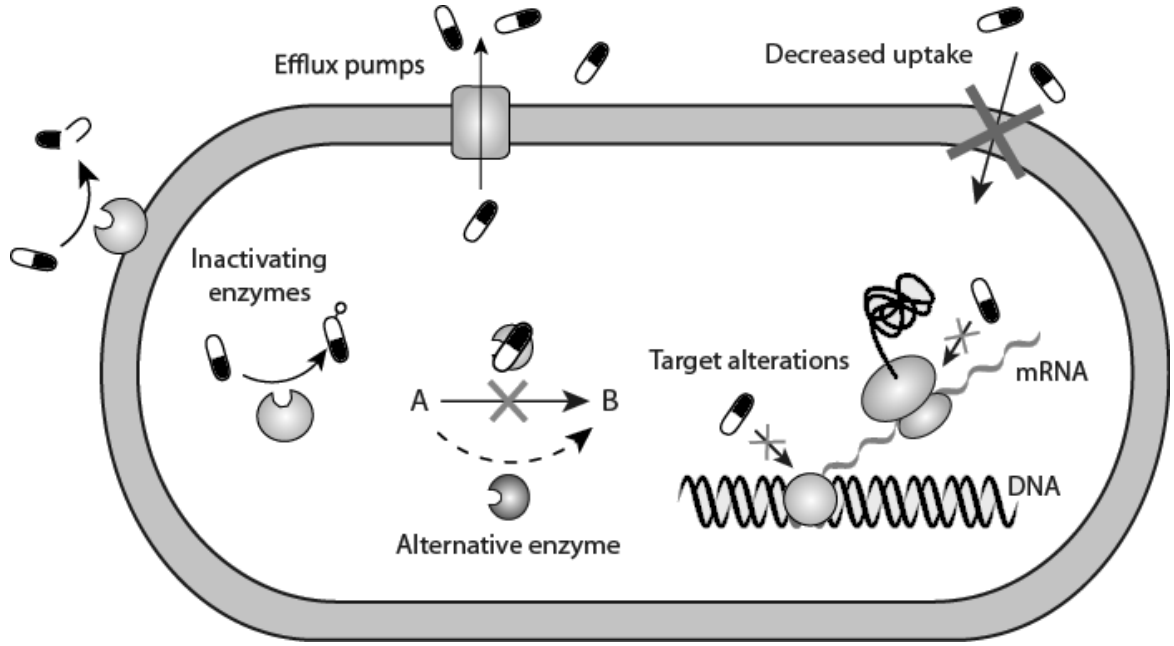


Fig. 2: Different mechanisms conferring antibiotic resistance to a bacterial cell (adapted from: Pal 2017).

Plasmids are self-replicating circular pieces of DNA that are smaller than the bacterial genome. While the chromosomes are big and need to contain all the essential genetic information for living under normal conditions, plasmids usually are very small and only contain additional genes that may be useful in certain situations or conditions. A few types of plasmids can also insert into the host chromosome. Some plasmids have a broad host range and can transfer between different species whereas others have a much narrower host range and are confined to one genus or species (Carattoli 2009). There are also plasmids that have the capability of transferring to a particular host but cannot replicate in the new host or do not replicate well. In these circumstances the plasmid may be lost, however if it contains a resistance gene on a transposon this genetic element can translocate to the bacterial chromosome and be maintained in the absence of the plasmid. Therefore a plasmid does not necessarily need to be maintained in a particular host in order to contribute to the spread of resistance.

Other ways of transmitting resistance are through bacteriophages which are viruses that infect bacteria or through a process known as transformation, in which bacteria capable

of taking up DNA from the environment absorb DNA released by dying bacteria (Hawkey 1998).

1.3 Beta-Lactamases

Beta-lactamases are among the best studied enzymes. They break four-atom rings known as β-lactam rings open by hydrolysis. Beta-lactam rings are part of the core structure of several antibiotic families, the principal ones being the penicillins, cephalosporins, monobactams and carbapenems, which are, therefore, also called β-lactam antibiotics.

1.3.1 Beta-Lactam Antibiotics

Beta-lactam antibiotics are arguably the most important family of microbial natural products ever applied to human medicine. Even now that our reliance upon antibiotics is threatened by the increasing threat of antibiotic resistance, the β-lactam class of compounds, antibiotics that contain a beta-lactam ring in their molecular structure (see Fig. 3) accounts for more than half of all antibiotic prescriptions (Elander 2003).

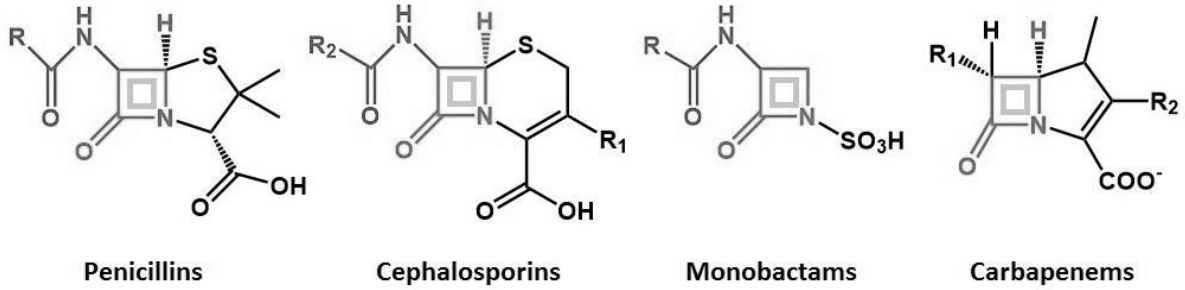


Fig. 3: Core structure of beta-lactam antibiotics (adapted from: Ringbio 2018).

Beta-lactam antibiotics act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. Peptidoglycan is a polymer consisting of sugars and amino acids. It forms a mesh-like layer on the outside of the plasma membrane of most bacteria forging the bacterial cell wall. The final step in the synthesis of the peptidoglycan is facilitated by enzymes called DD-transpeptidases. They form the bonds between oligopeptide

crosslinks in peptidoglycan. For a bacterial cell to reproduce more than a million peptidoglycan subunits must be attached to existing subunits. DD-transpeptidases bind to β-lactam antibiotics because they are similar in chemical structure to the modular pieces that form the peptidoglycan. When bound by the enzyme the β-lactam antibiotic ruptures and forms a covalent bond with a serine amino acid residue at the active site of the enzyme and thereby inactivates it irreversibly. Since these enzymes are the targets of β-lactam antibiotics they are also known as penicillin binding proteins (PBPs). During the terminal stages of cell wall biosynthesis, these enzymes are thus prevented from effecting the cross-linking of peptide chains to form peptidoglycan, resulting in bacterial cell death.

Carbapenems possess potent broad spectrum antibacterial activity and have a unique structure that is defined by a carbapenem coupled to a β-lactam ring (see Fig. 3) which confers protection against most β lactamases. They act against Gram-positive bacteria, Gram-negative bacteria and anaerobes. Consequently, carbapenems are considered one of the most reliable drugs for treating bacterial infections and are preferred over other types of antimicrobials in treating invasive or life-threatening infections (El-Gamal et al. 2017). Carbapenems are administered intravenously with little or no allergic cross reactions. Consequently the emergence and spread of resistance to these antibiotics constitute a major public health concern (Codjoe & Donkor 2017).

1.3.2 The Origins of Beta-Lactamases

The majority of β-lactamases contain an active-site serine that can be bound by β-lactam molecules just like penicillin binding proteins (PBPs). There are in fact structural and mechanistic similarities between the two sets of enzymes. Molecular modeling of various β-lactamases and PBPs structures has demonstrated three-dimensional similarities with conserved folding patterns and preservation of topology at the active site, in spite of low amino acid identities (Smith et al. 2013). It is commonly assumed that PBPs were the precursors of today's β-lactamases. Beta-lactamases are ancient enzymes that existed even in the absence of the pressure of therapeutic antibiotics. Although most of the β-lactam-containing agents in use today are synthetic or semisynthetic molecules, β-lactams as therapeutic agents originate from naturally occurring sources. All these iterations of unmodified β-lactams existed in natural

environments and placed selective pressure on the neighboring bacteria to evade a milieu of deleterious agents. When β-lactam-producing organisms compete with nonproducing bacteria in the same environmental niche, survival strategies are quickly developed (Bush 2018).

In Gram-negative bacteria, β-lactamases have played a critical clinical role and have served as the primary resistance mechanism. As β-lactam resistance began to be more frequently recognized in Gram-negative pathogens, it was shown that many enteric bacteria produced species-specific inducible chromosomal β-lactamases (Matthew & Harris 1976). However, it is the mobile β-lactamases in Gram-negative bacteria that have created a more insidious threat to the β-lactam antibiotics. They became the most prevalent mechanism leading to the emergence of β-lactam resistance among Gram-negative bacteria, with few species barriers existing for their transmission (Bush 2018).

1.3.3 The Classification of Beta-Lactamases

The variety of unique β-lactamases that have been identified in natural isolates now exceeding 2770 does not facilitate the creation of a reliable and easily understandable nomenclature to refer to these enzymes. Two major classification schemes exist for categorizing β-lactamase enzymes, the Ambler and the Bush-Jacoby systems. The Ambler classification scheme divides them into groups A, B, C and D based on their amino acid sequence homology, primarily on their active site. Classes A, C and D enzymes are serine β-lactamases, Class B enzymes are metallo-β-lactamases, which require a bivalent metal ion for activity, usually being a zinc ion. The Bush-Jacoby system instead divides them into group 1, 2, 3 and 4 based on their substrate hydrolysis and inhibitor profile (Bush 2010).

The most widely used classification of β-lactamases to date is the Ambler classification (see Tab. 1) and for the purpose of this thesis it is the one we will focus upon. Ambler originally specified two classes, class A, the active-site serine β-lactamases and class B, the metallo-β-lactamases that require a bivalent metal ion. Later a new class of serine β-lactamases was found that bore little sequence similarity to the then-known class A enzymes which was designated as class C. Later another class of serine β-lactamases, the OXA β-lactamases was found to bear little resemblance to either class A or class C

and was designated class D. The three classes of serine β-lactamases are sufficiently different that alignment programs find no detectable sequence similarity, yet there is sufficient structural similarity among the three classes of serine β-lactamases that it is clear that they are descended from a common ancestor.

Tab. 1: Ambler classification system of beta-lactamases.

Ambler Molecular Class	Type	Hydrolyze	Examples
A	Narrow-spectrum β-lactamases	Penicillins (Narrow-spectrum Cephalosporins)	Penicillinase, TEM, SHV
	Extended-spectrum β-lactamases	Penicillins Cephalosporins Aztreonam	TEM, SHV, CTX-M
	Serine carbapenemases	Penicillins Cephalosporins Carbapenems	**KPC**
B	Metallo-β-lactamases	Penicillins Cephalosporins Carbapenems	**VIM**, IMP, **NDM**
C	Cephalosporinases	Penicillins, Cephalosporins	AmpC,
D	OXA-type enzymes	Penicillins, Cephalosporins, Carbapenems	**OXA**

The class B metallo-β-lactamases instead are a group of enzymes with evolutionary origins that are independent of the common ancestor of the serine-β-lactamases. They hydrolyze β-lactams through an enzymic process that is distinctly different from that of the serine β-lactamases and, furthermore are structurally unrelated to them. However within class B itself there are enzymes that are alignable neither by DNA nor by protein sequence.

1.3.3.1 Ambler Class A Beta-Lactamases

Ambler class A enzymes can be subdivided into class A narrow-spectrum β-lactamases, extended-spectrum β-lactamases (ESBLs), and serine carbapenemases. Class A narrow-spectrum β-lactamases and ESBLs are generally inhibited in by sulbactam,

clavulanate, and tazobactam, three clinically important β-lactamase-inhibitors. They are generally not capable of hydrolyzing carbapenems.

Penicillinase, the first β-lactamase to be identified, is a narrow-spectrum β-lactamase. When penicillin entered clinical use penicillinase production quickly spread to bacteria that previously did not produce it or produced it only rarely (Abraham 1987).TEM and SHV β-lactamases have approximately 200 varieties each. Of these a few only hydrolyze penicillins and early cephalosporins and are hence considered narrow-spectrum β-lactamases whereas the great majority is counted to the extended-spectrum β-lactamases (Jacoby & Munoz-Price 2005). The TEM- and SHV- ESBL enzymes are thought to have evolved later and derive from point mutations from TEM- and SHV-enzymes that were incapable of hydrolyzing broad-spectrum cephalosporins (Bush & Jacoby 2010). TEM- and SHV-ESBLs were predominant in the ESBL landscape over the 1980s and 1990s, mainly associated with outbreaks in hospitals.

CTX-M β-lactamases are characterized by their greater activity against broad-spectrum cephalosporins. They did not reach prominence over the other ESBL enzymes until the first decade of the 2000s when accelerated evolution and extraordinary dispersion of these enzymes were observed. They were confined not only to the hospital setting but also to the community. They are the most common ESBL type worldwide (Cantón et al. 2012; Coque et al.2008).

The newly evolved class A carbapenemases are a major health concern. These enzymes possess the same drug-hydrolyzing activity as ESBLs as well as the additional ability to inactivate carbapenems (Touissant & Gallagher 2015). The most clinically important group of the Class A carbapenemases are the KPC enzymes. These enzymes reside on transmissible plasmids and hydrolyze all beta-lactams at varying rates.

1.3.3.2 Cephalosporinases

Class C enzymes are produced to some degree by virtually all gram-negative organisms and are usually chromosomally mediated, though plasmid-mediated class C β-lactamases exist and can be exchanged by conjugation. They are distinguishable from the common Class A enzymes by the phenotypic resistance to cephalosporins and β-lactam-β-lactamase inhibitor combinations they confer. The overuse of third-generation

cephalosporins has been associated with the selection and promotion of these β-lactamases (Rice & Bonomo 2000).

AmpC β-lactamases are clinically important cephalosporinases that are encoded on the chromosome of many *Enterobacteriaceae*, inducible, and can be expressed at high levels by mutation. Overexpression confers resistance to broad-spectrum cephalosporins and is a problem especially in infections where an isolate initially susceptible to these agents may become resistant upon therapy. AmpC enzymes encoded by both chromosomal and plasmid genes are also evolving to hydrolyze broad-spectrum cephalosporins more efficiently (Jacoby 2009). Hyperproduction of AmpC β-lactamases characteristically provides resistance to many extended-spectrum cephalosporins like ceftazidime, ceftriaxone and cefotaxime. High levels of AmpC production in combination with other mechanisms of resistance, such as porin channel changes, can lead to resistance to carbapenems as well (Touissant & Gallagher 2015).

1.3.3.3 OXA-type enzymes

Class D includes OXA enzymes, most of which are ESBLs and carbapenemases. Class D β-lactamase-mediated resistance to β-lactams has been increasingly reported during the last decade. These enzymes are characterized by an important genetic diversity and a great heterogeneity in terms of β-lactam hydrolysis spectrum. They were defined oxacillinases due to their ability to hydrolyze oxacillin faster than benzyl penicillin. This characteristic does not apply to more recently described members of the family. Class D β-lactamases are usually not inhibited by clavulanic acid, tazobactam, and sulbactam. While many gram-negative species naturally possess genes in their genomes coding for OXAs the genes can as well be acquired (Poirel et al. 2009).

OXA enzymes are given a unique number based on the chronological order in which they were discovered. The first OXA enzymes discovered had a lower specific activity against penicillin and first-generation cephalosporins when compared to oxacillin and methicillin. Their emergence coincided with the widespread introduction of flucloxacillin and methicillin for the treatment of staphylococcal infections. OXA-10 was the first to provide weak hydrolysis of cefotaxime, ceftriaxone, and aztreonam. OXA-11 was the first example of an OXA enzyme that had become, through mutation from OXA-10, an

extended-spectrum β-lactamase. Not much later more ESBLs derived from OXA-10 were discovered (Evans & Amyes 2014).

The emergence of OXA enzymes capable of conferring resistance to carbapenems has transformed these β-lactamases from a minor hindrance into a major problem. The first group of carbapenem-resistant OXA-type β-lactamases to be identified was the OXA-23 group in *Acinetobacter baumannii* isolates. Interestingly this happened not long after the carbapenem imipenem was approved for use. The OXA-23 group consists of OXA-23 as well as many other OXA enzymes that are functionally and or structurally closely related to it. Other important groups found in isolates of *Acinetobacter baumannii* are the OXA-40 group that shows weak activity against cephalosporins and the carbapenems, the OXA-51 group, representing the largest OXA enzyme group, and the OXA-58 and OXA-143 groups. The most important OXA enzymes found in *Enterobacteriaceae* belong to the OXA-48 group (Hamidian & Nigro 2019; Evans & Amyes 2014).

1.3.3.4 Metallo-Beta-Lactamases

Class B enzymes are metallo-β-lactamases (MBLs). They are characterized by one or two zinc ions at the active site. These enzymes are generally not inhibited by the currently available β-lactamase. They have a broad substrate spectrum and can catalyze the hydrolysis of virtually all β-lactam antibiotics with the exception of monobactams. MBLs are clinically the most relevant carbapenemases. Their discovery dates back more than forty years but until recently they were not considered a serious problem for antibiotic therapy because they were only found chromosomally encoded and in non-pathogenic organisms.

MBLs are divided into three subclasses, B1, B2 and B3, based on primary amino acid sequence homology. Subclass B1 contains the largest number of known MBLs and includes the clinically important and transferable IMP-, VIM-, and NDM-type enzymes. IMP-type MBLs were first recognized in the 1990s and currently up to 18 varieties have been identified. VIM enzymes are among the most widely distributed MBLs, with more than 40 reported varieties.The prevalence of NDM enzymes is increasing. They are easily transferable and additionally harbor other genes conferring resistance to other antibiotics (Codjoe & Donkor; Wei et al. 2015; Palzkill 2013).

1.4 Carbapenemase-Encoding Human Pathogens

Carbapenemase-encoding pathogens have spread worldwide, and the Centers for Disease Control and Prevention have categorized these organisms as an “*urgent threat*” due to the high mortality rates among infected patients. The most relevant carbapenemases include the previously discussed KPC, NDM, IMP, VIM, and OXA family enzymes. Bacteria producing these enzymes are frequently resistant to nearly all antibiotics due to additional resistance genes carried on those plasmids.

1.4.1 Clinically Relevant Carbapenemase-Encoding Bacteria

Pathogenic bacteria are those capable of causing disease. Pathogens are generally obligate or opportunistic. Pathogenic bacteria encoding carbapenemases fall in the latter category. The most clinically relevant to date are *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Most other gram-negative bacteria that carry or can acquire carbapenemase-genes are considered clinically less important by themselves due to their lower pathogenicity. However they might pass these resistance-genes to more pathogenic bacteria and should therefore as well be cautiously monitored and kept from spreading.

1.4.1.1 Enterobacterales

Carbapenemase-encoding *Enterobacterales* have been identified as one of the major threats to human health in recent years. The clinically most important families are the *Enterobacteriaceae* and the *Morganellaceae*, to a lesser degree the *Yersiniaceae* and the *Hafniaceae*. The *Enterobacteriaceae* that most frequently encode carbapenemases are members of the genus *Klebsiella*, *Escherichia*, *Enterobacter* and *Citrobacter*.

1.4.1.2 Non-Fermenter

The group of non-fermenter is a taxonomically non-uniform group of aerobic bacteria. These coccoid or rod-shaped bacteria that are non-sporulating are mostly found in soil and wet areas. Over the past decade, non-fermenters have emerged as important opportunistic pathogens in the growing population of patients whose immune systems have been weakened by their disease or medical treatment. Clinically the most important non-fermenter are *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

1.4.2 The Detection of Carbapenemase-Encoding Human Pathogens

Distinguishing carbapenemase-encoding bacteria from those resistant to carbapenems due to other mechanisms is important, as genes encoding for carbapenemases disseminate between patients more readily than the latter. They warrant implementation of more intensive infection control (Magiorakos et al. 2017). Colonization with multidrug resistant bacteria may as the term implies not manifest as a clinically overt infection. The general view has been that the drug-resistant strains are not necessarily more virulent per se, and that the difference in clinical outcome may be attributable to the restricted therapeutic options available for their treatment, failure of empirical treatment, and the delay in instituting effective therapy.

Active surveillance is useful if it is part of a multi-factorial intervention to control multidrug-resistant bacteria given that isolation of carriers is one strategy that can be used to limit the spread of these bacteria and even represents one of the most effective strategies to limit the spread of carbapenemase-encoding bacteria in health care settings (Zhou et al. 2019; Viau et al. 2016; Bhattacharya 2013).

Generally the principal approaches for the detection of antibiotic resistant bacteria in surveillance microbiology samples are culture-based or molecular.

1.4.2.1 Culture-Based Detection of Resistance to Carbapenems

For culture-based methods various samples from appropriate anatomical sites are inoculated onto suitable culture media and incubated at specific temperatures. Following growth after a definite incubation period of generally 24 to 48 hours bacterial colonies are identified by conventional phenotypic microbiological techniques. These include for example culture characteristics, Gram stain, biochemical reactions and MALDI-TOF mass spectrometry. Antibiotic susceptibility confirmation can then be performed using standardized criteria established by reputed organizations such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in our case.

Sensitivity of detection of carbapenemase-resistant gram-negative bacteria depends on several factors including among others the culture methodology and media used, the concentration and type of antibiotics used for screening as well as the technique of

inoculation. The minimum inhibitory concentration guidelines determine whether an antibiotic is susceptible or not. Standard methods for antibiotic susceptibility testing are the disk diffusion method, the micro- and macrodilution, epsilometer testing and automated systems (Khan et al. 2019). Culture-based methods for detecting carbapenemase-encoding organisms are convenient due to their ready availability and low cost, but their limited sensitivity and long turnaround time may not always be optimal for infection control practices (Viau et al. 2016).

The most frequently tested carbapenems in our laboratory are ertapenem, imipenem and meropenem. When gram-negative bacterial isolates show some degree of resistance to carbapenems in one or more of the standard methods for antibiotic susceptibility testing, the isolate is further tested molecularly for the presence of the most common and most important carbapenemase-encoding genes.

1.4.2.2 Molecular Detection of Resistance to Carbapenems

Molecular techniques, such as real-time polymerase chain reaction (PCR) assays are faster in contrast to phenotypic methods that are very time consuming and allow for the quick identification of carbapenemase genes. The PCR is considered as the reference standard for carbapenemase detection, but it requires additional equipment, skilled staff and is not available in many laboratories, especially beyond core working hours. Additionally, only targeted genes can be detected, with new enzyme variants possibly being missed (Baeza et al. 2019). Contemporary nucleic acid amplification techniques or a combined culture and nucleic acid amplification techniques approach may provide fast results and added sensitivity and specificity (Viau et al. 2016).

1.5 Issue Summary and Aim of the Thesis

On a Friday morning, the 28th of September in 1928, Alexander Fleming by accident found a mold inhibiting staphylococcal growth. What he witnessed was nothing less but the ongoing war between microorganisms that had all along been raging behind closed curtains. With his discovery Alexander Fleming did not only pave the way for the purification and production of penicillin and other antibiotics, but he had given humanity

a powerful weapon to finally take arms against an enemy that had tormented mankind since the dawn of time.

Unexpectedly and yet as expected, these foes soon started to respond to this new threat by arming their defense. After all, only the fittest survive in this harsh world.

The introduction of penicillin in 1943 was followed by resistances in 1967 and so did every other antibiotic that was marketed thereafter. The war against an evolving foe will always be one demanding adjustment and enhancement. In our timeline, we humans have reached a point where infectious disease is considerably better controlled than in past centuries with the currently available antibiotics and hygienic measures. Nevertheless, while bacteria continue to evolve crafty mechanisms to survive inside and on the human body even in times of antibiotic exposure, the development of new antibiotics has come to a perceived halt.

Carbapenems play a critically important role in our antibiotic armamentarium and are often referred to as the last resort in the treatment of bacterial strains that display broad antibiotic resistance. Carbapenemases inactivate these reserve antibiotics. These tremendously ominous enzymes are encoded on DNA segments that can in many cases easily be transferred from one bacterium to another and therefore swiftly confer resistance to a whole bacterial population. As if this wasn’t enough, they are in many cases neither species- nor genus-specific and can hence spread at tremendous pace in the presence of selective pressure. To date bacteria carrying genes encoding for carbapenemases cause an unacceptably high number of deaths and complications worldwide.

In this thesis we will deal with the spread of genes encoding for carbapenemases and their detection with the lead question of what the data on detected carbapenemase-encoding bacteria we have collected at the Institute of Medical Microbiology of the University Hospital Bonn during the last six years can teach us by itself and combined with new experimentally acquired data through genotypic typing and an alternatively designed step-by-step diagnostic workflow.

Molecular in-institute tests to scan bacterial isolates for the presence of carbapenemase-encoding genes have been introduced in late 2014 at the Institute of Medical

Microbiology of the University Hospital Bonn. Since then more than 600 isolates from clinical samples tested positive for these genes. We collected the data for the years 2014 to 2019 and linked test results to clinical information and patient history. The first part of the results section deals with the evaluation of this data and works out correlations and causalities between different parameters as well as new insights on variables that may be taken into account when optimizing preventive sanitary precautions and developing safe hospital environments.

Routine cryo-conservation of carbapenemase-encoding isolates gave us access to all isolates under study and enabled us to re-culture selected isolates for whole-genome sequencing purposes. The second part of the result section will deal with the outbreak- and in depth analysis of mentioned isolates

In the last part of the result section we concentrate on an evaluation of the current stepwise diagnostic approach, in which only clinically relevant bacteria displaying phenotypic resistance to carbapenems are genotypically examined for the presence of carbapenemase-encoding genes, as well as possible alternatives to it. For this purpose we collected more than 300 *Enterobacterales* and *Pseudomonas species* isolates, isolated from urine and screening samples, that arrived during a randomly selected week to the microbiology laboratory and genotyped all isolates that would not have been candidates for genotyping.

2. Materials and methods

2.1 Data

We analyzed retrospective data on CEB isolates that could be retrieved from the laboratory and hospital information systems of our institute, which is part of the University Hospital of Bonn, Germany (UHB). The UHB is a tertiary referral and maximum care hospital with 1300 beds. Every year about 50 000 inpatients and 35 000 emergencies are treated and over 350 000 outpatient procedures are provided. The UHB serves mainly German residents but also attracts patients living outside of Germany, mainly in the Arabian Peninsula. Our microbiological diagnostic unit services the University Hospital Bonn and other hospitals in the area and receives an average of 178.000 clinical samples each year.

All CEB isolates from September 2014 till December 2019 were traced in the laboratory information system using species and resistance keywords by one operator. Only first isolates were selected for each pathogen-patient combination. The isolate information was complemented by accessible clinical patient information including gender, age, co-colonization by methicillin-resistant Staphylococcus aureus (MRSA) or vancomycin-resistant Enterococcus (VRE), date of hospital admission, hospitalization stay, oncological or intensive-care ward (ICU) stay, place of residency and ethnicity.

We constructed a database that was password protected and accessible by only 1 operator who ensured that all patient data was delinked from any results other than CEB and was fully de-identified prior to analysis after the establishment of residency and ethnicity. To infer patient ethnicity, we utilized the free version of the validated software tool Onolytics (Version 2020, San Jose, California, US). Onolytics classifies names into 189 cultural ethnic and linguistics groupings. Where available, the results were further controlled for plausibility and accuracy using additional information such as patients' nationality.

The ethics committee of the University Hospital Bonn confirmed that no ethics approval was required for this study.

2.2 Screening and Surveillance Policy

Following German guidelines, multi-resistance of gram-negative rods (MRGN) was defined on the basis of resistance of a pathogen against three (3MRGN) or four (4MRGN) of the following antibiotic groups: acylureidopenicillins, third and fourth generation cephalosporins, carbapenems and fluoroquinolones.

At our UHB and serviced hospitals primary MRGN screening is performed on all patients who had been hospitalized abroad within the past year, on all transfers without current MRGN screening as well as on all patients that were in the same room with a patient with a 4MRGN. Screening and surveillance body sites include the anal region, the inguinal region, the throat as well as wounds if present. Surveillance after admission is ward-dependent but generally performed at least weekly.

Screening and surveillance samples for CEB were routinely cultured by the laboratory on selective ESBL-media, identified via MALDI-TOF MS (VITEK MS, Biomerieux, Marcy-l'Etoile, France) and susceptibility-tested with the VITEK 2 system (Biomerieux, Marcy-l'Etoile, France). Carbapenem-resistant *Enterobacterales*, *A. baumannii* and *P. aeruginosa* or isolates with an unusual carbapenem-susceptibility profile (Ertapenem/ Imipenem/ Meropenem) are routinely genotyped in-institute for the presence of common resistance-genes, using the Allplex Entero-DR (Seegene, Seoul, South Korea) and eazyplex SuperBug Acineto Assays (AmplexBiosystems, Giessen, Germany).

2.3 Statistical Analysis

We used regression techniques to assess the association between age, sex, ethnicity, place of residency, and other parameters with the occurrence and/or type of CEB colonization in patients. This allowed to construct patient and/or hospitalization driven risk profiles. First, we report the average prevalence of different types of CEB in various patient populations. Then, using multivariate regressions, we test whether the uncovered differences between groups persist when we take into account the differentials in observable characteristics (e.g. age, sex, days of hospitalization, etc) among individuals in these groups. To run the regressions we use the statistical software package Stata.

Our main results, showing the relationship between demographic and hospitalization related characteristics with CEB colonization, are shown in Tables B1-B4 in Appendix B. Figures showing the absolute number and share of patients with a certain type of CEB colonization are shown throughout the text. The p-values marked with an asterisk (*) indicated in these Figures, and throughout the text, refer to the significance (at the 0.05, or 0.01 level) of the point estimates obtained in the multivariate regression analysis (shown in Tables B1-B4). To test the robustness of our results, we performed some additional sensitivity analyses. Results from univariate regression analysis (i.e. including only ethnicity or place of residence as independent variables and excluding all other covariates) are shown in Tables B9-B12 in Appendix B. Furthermore, due to the binary nature of the dependent variables (e.g. the prevalence of specific CEB in the patient), we also estimated the same specifications as in the multivariate analysis applying logistic models. The results of this application are shown in Tables B13-B16 in Appendix B. These sensitivity analyses show the same patterns of statistical significance and, hence, confirm our baseline results obtained with multivariate linear models.

2.4 Isolate Selection for the Sensitivity Analysis and Molecular Testing

In the process of evaluating the sensitivity of our step-by-step diagnostic approach when testing gram negative bacteria for the presence of carbapenemases we tested for the length of one randomly selected week all bacteria documented to be able to carry carbapenemases from urine and screening specimens, that would not have been tested for the presence of carbapenemase-encoding genes due to the lack of phenotypically displayed resistance to carbapenems, molecularly for the presence of carbapenemase-encoding genes. These were 359 different bacterial isolates, consisting of 301 isolates belonging to the family of *Enterobacteriaceae*, 38 *P. aeruginosa* isolates, one *A. baumannii* isolate and 19 isolates of other non-fermenters.

The molecular biological detection of carbapenemase-positive strains was performed by real-time Polymerase-Chain-Reaction (PCR) with subsequent melting curve analysis. As for previously tested isolates, carbapenemase-production was evaluated using the

Allplex Entero-DR (Seegene, Seoul, South Korea) and eazyplex SuperBug Acineto Assays (AmplexBiosystems, Giessen, Germany).

2.5 Whole Genome Sequencing and Bioinformatic Methods

Re-cultivation of selected cryo-conserved isolates for whole-genome sequencing purposes occurred twice on on Columbia 5 % sheep blood agar (Becton Dickinson) prior to testing. Highly purified DNA was extracted from all strains using the column-based DNeasy UltraClean Microbial Kit (Qiagen GmbH). The isolation was performed according to the manufacturers instructions with the exception that at the end of the extraction process the DNA was eluted to 100 µl volume. The obtained DNA was qualitatively and quantitatively evaluated using the NanoDrop OneC from Thermo Fisher Scientific Inc.

Dual-indexed Illumina sequencing libraries were constructed from each sample using the NexteraXT kit (Illumina), pooled, and sequenced on the Illumina MiSeq platform. For de novo assembly, paired-end reads were trimmed and filtered with BBDuk Trimmer, a Q value of 20 was chosen. De novo assembly was then performed using Geneious Prime (2020.1 Biomatters, Auckland, New Zealand) and multi-contig draft genomes were generated for each isolate, by Mauve-aligning the contigs to the reference genome sequence NC_014121.1 of *Enterobacter cloacae subsp. cloacae* strain ATCC 13047 and the reference genome sequence NC_016845.1 of *Klebsiella pneumoniae subsp. pneumoniae* strain HS11286, respectively, and subsequent concatenation. Genome analysis was performed with software tools of the CGE Server (Update June 8th 2020, Center for Genomic Epidemiology, DTU, Denmark).

3. Results

3.1 Detected CEB from 2014 to 2019

Between September 2014 and December 31st, 2019, 1917 isolates from 1384 patients had been genotyped with the Allplex Entero-DR and eazyplex SuperBug Acineto Assays (See Tab. 2), and had revealed 301 CEB isolates from UHB patients and 96 CEB isolates from patients of neighboring clinics.

Demographic and clinical summary information for patients with detected CEB are depicted in Tab. 3. Male patients (69.72 %; 221/317) contributed more than twice the amount of CEB that female patients did (30.28 %; 96/317). There were more CEB isolates during June, August and October than during other months. However, clusters were mostly linked to small outbreaks rather than to seasonality.

Scanning multidrug resistant gram-negative bacteria for carbapenemase-production in the Institute for Medical Microbiology of the University Hospital Bonn has been started in the middle of September 2014. In the remaining three and a half months of the year 96 bacterial isolates from clinical samples were tested for carbapenemase-production among which 21 tested positive. 20 patients of which only six were female were shown to be infected and or colonized by carbapenemase-encoding gram-negative bacteria.

In 2015 scanning multidrug resistant gram-negative bacteria for carbapenemase-production was established and performed regularly on clinical isolates that showed suspicious resistance patterns from the very beginning of the year. Three VIM-encoding *P. aeruginosa* were detected in blood cultures. Testing was generally performed at irregular intervals every one to three weeks, resulting in an average duration of 10 (9.8) days until proof of carbapenemase-production from the date of collection of the clinical sample.

Similarly to 2014, in 2015 OXA enzymes were the most prominently detected carbapenemases. In 2016 we witnessed a decrease of nearly 15 % in the amount of isolates that were screened for carbapenemase-genes compared to the previous year and a 7 % decrease in the amount of detected carbapenemases. Testing was now performed every five to fourteen days, resulting in an average duration of eight (8.19)

days until evidence of carbapenemase-production. In five blood cultures four *K. pneumoniae* isolates encoding OXA-48 and one *E. coli* isolate encoding an NDM were detected.

Tab. 2: Number of genotyped isolates and detected CEB each year.

Year	Number of Genotyped Isolates	Number of Detected CEB
2014	96	21
2015	406	88
2016	325	79
2017	309	61
2018	349	56
2019	432	92
Total	1917	397

Tab. 3: Summary demographics for patients with detected CEB isolates.

Demographic parameters	Patients n=317 (%)
Age (years)	
Mean (Min, Max)	56.95 (0, 97)
Sex	
Female	96 (30.28%)
Male	221 (69.72%)
Ethnicity	
German	205 (64.67%)
Arabic	33 (10.41%)
Turkish	15 (4.73%)
Punjabi	13 (4.10%)
Somalian	11 (3.47%)
Kashmiri	4 (1.26%)
Other/Not avaiable	36 (11.36%)
Residency	
Germany	209 (65.93%)
Arabian Peninsula	32 (10.09%)
Other/Not avaiable	76 (23.98%)
Ward	
ICU (but not oncological)	76 (23.98%)
Oncological (but not ICU)	40 (12.62%)
Oncological ICU	13 (4.10%)
Other	188 (59.31%)

In the year of 2017 another decrease in the amount of scanned isolates (6 %) and in the amount of detected carbapenemase-producing isolates (18 %) was witnessed. Average duration until evidence of carbapenemase-production from the date of collection of the clinical sample had not notably changed (7.97 days). No carbapenemase-encoding organism was found in blood cultures in 2017, three were found however in body tissue samples.

The trend of a decrease in detected carbapenemases continued in 2018, the trend of a decrease in the number of scanned isolates did not. In 2018 349 bacterial isolates were scanned for the potential of carbapenemase production and 56 tested positive. More scanned isolates than during the previous two years and fewer detected carbapenemases.

The average duration until evidence of carbapenemase-production from the date of collection of the clinical sample had decreased to 7 days (7.36). From two patients, strains of *K. pneumoniae*, encoding NDM and OXA-48 enzymes each, were isolated in blood cultures. Altogether notable was an increase in gram-negative bacteria encoding NDM enzymes as compared to all previous years. Six bacterial strains encoded both NDM and OXA-48 enzymes.

The number of detected carbapenemases in 2019 reached its highest ever peak. 432 isolates were scanned, more than ever previously, and 92 tested positive. After a steady decline in detections along the years in which multidrug resistant isolates were scanned for the presence of carbapenemase-encoding genes, in 2019 we witness a striking increase. Three carbapenemase-encoding isolates were detected in blood cultures and one in a tissue sample.

Testing was now performed every day from Monday to Friday and occasionally on Saturdays, too. As soon as a suspiciously looking isolate emerged through the antibiotic susceptibility testing the isolate was now tested on the same day. This reduced the time period from the day the sample was taken to the moment the genes encoding for carbapenemases were detected to less than five (4.86) days. Comparing 2019 to the first year in which molecular carbapenemase-testing was routinely performed, 2015, the time to detection halved from 10 to 5 days (see Fig. 4).

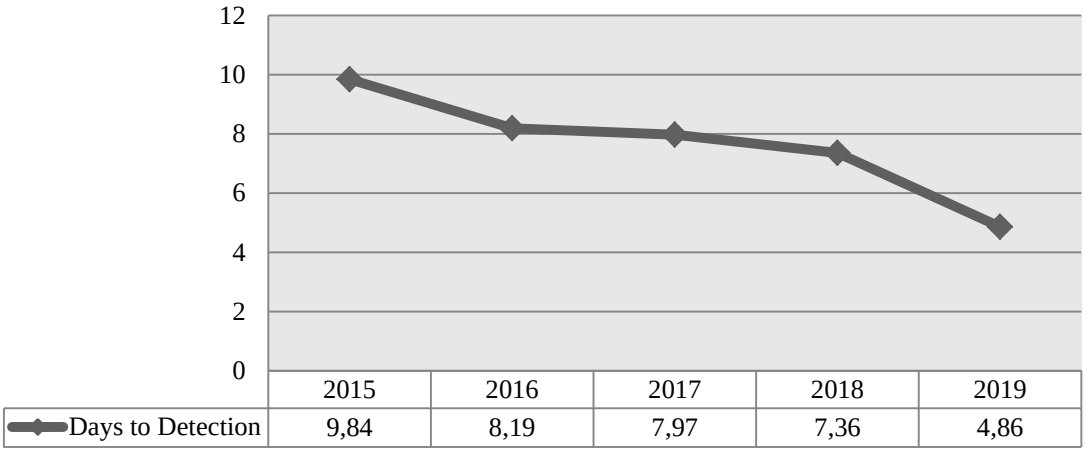


Fig. 4: Average amount of days passed between the day of sample collection and the day of carbapenemase-detection in the years 2015 to 2019.

Examining the months of the year in which carbapenemase-encoding bacteria were detected, we see that during some months there were more cases than during others (see Tab. 4). In 2015 and 2016 we have a higher prevalence of detections during the warmer months (May, June, July, September) and generally in the 5 year period, detections were lowest in February.

Tab. 4: Number of CEB-detections each month in the years 2015 to 2019 and in total. Larger numbers during the years are highlighted in darker shades.

	J	F	M	A	M	J	J	A	S	O	N	D
2015	5	7	5	9	12	15	10	6	11	5	5	5
2016	6	2	9	13	8	11	8	10	3	4	4	4
2017	4	4	3	2	3	9	2	6	13	8	7	4
2018	6	5	4	1	4	4	2	6	2	7	5	10
2019	10	4	2	4	4	3	2	12	5	20	7	5
Total	31	22	23	29	31	42	24	40	34	44	28	28

Detected species and encoded carbapenemases are displayed in Tab. 5. Two hundred and fifty-three patients carried a single CEB species and 64 patients carried multiple CEB species. Of these 64 patients, 51, 11, 1 and 1 patients carried respectively 2, 3, 4 and 5 different CEB, 144 species in total.

Tab. 5: Number and type of carbapenemases the most prevalent CEB species encoded.

Species	Total	KPC	VIM	NDM	OXA23	OXA48	Others
K. pneumoniae	117	12	7	28	2	85	0
Enterobacter cloacae complex	46	11	21	6	0	7	1
E. coli	40	3	9	11	0	18	0
Other *Enterobacterales*	55	4	13	11	0	27	0
P. aeruginosa	74	0	63	5	1	3	4
A. baumannii	58	1	0	5	42	0	12
Total	390	31	113	66	45	140	17

Among patients colonized by only 1 CEB, *P. aeruginosa* made up 26 % (66/253) of all. Among patients that were detected to carry 2 or more CEB, *P. aeruginosa* made up only 8 % (11/142) $p < .01$), and *Enterobacteriaceae* made up more.

The number of KPC, VIM, NDM, OXA-23, and OXA-48 like carbapenemases that had been detected as colonizers fluctuated each year between 2015 and 2019 (see Fig. 5). The majority of the KPC enzymes were detected in 2019 between June and August, due to an outbreak. The source of the outbreak had been timely traced, and no patients developed clinical CEB infections. The outbreak was mainly characterized by a monoclonal *Enterobacter cloacae complex*. In contrast, all KPC-encoding bacterial isolates detected between 2015 and 2018 were *K. pneumoniae* isolates and were detected only sporadically. Of all KPC-encoding isolates, 84 % (26/31) were detected in screening and surveillance samples.

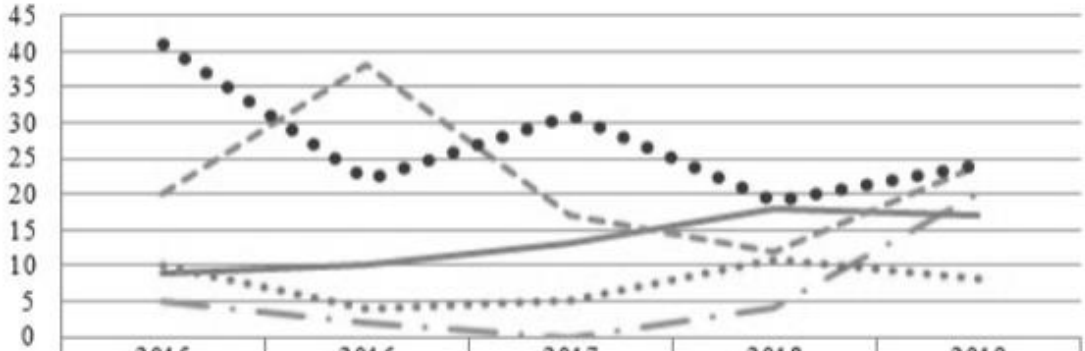
Of VIM-encoding isolates, 31 % (36/116) belonged to oncological and 35 % (40/116) to ICU patients, making them the most frequently detected carbapenemases in both patient populations.

The number of NDM carbapenemases grew steadily until 2018. ICU patients carried 26 % (18/67) of the isolates and oncological patients carried 29 % (20/67).

Numbers of OXA-23 carbapenemases showed only minimal variations, and only 22 % (10/46) of the OXA-23-encoding bacterial strains belonged to female patients.

K. pneumoniae made up 61 % (85/140) of OXA-48-encoding isolates. Of interest was that, every 10th patient colonized by an OXA-48-encoding isolate was colonized by at least two different OXA-48-encoding species, all belonging to the family of *Enterobacteriaceae*.

Sixteen isolates, including 14 *K. pneumoniae* isolates, encoded OXA-48-like carbapenemases in addition to other carbapenemases. 14 % (19/140) of the OXA-48-encoding isolates belonged to oncological, and 24 % (34/140) to ICU patients.



	2015	2016	2017	2018	2019
KPC	5	2	0	4	20
VIM	20	38	17	12	24
NDM	9	10	13	18	17
OXA-23	10	4	5	11	8
OXA-48	41	22	31	19	24

Fig. 5: Number of detected carbapenemases each year between 2015 and 2019 highlighting the overall trend.

3.2 Clinical Specimens

The relative and absolute quantities of carbapenemase-encoding species that were isolated from the respective type of clinical specimen are depicted in Fig. 6. Urine samples made up the majority (104) of all the specimen types in which CEB isolates were detected, followed by inguinal swabs (77), anal swabs (77), stool samples (53), wound swabs (49), throat swabs (41), tracheal secretions (36), and blood cultures (14). Less were found in other clinical specimens like genital swabs, tissue, bile, skin and nose swabs.

One isolate was detected in a urethral swab, one in a sputum sample, one in a prosthetic-joint sonication and one in a bronco-alveolar lavage. Nearly half (46 %, 183/397) of all CEB were detected in screening and surveillance samples.

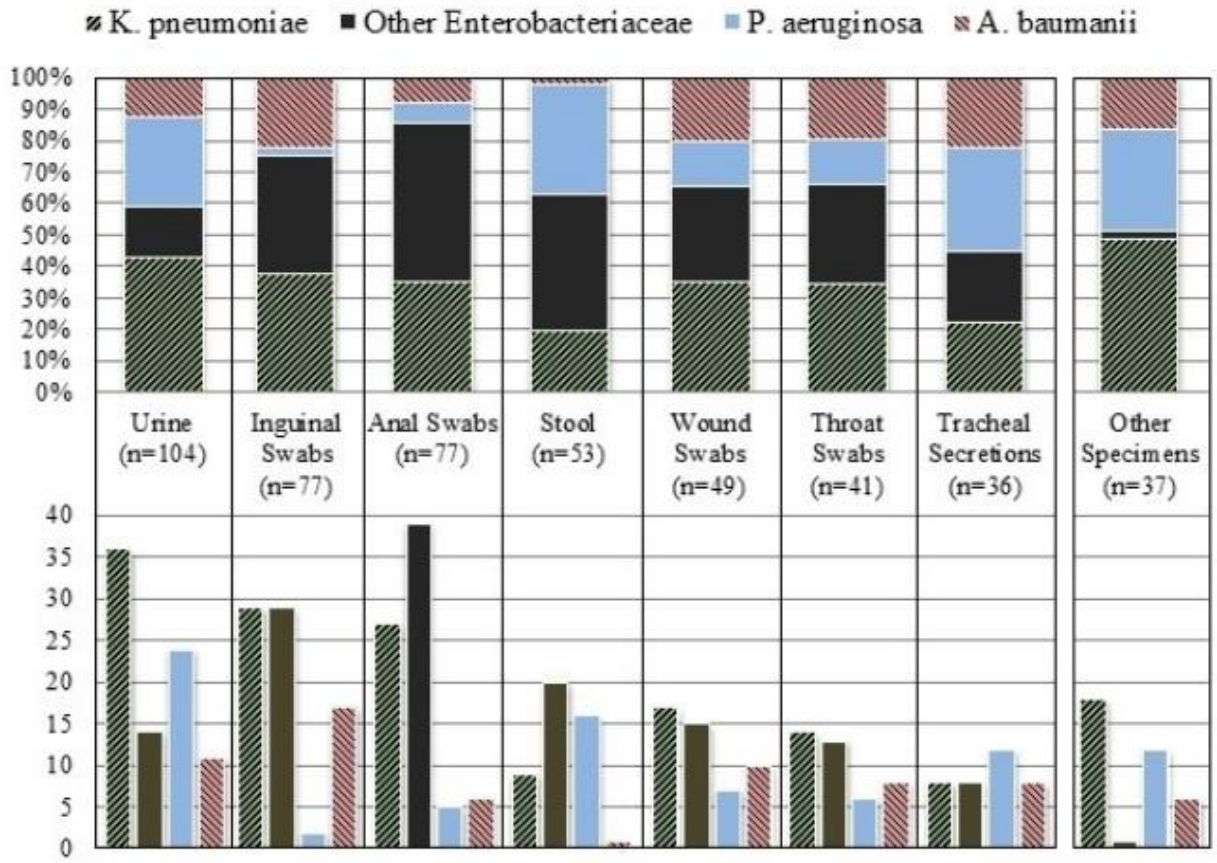


Fig. 6: Relative (above) and absolute (below) quantities of carbapenemase-encoding species that were isolated from the respective type of clinical specimen.

More than 22 thousand urine samples reached the microbiology laboratory each year between 2015 and 2017, more than 25 thousand in 2018 and more than 28 thousand in 2019. Of these approximately 120-thousand samples that arrived since 2015 approximately 330 have been further tested for the presence of a carbapenemase-producing gram-negative organism. Of these 98 tested positive, and only 34 belonged to female patients.

The bacterial species that made up most of the ones detected to carry a carbapenemase-encoding gene was *K. pneumoniae* (36) followed by *P. aeruginosa* (24)

and the most commonly found carbapenemase was OXA-48 (42), followed by carbapenemases of the VIM (31) and NDM type (24).

Anal swabs and stool samples make up a large amount of clinical specimens in which gram negative bacteria with high-resistance profiles are detected. Since the introduction of the molecular test scanning for genes encoding for important carbapenemases slightly more than 400 anal swabs and nearly 200 stool samples have been tested at the molecular level for the presence of bacteria encoding these. Fig. 6 displays an unexpectedly differing spectrum of CEB detected in anal swabs and stool specimens.

Screening materials reach the microbiology laboratory in very large numbers. Beside the previously discussed anal swabs that account for approximately 10 % screening samples consist in throat, nose and inguinal swabs and in a much smaller amount in unspecified swabs or such labeled as skin swabs. More than 70 000 screening specimens are sent to the microbiology lab each year.

Of the specimens that were further tested, approximately 150 were throat swabs, 330 inguinal swabs, 20 skin swabs and 10 nose swabs. Carbapenemases were found in approximately every 4^{th} to 5^{th} sample. In the 330 inguinal swabs 77 carbapenemase-encoding strains were found, in the 150 throat swabs 41, in the 20 skin swabs four and in the 10 nose swabs two. 3.2.4 Tracheal Secretions and Wound Swabs

Since carbapenemase-testing started in the end of 2014, slightly more than 200 tracheal secretions were scanned for the presence of genes encoding for carbapenemases. 36 tracheal secretions were detected to contain carbapenemase-producing bacteria. The bacterial species most frequently found was *P. aeruginosa* (12), all but one carrying a VIM carbapenemase. All *A. baumannii* (8), but one carried an OXA-23 carbapenemase. All *K. pneumoniae* (8), all but one carried an OXA-48 enzyme. The remaining 8 strains were other *Enterobacterales*.

Of the slightly more than 120 wound swabs scanned for carbapenemase-producing bacteria, 49 were proven to contain some, more than one out of three.

Multidrug-resistant gram-negative bacteria are most commonly found in the clinical specimens we previously discussed, be it because those materials often have the

purpose to detect them or be it because of the large quantities they arrive in. Occasionally however, CEB are as well detected in other clinical specimens.

14 CEB isolates have been found in blood cultures since testing began, every year at least one, except in 2017. K. pneumonia was the most important pathogen isolated in these circumstances, mainly carrying an OXA-48 enzyme or additionally an NDM enzyme. Four VIM enzymes were detected; three of them were encoded by *P. aeruginosa*. The remaining isolates were *E. coli* carrying an NDM enzyme and *A. baumannii* carrying an OXA-23 enzyme.

Carbapenemase-producing bacteria were as well detected in five tissue samples that belonged to five male patients. Two isolates were OXA-48-encoding *K. pneumoniae*, two were VIM-encoding *P. aeruginosa* and one was an OXA-23-encoding *A. baumannii*.

Five more carbapenemases were detected in genital swabs among which only one was male. Three OXA-48 encoding *K. pneumoniae* strains were detected in bile.

One *Acinetobacter* with an OXA-23 carbapenemase was detected in a prosthetic joint sonication and one *P. aeruginosa* carrying a VIM enzyme was detected in a bronco-alveolar lavage and another one in a sputum sample.

3.3 Age, Gender and Co-colonization with other Resistant Bacteria

In the year of 2015 two infants below the age of one year were detected to carry carbapenemase-encoding gram-negative bacterial strains. In 2016 one infant below the age of one year was shown to carry three different *Enterobacteriaceae* encoding the same OXA-48 carbapenemase. In 2019 a one year old child was found to carry two NDM-encoding *Enterobacteriaceae*.

Four more different carbapenemases were detected in children below the age of ten years, all but one in 2019. One of the clinical samples was a blood culture. Another four were found in clinical specimens of four young patients aged between ten and 18.

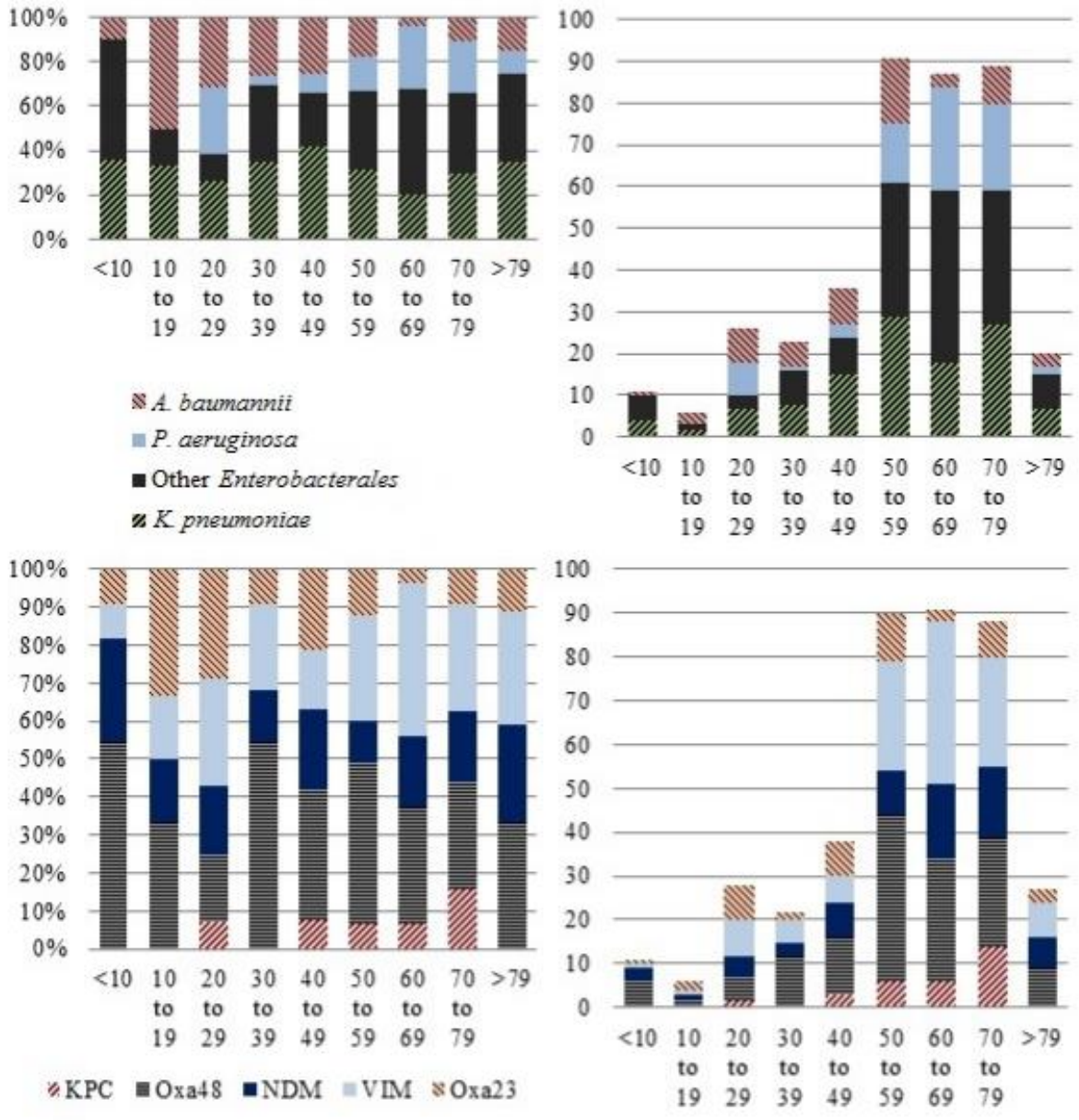


Fig. 7: Relative (left) and absolute (right) quantities of the most clinically relevant species (above) and carbapenemases (below) detected among patients of different age groups.

The total and relative frequency with which certain carbapenemases and species were detected across different age groups is shown in Fig. 7. More than 83 % (265/323) of the patients colonized by CEB were aged 40 or older, 51 % (163/323) were 60 or older. No carbapenemase-encoding *P. aeruginosa* isolates were detected in patients

below the age of 25 (n=25). Among patients aged 25-29 years, including 4 oncological patients, a disproportionately high number (n=8/19) was found.

While there are no gender differences in the average age, we found significant differences in the occurrence of CEB isolates in male and female patients. As the results of the multivariate regressions in Tables B1-B4 show, VIM carbapenemases (p<.05*) and *Citrobacter* isolates (p<.05*) were significantly more common in female patients.

Thirty-four and 67 patients were co-colonized with methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE), respectively, and 5 of these patients with both. There were significantly less women co-colonized with MRSA, 5/34 (14.7 %) (Vs male, p= .04), compared to co-colonization with VRE, 22/67 (32.8 %) (Vs male p= .61).

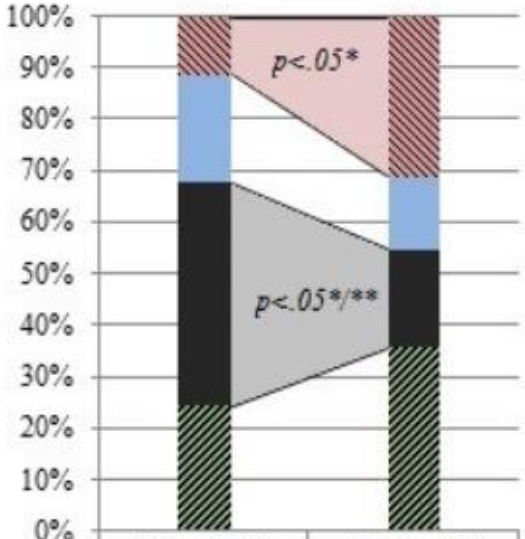
3.4 The Role of Residency and Ethnicity

3.4.1 Country of Residency

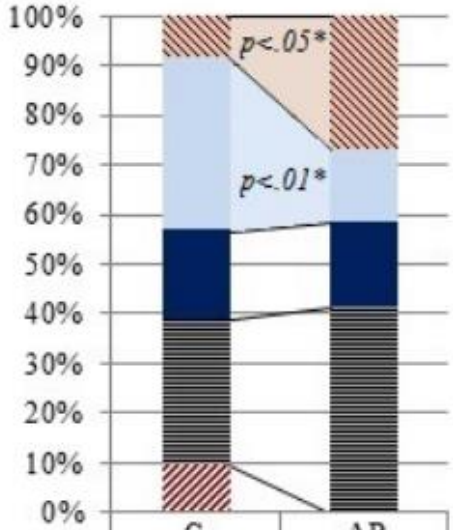
Two groups were considered for residence, patients residing in Germany (G-residents), and patients residing in the Arabian Peninsula (AP-residents) (See Tab. 3). AP-residents colonized with CEB were substantially younger (average age, 39 yrs) compared to G-residents (average age, 61 yrs) ($t = 7.62$, $p < .01$).

Forty-four percent (14/32) of AP-residents were detected to carry CEB on admission, compared to only 23 % (48/209) G-residents ($p = .01$). The relative frequencies of species and carbapenemases among the two groups are displayed in Fig. 8. The indicated p-values derive from the multivariate regressions shown in Tables 8-19 in the Appendix.

Enterobacter isolates ($p < .05$*) and VIM enzymes ($p < .01$*) were more frequent among G-residents, while KPC enzymes were solely detected in patients in this group. In contrast, *A. baumannii* isolates ($p < .05$*) and OXA-23 carbapenemases ($p < .05$*) were more frequent among AP-residents.



	G (n=250)	AP (n=42)
A. baumannii	27	13
P. aeruginosa	51	6
Other *Enterobacterales*	105	8
K. pneumoniae	59	15



	G (n=259)	AP (n=41)
Oxa23	21	11
VIM	91	6
NDM	46	7
Oxa48	76	17
KPC	25	0

* P-values refer to the significance of the point estimates in the regression analysis (see Appendix B)
** P-value refers to Enterobacter isolates

Fig. 8: Species and carbapenemases patients resident in Germany (G) and on the Arabian Peninsula (AP) were detected with.

3.4.2 Ethnicity

The classification of people into ethnic groups is an essential part of studies in a wide range of areas. A common denominator for the vast majority of these studies is the absence of any direct information about the racial or ethnicity of the individual and the need to derive this information from other, readily available parameters. The ethnicity of patients has been retrieved, as explained in Section 2 (see Tab. 3).

Figure 9 shows the relative frequencies of species and carbapenemases for each group. Again, p-values derive from the multivariate analysis. Although we report the uncovered differences among all ethnic groups in our sample, only German and Arabic ethnicity

have a sufficiently high number of observations. Hence, results for the other groups should be red cautiously.

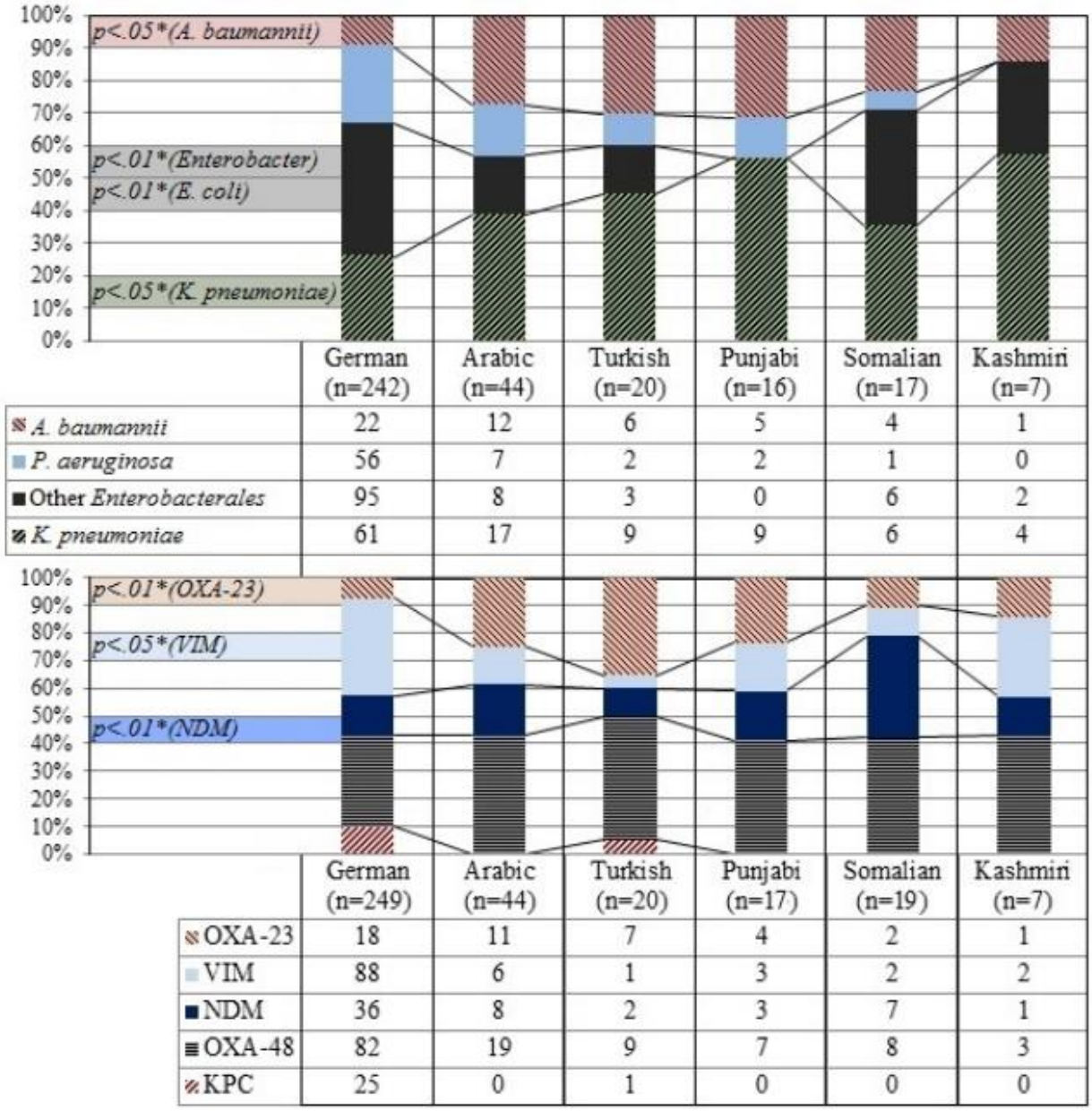
Controlling for all differential characteristics among groups, as age, sex, hospital stay etc., patients of German ethnicity were less frequently colonized by *A. baumannii* than patients of Arabic ethnicity ($p < .05$*), and by *K. pneumoniae* than patients of Punjabi ethnicity ($p < .05$*). Conversely, they were more frequently colonized by *Enterobacter* isolates than patients of Arabic ethnicity ($p < .01$*). VIM enzymes were more frequently detected among patients of German ethnicity rather than among patients of Arabic ($p < .05$*), Somali ($p < .05$*), and Turkish ethnicity ($p < .05$*). On the other hand, patients of German ethnicity were less frequently colonized with bacteria harboring OXA-23 carbapenemases than patients of Arabic ($p < .01$*) and Turkish ethnicity ($p < .01$*) and by bacteria harboring NDM carbapenemases than patients of Somali ethnicity ($p < .01$*).

3.4.3 Residence vs. Ethnicity

We performed two further analyses. Firstly, we compared isolates of patients of German ethnicity residing in Germany with isolates of patients of Arabic, Somali, Turkish, Punjabi, or Kashmiri ethnicity residing in Germany (Tab. 18-19 in the Appendix). Secondly, we compared isolates of patients of Arabic ethnicity residing in Germany with isolates of patients of Arabic ethnicity residing on the Arabian Peninsula (Tab. 15 in the Appendix).

German ethnicity patients residing in Germany were less frequently colonized by OXA-23-encoding isolates compared to Turkish ($p < .05$*) and Arabic ethnicity patients residing in Germany ($p < .05$*), by OXA-48-encoding isolates compared to Somali ethnicity patients residing in Germany ($p < .05$*) and by VIM-encoding isolates compared to Kashmiri ethnicity patients. At the same time, they were detected more frequently with VIM enzymes than patients of Turkish ethnicity residing in Germany ($p < .05$*).

Patients of Arabic ethnicity residing in Germany were more frequently detected with carbapenemase-encoding *E. coli* isolates compared to patients of Arabic ethnicity residing in the Arabian Peninsula ($p < .05$*).



	German (n=242)	Arabic (n=44)	Turkish (n=20)	Punjabi (n=16)	Somalian (n=17)	Kashmiri (n=7)
A. baumannii	22	12	6	5	4	1
P. aeruginosa	56	7	2	2	1	0
Other *Enterobacterales*	95	8	3	0	6	2
K. pneumoniae	61	17	9	9	6	4

	German (n=249)	Arabic (n=44)	Turkish (n=20)	Punjabi (n=17)	Somalian (n=19)	Kashmiri (n=7)
OXA-23	18	11	7	4	2	1
VIM	88	6	1	3	2	2
NDM	36	8	2	3	7	1
OXA-48	82	19	9	7	8	3
KPC	25	0	1	0	0	0

* P-values refer to the significance of the point estimates in the regression analysis, baseline category for Ethnicity is German (see Appendix)

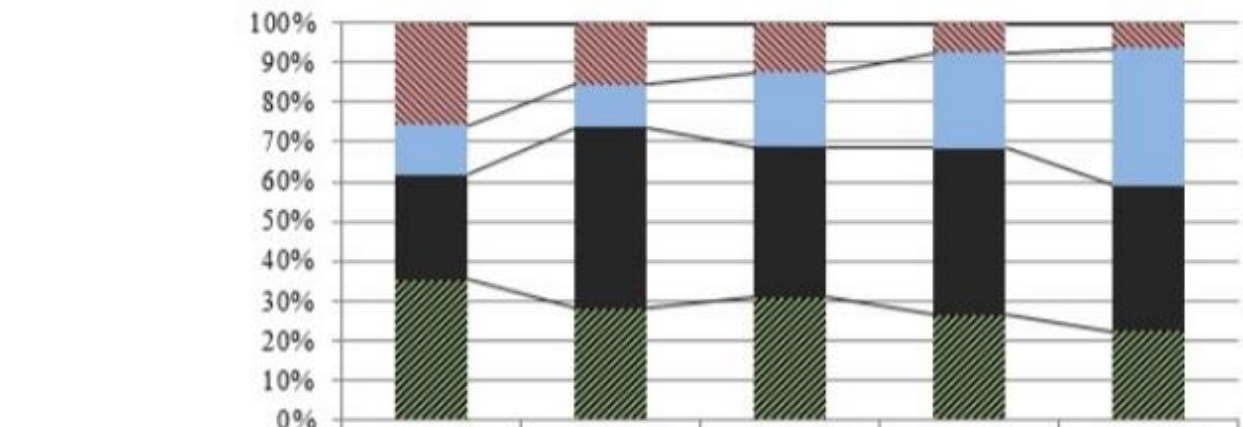
Fig. 9: Summary of patient ethnicity and frequency of species and carbapenemases.

3.5 Introduced vs. Hospital-Acquired

In order to get a picture of which CEB are frequently introduced into the hospital and which ones are more likely hospital-acquired, we studied CEB detection and the length of stay in the hospital (LOS). Fig. 10 displays that carbapenemase-encoding *A.*

baumannii isolates were more frequently detected in patients on hospital admission. Carbapenemase-encoding *P. aeruginosa* isolates on the other hand were more frequently detected with longer LOS. In more than half of the cases *P. aeruginosa* was detected after more than four weeks LOS.

The results of the multivariate analysis confirm the existence of a statistically significant and negative relationship between the LOS and *P. aeruginosa*, *Proteus, Serratia, Klebsiella oxytoca*, and *Klebsiella aerogenes* as well as with OXA-48-like enzymes. *P. aeruginosa* was the most frequently isolated CEB from oncological and ICU patients. Especially oncological patients were colonized significantly more frequently by carbapenemase-encoding *P. aeruginosa* than by *A. baumannii* ($p < .01$).



	0 days	1 to 7 days	8 to 30 days	30 to 90 days	>90 days
A. baumannii	31	7	11	6	3
P. aeruginosa	15	5	17	20	17
Other *Enterobacterales*	32	21	34	35	18
K. pneumoniae	43	13	28	22	11

Fig. 10: Total (below) and relative (above) amounts of species by Length of hospital-stay (LOS).

3.6 Outbreak Analysis

Whole genome sequencing is the process of determining the complete DNA sequence of an organism's genome. The sequencing of the entire genome can provide the raw nucleotide sequence of the DNA of a single organism. For this work ten clinical KPC-encoding *Enterobacter cloacae complex* isolates and four clinical OXA-48 encoding *K.*

pneumoniae isolates from 2019 were whole-genome sequenced by NGS methods. The MLST and OXA Variant data of eight additional OXA-48 encoding *K. pneumoniae* strains from 2019 were available from previous projects in our institute. All tested isolates had an average coverage rate of over 30. Altogether, WGS data of 12 *K. pneumoniae* isolates and 10 *Enterobacter cloacae complex* isolates from 2019 was available.

3.6.1 KPC-encoding *Enterobacter cloacae complex*

The ten *Enterobacter cloacae complex* genomes were scanned with Resfinder (Bortolaia et al. 2020) for known acquired resistance genes. A large number of resistance genes for each isolate was revealed, listed are nevertheless only detected beta-lactamases (see Tab. 6). MLST matching was also performed for the isolates (Larsen et al. 2012). We see that nine of the ten tested isolates are directly related. Four clinical samples in which the KPC-encoding *Enterobacter cloacae complex* isolates were detected came from the same ward (D) and within two weeks. The other five patients had stayed on that ward before being moved to other wards (B,C,E, and F) from which the respective clinical samples came. This is an example of a monoclonal outbreak.

3.6.2 OXA-48-encoding *Klebsiella pneumoniae*

The Four *Klebsiella pneumoniae* genomes were analyzed in regard to their MLST type and their OXA Variant. Additionally genomes were analyzed with Resfinder for known acquired resistance genes. Common to all four genomes were genes encoding for OXA and TEM beta-lactamases. On three genomes SHV enzymes were encoded. One genome encoded an NDM enzyme. Three isolates carried the OXA-48 variant, one the OXA-232 variant. Additionally three OXA-1 and one OXA-9 enzymes were encoded.

Tab. 7 shows all OXA-48-encoding *K. pneumoniae* isolates between January and October 2019, their MLST type, their OXA variant and the wards the clinical samples were collected at.

Tab. 6: Table summarizing sample date, MLS-type, ward and acquired resistance genes conferring decreased susceptibility against beta-lactam class antibiotics of sequenced KPC-encoding *Enterobacter cloacae complex* isolates.

Isolate	Sample Date	MLST	Ward	BLRG
Enterobacter cloacae complex	15.05.2019	165	A	blaTEM-1A ; blaMIR-1, -5, -6 ; blaOXA-9, -10; blaKPC-2
Enterobacter cloacae complex	26.06.2019	419	B	blaCTX-M-9 ; blaTEM-1B ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	30.06.2019	419	C	blaCTX-M-9 ; blaTEM-1B ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	06.07.2019	419	D	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	12.07.2019	419	D	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	14.07.2019	419	B	blaCTX-M-9 ; blaTEM-1B ; blaDHA-12 blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	20.07.2019	419	E	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	21.07.2019	419	D	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	21.07.2019	419	D	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2
Enterobacter cloacae complex	01.08.2019	419	F	blaCTX-M-9 ; blaTEM-1B ; blaACT-14 ; blaOXA-1 ; blaKPC-2

Tab. 7: Table summarizing sample date, MLS-type, ward and OXA variant of sequenced OXA-48-like encoding *Klebsiella pneumoniae* isolates.

Isolate	Sample Date	MLST	OXA Variant	Ward
Klebsiella pneumoniae	02.01.2019	466	48	G
Klebsiella pneumoniae	25.01.2019	101	181/32	H
Klebsiella pneumoniae	25.01.2019	395	48	I
Klebsiella pneumoniae	28.01.2019	16	181/32	J
Klebsiella pneumoniae	20.02.2019	16	181/32	K
Klebsiella pneumoniae	27.02.2019	1198	48	L
Klebsiella pneumoniae	15.04.2019	16	48	M
Klebsiella pneumoniae	13.05.2019	78	48	O
Klebsiella pneumoniae	16.07.2019	48	48	D
Klebsiella pneumoniae	17.08.2019	14	48	P
Klebsiella pneumoniae	16.10.2019	3440	48	B
Klebsiella pneumoniae	25.10.2019	2096	232	L

3.7 Evaluation of Routine-Sensitivity

Lastly we wanted to address the question of the sensitivity of our step-by-step diagnostic approach when screening for carbapenemase-encoding bacteria. Between September 2014 and December 31st, 2019 397 CEB were detected. The question at this point was how many might have been missed.

3.7.1 Routine Diagnostic Workflow for CEB Detection

To evaluate the sensitivity of our step-by-step diagnostic approach in differentiating carbapenemase-encoding gram negative bacteria from gram negative bacteria that do

not carry such genes we designed an experimental setup. Our standard diagnostic workflow is displayed in Fig. 11.

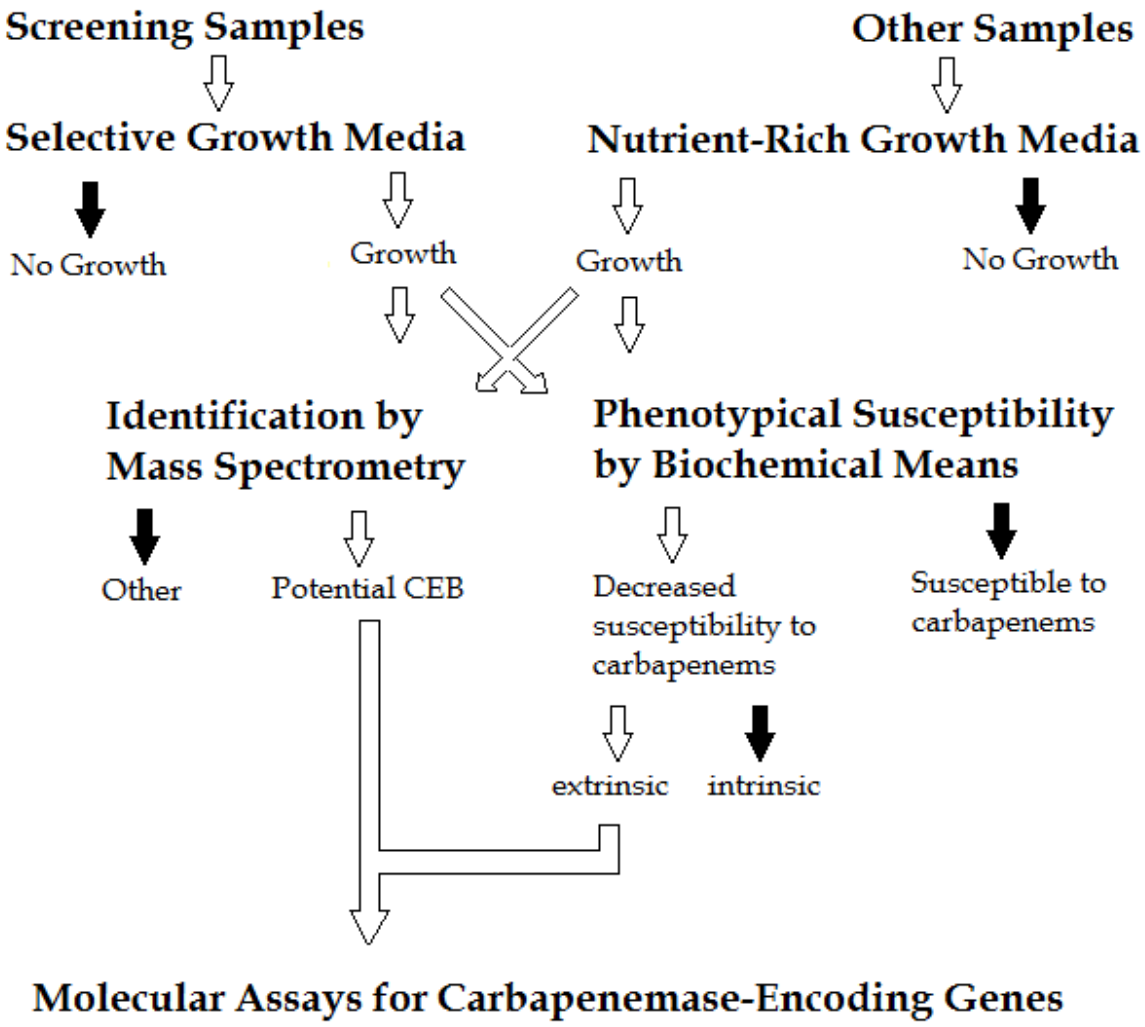


Fig. 11: Routine diagnostic microbiological laboratory workflow in regard to CEB detection.

Screening samples are inoculated onto selective media. Selective media for gram negative bacteria are ESBL type media. If growth occurs, for each morphologically distinct colony, identification by mass spectrometry is carried out, as well as a phenotypical susceptibility profile is determined by biochemical means. If identification reveals bacteria capable of carrying carbapenemase-encoding genes and susceptibility

testing reveals a decreased susceptibility to carbapenems that cannot be traced back to intrinsic resistances, the bacterial culture is brought to the molecular laboratory unit in which genotypic testing occurs. Excluded from molecular testing are also suspiciously looking isolates from patients in which the same species has recently already been detected to carry a carbapenemase.

The molecular assays scan the bacterial genome for the most commonly encountered acquired resistance genes conferring resistance co carbapenems. Hence, the molecular assay is used to differentiate between bacteria that are resistant to carbapenems due to acquired resistance genes, frequently carried on plasmids, and others. Other frequently encountered resistance mechanisms in gram-negative bacteria are the up- or downregulation of certain cell-membrane transporters or porins.

3.7.2 Experimental Setup for CEB Detection

Information we lack is on the sensitivity of our routine diagnostic workflow in detecting CEB. The main question is how many CEB might have been missed during the five year period because of the selection process bacterial isolates undergo before being selected for genotyping. Hence, for the length of one week we collected and genotyped all isolates from screening and urine samples that would otherwise have been excluded from testing.

Weekly approximately 300-400 potential CEB isolates from screening and urine specimens are excluded from molecular testing and only 5-15 included. The bacterial isolates that we collected and genotyped during the course of one week were 359 (see Fig. 12). These would all have been excluded by the routine stepwise approach (see Fig. 11). Five isolates instead would have been included according to the same. Of these five isolates, one isolate turned out to encode an NDM carbapenemase.

The 359 isolates divided as follows: 301 were *Enterobacterales*, 38 *P. aeruginosa*, one *A. baumannii* and 19 other *Pseudomonas* or *Acinetobacter* species. None of these isolates was detected to carry a carbapenemase-encoding gene. One *Acinetobacter pittii* isolate at first tested VIM-positive, but further analyses showed the bacterial culture to be contaminated with an already known VIM-encoding *P. aeruginosa* belonging to the same patient.

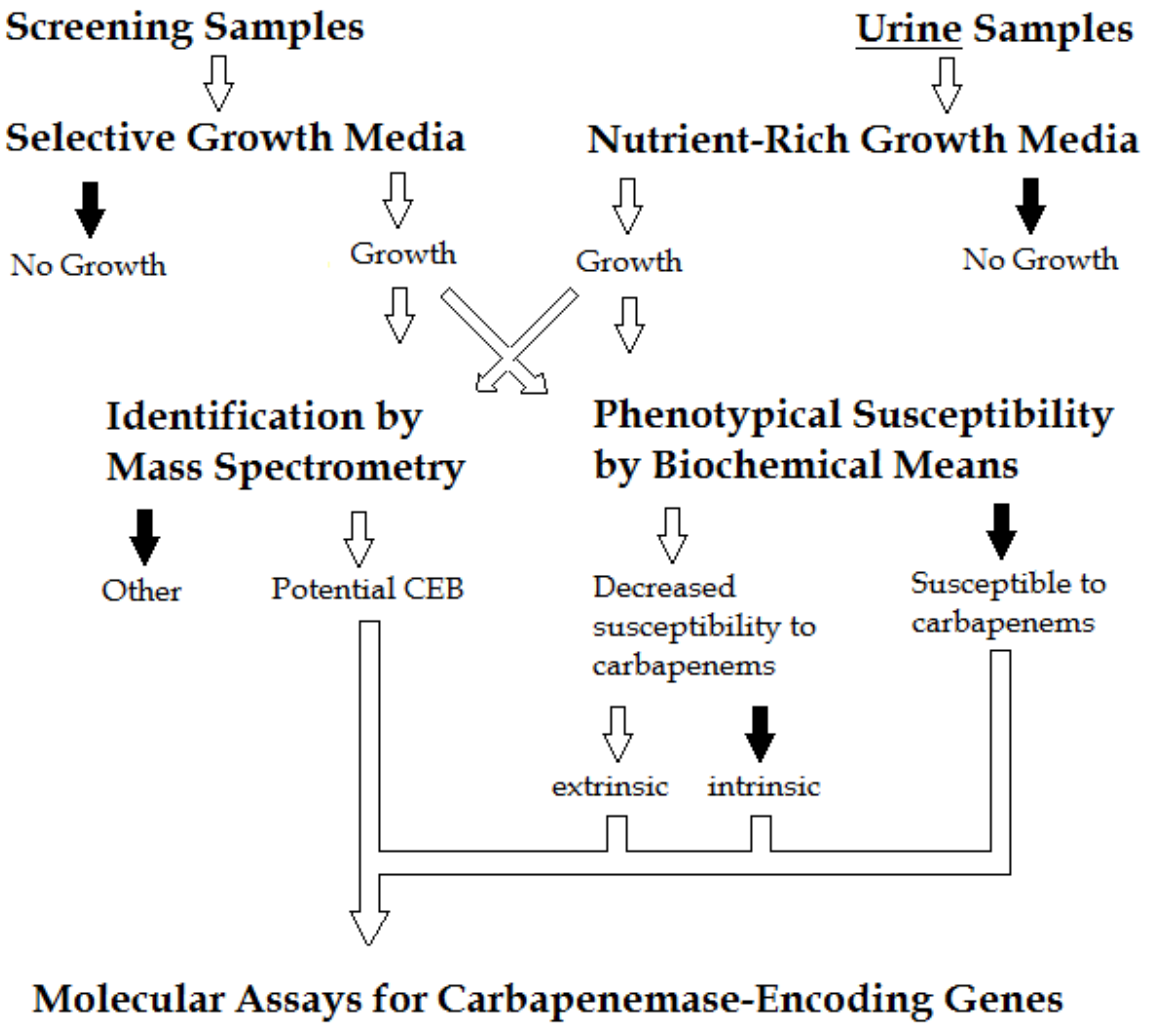


Fig. 12: Experimental workflow designed to maximize sensitivity in regard to CEB detection.

4. Discussion

4.1 The Gender Imbalance

The demographic and clinical summary information for patients with detected CEB (see Table X) gives the impression that male patients (69.72 %; 221/317) are more than twice as likely to be colonized with clinically-relevant CEB compared to female patients (30.28 %; 96/317), based on the assumption that there is no gender imbalance regarding the overall amount of specimens received by our diagnostic unit. We find confirmation on this hypothesis in other studies on multidrug-resistant gram-negative bacteria (GNB) (Nicolas-Chanoine et al. 2019; Kaase et al. 2016).

Methicillin-resistant Staphylococcus aureus (MRSA), but not vancomycin-resistant Enterococcus (VRE), more frequently colonizes male patients (Markwart et al. 2019; Humphreys et al. 2015). The higher incidence of each, CEB and MRSA, in male patients explains the intensified gender imbalance in patients co-colonized by both. Higher MRSA-prevalence among men has been postulated to be linked to hand-hygiene and other behavioral factors as well as to immunological and endocrinological differences (Humphreys et al. 2015). If the same mechanisms apply for the higher occurrence of multidrug resistant gram-negative bacteria in males, too, remains uncertain.

4.2 Age and Length of Hospital-Stay as Risk Factors

Infections with antibiotic-resistant bacteria affect all age groups. Their burden however is significantly higher among infants than in any other age group (Cassini et al. 2019). Among adults, the burden and relative frequency increases with age. The apparent decline in occurrence of CEB after the age of 70 observed in our study (see Fig. 7) is solely due to the decreasing number of people in that age group. Colonization with multidrug-resistant bacteria is generally antibiotic- and healthcare associated (Segagni Lusignani et al. 2020; Nicolas-Chanoine et al. 2019; Logan & Weinstein 2017) and hence more prevalent among the older population.

Nearly one third of CEB detected was detected on hospital admission and can hence safely be assumed to have been introduced to the hospital by patients. Another third was detected within one month of hospital stay and another third after more than a month of hospital stay. In order to get a picture of which CEB are frequently introduced into the hospital and which ones are more likely hospital-acquired, we studied CEB detection and the length of stay in the hospital (LOS).

A. baumannii has been documented to swiftly spread within hospital environments if effective preventive measures are inadequate (Teare et al. 2019). *A. baumannii* mainly being detected in patients on hospital admission, we assume hospital-acquired cases in our study to be low due to effective screening, hygiene and isolation measures (Luebbert et al. 2013). *P. aeruginosa* is documented to predominantly infect critically ill as well as immunosuppressed patients (Tsao et al. 2018, Aloush et al. 2006) and its infection risk increases with longer LOS. Our study confirms these findings. Given its frequent presence in hospital-wastewater, hotspots such as the drains, traps, sinks, faucets and toilets have undergone extensive remodeling. Nevertheless, our results and previous findings of the biofilms in the wastewater networks (Sib et al. 2019) further highlight the need to continuously rethink hospital-built environments for safety (Hopman et al. 2019; Kizny Gordon et al. 2017).

4.3 The Role of Residency, Ethnicity and the Microbiome

The human microbiome is very individual and has been correlated with factors such as ethnicity and geography (Reinheimer et al. 2019; Brooks et al. 2018; Gaulke & Sharpton 2018). Hence, we investigated the presence of certain types of CEB in particular groups within our sample, identified by their ethnicity and country of residency. Our data suggests that besides the place of residence, ethnicity plays a role in terms of frequency with which certain CEB are detected in patients.

Even tough statistically significant in our study, confirmation by further studies is required due to the possibility of bias with smaller sample sizes. If confirmed, global distribution patterns of carbapenemases should be considered with these new insights. A higher likelihood of certain CEB in certain patient groups would offer the possibility to

further tailor screening and diagnostic approaches as well as patient care, aiming for personalized and efficient precision medicine. On the other hand there might currently be patients disadvantaged in healthcare settings because of their ethnicity, OXA-enzymes being the most challenging carbapenemases to detect with routine screening processes (Koroska et al. 2017).

Regarding the differences related to age and CEB colonization between the two residency-groups: Patients resident in foreign countries in need of medical attention coming to Germany for treatment likely more frequently have a long history of hospital stays and antimicrobial therapies which explains the higher amount of patients already colonized with CEB at hospital admission among AP-residents compared to G-residents, even though endemicity might as well play a role to a certain degree (van der Bij & Pitout 2012). The age difference between the two groups may be linked to the more than 15 years higher life expectancy in Germany compared to several countries on the Arabian Peninsula.

4.4 CEB detection

To address the question of how many CEB might have been missed along the years due to the selection process bacterial isolates undergo before being selected for genotyping, we designed an experimental alternative workflow in order to increase sensitivity. In laboratory routine following our step-by-step diagnostic workflow only very small minorities of bacterial isolates that grow from clinical samples are finally tested for acquired resistance-genes to carbapenems. Detection of plasmid-encoded resistance mechanisms is fundamentally important for the containment of multidrug-resistant bacteria, since these resistance mechanism belong to the most easily transferred ones.

The routine workflow in this regard is designed to avoid testing an isolate if the probability of it to carry such genes is not over a certain threshold. This is advantageous under financial aspects and to avoid unnecessary testing. Looking at the numbers of the last five years we see that one isolate in five that reached molecular testing turned out to be a CEB. Hence, probability of an isolate to be a CEB once it reaches molecular testing can on average be assumed to be around 20 % with this workflow in place.

The experimental setup and the altogether 364 tested isolates spoke in favor of the stepwise approach. Keeping costs and workload at a minimum, the only CEB that could have been detected that week was in fact detected. This study would of course have benefited to be continued over courses longer than a week, to paint a brighter picture on the sensitivity of routine diagnostics in detecting CEB. Costs associated to testing and the workload of testing such numerous amounts of isolates however restricted the study length to one week.

Limiting factors are also the carbapenemase-genes the molecular assays scan for. These only include the clinically most important and the most frequent carbapenemase-genes, while many more exist. Also we only collected samples from screening and urine samples and ignored other specimen types that constitute a much smaller fraction of samples CEB are detected in. Further, decision on which isolates are chosen for molecular examination is generally up to the laboratory physician specialized or specializing in microbiology or similarly trained staff. While there are some general guidelines on when to scan or when not to scan an isolate for carriage of a carbapenemase-encoding gene, often there are no categorical imperatives. This of course is a factor that can potentially bias detections along the years since medical staff frequently rotates into and out of the laboratory.

While concluding that we do not have missed any CEB between September 2014 and December 31st, 2019 would probably be wrong, we assume to have detected the vast majority of isolates with common and clinically important carbapenemases. Our Study, hence, gives a good estimation of CEB in the UHB and some surrounding clinics in mentioned period.

Comparing 2019 to the first year in which molecular carbapenemase-testing was routinely performed, 2015, the time to detection halved from 10 to 5 days. A timely detection is desirable but in most instances not crucial. Evidence of carbapenemase-production only in rare occasions leads to a change in antibiotic regimen and isolation precautions. The reason for this is that antibiotic therapy is generally dictated by the phenotypical susceptibilities and isolation precautions are taken as soon as phenotypically multi-resistant bacterial isolates are detected. Only in a small minority of cases pathogens are phenotypically susceptible to carbapenems but carry a

carbapenemase. In even less cases patients are really switched to another substance after carbapenemase-detection.

These cases however exist and for those carbapenemase-testing remains of great importance. The same is valid for isolation precautions. Even though in the majority of cases patients shown to carry a carbapenemase-encoding strain are already isolated in the few cases in which they are not carbapenemase-testing is highly relevant for preventing dissemination. Independent from timely detection, carbapenemase-testing remains vitally important from a research and epidemiological point of view.

4.5 Seasonality, Outbreaks and Trends

That February has fewer days than the other months might bias the observation that during this month detections are lowest on average, but cannot account for the difference by itself. Another potentially biasing parameter is that medical tourists, which are main drivers of the global spread of carbapenemases (Frost 2019; Kracalik et al. 2019; Kaul & Chhina 2010; So et al. 2010) might be more prone to visit Germany for treatment outside of the cold season.

This 5 year period does, nevertheless, not give us a clear clue if infections or colonization with mentioned organisms are generally more likely during certain times of the year. In our case, clusters more likely represented small outbreaks as was the case in October 2019 with KPC-encoding bacteria. This KPC outbreak highlights how explosively KPC-encoded resistance genes can spread in hospital settings despite surveillance and preventive measures.

Understanding the transmission of pathogens by genotyping methods is an important tool for outbreak management. We confirmed the KPC-encoding *Enterobacter cloacae complex* outbreak in 2019 to be of monoclonal origin. Thanks to regular surveillance and thorough microbiological diagnostics the outbreak was timely detected. It was traced back to a sponge with which patients were washed. What facilitated the spread was improper implementation of hospital hygiene regulations in the ward by many new still untrained health personnel. Hygiene schooling and outbreak management quickly

solved the problem and contained the outbreak. No patient developed an infection succeeding colonization by the KPC-encoding *Enterobacter cloacae complex*.

Regarding OXA-48-encoding *K. pneumoniae* isolates in 2019, the large spread of MLST types (10 MLST types with 12 isolates) instead indicates that these infections are not directly related and suggests effective hygiene management in the spread-prevention. A large number of samples within a short period of time with the same MLST type would indicate that these infections are directly related, as was the case with the analyzed KPC-encoding *Enterobacter* isolates.

In our study OXA-48-like and VIM carbapenemases were the first and second most prevalent carbapenemases. However in 2018 NDM-carbapenemases were firstly detected more commonly than VIM-enzymes. The steadily growing number of NDM carbapenemases follows a countrywide trend (RKI 2019). The interchangeable and compatible nature of OXA-48-like enzymes has been reported before (Hamprecht et al. 2019; Pulss et al. 2018). In our study these enzymes were encoded by many *Enterobacterales* and even by *P. aeruginosa*.

K. pneumoniae confirmed its versatility in taking up resistance genes (Navon-Venezia et al. 2017). That *P. aeruginosa* is more frequently the only carbapenemase-encoding species carried by patients compared to *Enterobacteriaceae*, might be caused by the fact that the plasmid-subtypes generally carried by *P. aeruginosa* are less conjugative to other species, whereas plasmid-subtypes encoded by *Enterobacteriaceae* are more readily transferable to other *Enterobacteriaceae* (Sawa et al. 2020; Hamprecht et al. 2019; Pulss et al. 2018).

4.6 CEB Spectrum across Different Specimen Types

The frequency, with which CEB carrying species were detected, differed substantially with specimen type. In our setting, inguinal swabs were the most efficient specimen for detecting carbapenemase-encoding *A. baumannii*, which is most frequently carried at hospital admission. *P. aeruginosa* was more evenly found in the various specimen types, the least efficient recovery was in anal and inguinal swabs. *P. aeruginosa* was

most prominently found in humid specimens like urine, stool and tracheal secretions. The large number of *P. aeruginosa* in stool samples and urine can be biased by a few factors like the way stool and urine samples are collected, that is mostly from the bedpan, the toilet, or plastic cups stored in the bathrooms.

We hypothesize several factors for the different spectrum of CEB detected in anal swabs and stool specimen. In our experience, anal swabs are frequently erroneously collected only swabbing the superficial area surrounding the anal sphincter. Such samples are less representative of the bacterial colonization in the terminal part of the gastrointestinal tract but rather of the skin surrounding the anal sphincter. As such they would be more similar to inguinal swabs in terms of detected species, which very well matches our results; we have a higher relative amount of *A. baumannii* isolates and a higher relative amount of *K. pneumoniae* isolates in comparison to other *Enterobacteriaceae* on the one hand and a much smaller relative amount of *P. aeruginosa* isolates on the other hand.

Secondly and most importantly stool samples are not routinely screened for bacteria that potentially carry carbapenemases but rather those that cause symptoms of gastroenteritis. The only stool samples that are routinely inoculated on growth media on which carbapenemase-encoding organisms reliably grow are stool samples belonging to patients lying on oncological wards. More than 80 % of the stool samples in which carbapenemase-encoding bacteria were detected, belonged to oncological patients.

Worth underlining at this point seems to be the seemingly low sensitivity of stool samples for detecting *A. baumannii* and the seemingly low sensitivity of anal swabs for detecting *P. aeruginosa*. It might therefore be advisable when patient samples are collected for screening purposes that a stool sample and an inguinal swab are collected and sent to the microbiology lab rather than a stool sample alone or the combination of an anal and inguinal swab which is frequently done.

Among all clinical specimens that we received, inguinal swabs were the best for detecting carbapenemase-encoding *A. baumannii*. The amount of *A. baumannii* strains among inguinal swabs was 22 %, among all other samples taken together it was less than 13 %.

Tracheal secretions are obtained by the aspiration of secretion from deep sections of the bronchial tree using a sterile catheter, if possible after changing the tracheal tube. The indication is generally the suspicion of pneumonia in ventilated patients. This specimen is therefore mostly collected in patients with a reduced health condition. Wound swabs are sent in for testing to the microbiology laboratory if a wound infection is suspected or to rule it out. In Germany, superficial bacteriological smears taken for screening examinations in patients with chronic wounds are often taken in daily routine, especially for the detection of multi-resistant pathogens. As in the case of tracheal secretions, wound swabs usually belong to patients with a reduced general condition. CEB detections in these materials are therefore often of even greater significance. Even more significant are CEB detections in blood cultures. The quantity of CEB detected in screening and surveillance samples highlights the importance of screening and surveillance practices (Lübbert et al. 2013) to prevent and contain outbreaks.

5. Summary

We analyzed 397 carbapenemase-encoding isolates that were detected between 2014 and 2019 in our laboratory with the associated demographic and clinical patient information. Two bacterial clusters were whole-genome sequenced to establish pathogen-clonality and analyze the spread-dynamics. Further we designed and tested an experimental workflow to evaluate conclusiveness and validity in transferability of our dataset.

We confirmed a significantly higher prevalence of CEB among men and discovered evidence on a role for both, residency and ethnicity in the type of CEB colonization patients were more likely to be detected with. Residency on the Arabian Peninsula was associated with a higher likelihood of carrying OXA-23-encoding A. baumannii, whereas living in Germany rather predisposed to colonization with other CEB- types such as VIM-encoding Enterobacter cloacae complex. Ethnicity was a determinant variable even among patients within the same area of residency. The way global distribution patterns of carbapenemases are seen and studied may be substantially influenced if further studies confirm ethnicity to be a variable in this regard. Screening patients from countries with a higher prevalence of A. baumannii with more sensitive than routinely used culture-media, and very importantly, with the correct specimen, given that A. baumannii is more readily detected for example in inguinal swabs, may be appropriate. The important role of P. aeruginosa among complications of prolonged hospital stays was confirmed. Patients that were colonized by VIM-encoding P. aeruginosa largely contracted it during their hospital stay; oncological patients were particularly at risk.

Our diagnostic step-by-step workflow was found to be sensitive and effective in detecting CEB. The Outbreak Analysis has highlighted two different common spread patterns and the importance of timely diagnostics and hospital-hygiene intervention. Nevertheless, our study has also shown that even when extensive safety precautions are in place, not all hospital-acquired pathogens can be equally well contained, which prompts to continuously rethink hospital-built environments such as the sinks, faucets and wastewater-network and further optimize all precautions according to risk factors and the spectrum of expected pathogens.

6. Appendix

Tab. 8: Likelihood of colonization with carbapenemase-encoding Species for different Residency – multivariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.acaruginosa b/p-value/CI95
Residency								
Arabian Peninsula	0.136**	-0.027	-0.014	-0.122**	0.095	-0.056	-0.026	0.013
	0.0289	0.5423	0.8012	0.0354	0.2464	0.2548	0.2657	0.8423
	[0.014,0.258]	[-0.112,0.059]	[-0.120,0.093]	[-0.236,-0.008]	[-0.066,0.255]	[-0.152,0.040]	[-0.071,0.020]	[-0.119,0.146]
Other	-0.026	0.021	-0.068*	-0.049	0.122**	0.006	-0.028	0.022
	0.5759	0.5306	0.0981	0.2665	0.0469	0.8671	0.1044	0.6714
	[-0.118,0.066]	[-0.044,0.085]	[-0.148,0.013]	[-0.134,0.037]	[0.002,0.243]	[-0.066,0.078]	[-0.062,0.006]	[-0.078,0.121]
Control variables								
Age	-0.000	-0.000	0.000	0.002	0.001	-0.005	-0.003**	0.005
	0.9613	0.8793	0.8916	0.6454	0.7751	0.1325	0.0290	0.2572
	[-0.008,0.007]	[-0.006,0.005]	[-0.006,0.007]	[-0.005,0.009]	[-0.009,0.011]	[-0.011,0.001]	[-0.006,-0.000]	[-0.003,0.013]
Age × Age	-0.000	0.000	0.000	-0.000	-0.000	0.000	0.000***	-0.000
	0.4668	0.7497	0.8934	0.7263	0.6860	0.1732	0.0097	0.4641
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]
Female	-0.024	-0.053**	0.056*	0.020	0.067	0.002	-0.009	-0.058
	0.5336	0.0495	0.0972	0.5832	0.1882	0.9417	0.5216	0.1641
	[-0.100,0.052]	[-0.106,-0.000]	[-0.010,0.122]	[-0.051,0.091]	[-0.033,0.166]	[-0.057,0.062]	[-0.037,0.019]	[-0.141,0.024]
Oncological:yes	-0.148***	0.033	0.013	-0.016	-0.116*	-0.014	0.071***	0.176***
	0.0031	0.3410	0.7600	0.7346	0.0749	0.7227	0.0001	0.0012
	[-0.245,-0.050]	[-0.035,0.102]	[-0.072,0.098]	[-0.107,0.075]	[-0.245,0.012]	[-0.091,0.063]	[0.035,0.108]	[0.070,0.282]
History of ICU:yes	0.026	0.083*	0.015	0.011	-0.061	-0.041	-0.046*	0.013
	0.7079	0.0917	0.8096	0.8657	0.5064	0.4541	0.0791	0.8603
	[-0.111,0.163]	[-0.013,0.179]	[-0.105,0.135]	[-0.117,0.139]	[-0.242,0.119]	[-0.149,0.067]	[-0.097,0.005]	[-0.136,0.162]
ICU:yes	-0.119*	-0.058	-0.017	0.072	0.046	0.082	0.013	-0.021
	0.0903	0.2413	0.7853	0.2707	0.6159	0.1370	0.6081	0.7839
	[-0.256,0.019]	[-0.154,0.039]	[-0.137,0.103]	[-0.056,0.201]	[-0.135,0.227]	[-0.026,0.190]	[-0.038,0.065]	[-0.170,0.128]
Days hospitalised	-0.001	-0.000	-0.000	-0.000	-0.000	0.001**	0.000	0.001***
	0.1017	0.3897	0.2087	0.6145	0.3989	0.0138	0.8415	0.0078
	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.001]	[0.000,0.001]	[-0.000,0.000]	[0.000,0.002]
Constant	0.332***	0.055	0.071	0.072	0.266*	0.166**	0.077**	-0.039
	0.0018	0.4603	0.4417	0.4687	0.0556	0.0462	0.0495	0.7354
	[0.124,0.539]	[-0.091,0.200]	[-0.110,0.252]	[-0.122,0.265]	[-0.006,0.539]	[0.003,0.329]	[0.000,0.155]	[-0.264,0.187]
Observations	390	390	390	390	390	390	390	390
R^2	0.096	0.022	0.026	0.036	0.045	0.029	0.095	0.080

Notes: Values of b show the estimated coefficients of linear probability models on the likelihood that the respective Species were detected, together with their p-value and confidence interval (95%). Baseline category for Residency is Germany. Statistical significance level * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

Tab. 9: Likelihood of colonization with carbapenemase-encoding Species for different Residency – univariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.aeruginosa b/p-value/CI95
Residency								
Arabian Peninsula	0.202***	-0.040	-0.029	-0.124**	0.117	-0.036	-0.028	-0.061
	0.0006	0.3137	0.5661	0.0198	0.1208	0.4180	0.2021	0.3480
	[0.087,0.316]	[-0.119,0.038]	[-0.127,0.070]	[-0.229,-0.020]	[-0.031,0.265]	[-0.125,0.052]	[-0.071,0.015]	[-0.189,0.067]
Other	0.063	0.003	-0.076**	-0.072*	0.160***	-0.008	-0.028*	-0.042
	0.1188	0.9236	0.0293	0.0530	0.0025	0.8031	0.0677	0.3541
	[-0.016,0.143]	[-0.052,0.057]	[-0.145,-0.008]	[-0.145,0.001]	[0.057,0.263]	[-0.069,0.054]	[-0.058,0.002]	[-0.131,0.047]
Constant	0.108***	0.064***	0.124***	0.148***	0.240***	0.084***	0.028***	0.204***
	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000
	[0.065,0.151]	[0.034,0.094]	[0.087,0.161]	[0.108,0.188]	[0.184,0.296]	[0.051,0.117]	[0.012,0.044]	[0.155,0.253]
Observations	397	397	397	397	397	397	397	397
R^2	0.031	0.003	0.012	0.019	0.025	0.002	0.011	0.004

Notes: Values of b show the estimated coefficients of linear probability models on the likelihood that the respective Species were detected, together with their p-value and confidence interval (95%). Baseline category for Residency is Germany. Statistical significance level * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

Tab. 10: Likelihood of colonization with Carbapenemase-encoding Species for different Ethnicity – multivariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.acaruginosa b/p-value/CI95
Ethnicity								
Arabic	0.155**	-0.041	-0.002	-0.162***	0.105	-0.027	-0.023	-0.005
	0.0104	0.3285	0.9706	0.0040	0.1851	0.5689	0.3155	0.9393
	[0.037,0.274]	[-0.124,0.042]	[-0.106,0.102]	[-0.272,-0.052]	[-0.050,0.260]	[-0.120,0.066]	[-0.067,0.022]	[-0.134,0.124]
Kashmiri	-0.017	-0.073	-0.091	0.004	0.283	0.091	-0.008	-0.189
	0.8962	0.4332	0.4354	0.9764	0.1033	0.3823	0.8755	0.1878
	[-0.278,0.243]	[-0.255,0.110]	[-0.319,0.138]	[-0.238,0.246]	[-0.058,0.624]	[-0.114,0.296]	[-0.105,0.090]	[-0.472,0.093]
Punjabi	0.140	-0.078	-0.059	-0.131	0.292**	-0.072	-0.021	-0.070
	0.1313	0.2276	0.4666	0.1269	0.0163	0.3196	0.5400	0.4881
	[-0.042,0.322]	[-0.206,0.049]	[-0.218,0.100]	[-0.300,0.037]	[0.054,0.530]	[-0.215,0.070]	[-0.089,0.047]	[-0.267,0.127]
Somalian	0.103	-0.076	0.152*	-0.118	0.101	0.063	-0.050	-0.175*
	0.2497	0.2257	0.0518	0.1549	0.3855	0.3662	0.1314	0.0698
	[-0.072,0.277]	[-0.198,0.047]	[-0.001,0.305]	[-0.280,0.045]	[-0.128,0.330]	[-0.074,0.201]	[-0.116,0.015]	[-0.365,0.014]
Turkish	0.132	-0.086	-0.031	-0.137*	0.176	0.054	-0.024	-0.084
	0.1106	0.1367	0.6705	0.0738	0.1038	0.4046	0.4416	0.3475
	[-0.030,0.294]	[-0.200,0.027]	[-0.173,0.111]	[-0.288,0.013]	[-0.036,0.388]	[-0.073,0.181]	[-0.085,0.037]	[-0.259,0.092]
Other	0.025	0.030	0.005	0.034	-0.050	0.078*	-0.025	-0.097
	0.6489	0.4452	0.9169	0.5064	0.4887	0.0727	0.2306	0.1058
	[-0.083,0.133]	[-0.046,0.105]	[-0.090,0.100]	[-0.067,0.135]	[-0.192,0.092]	[-0.007,0.163]	[-0.065,0.016]	[-0.214,0.021]
Control variables								
Age	-0.000	-0.001	0.001	0.001	0.002	-0.004	-0.003**	0.004
	0.9403	0.8084	0.7950	0.6874	0.7094	0.1622	0.0260	0.3138
	[-0.008,0.007]	[-0.006,0.005]	[-0.006,0.007]	[-0.006,0.008]	[-0.008,0.012]	[-0.010,0.002]	[-0.006,-0.000]	[-0.004,0.012]
Age × Age	-0.000	0.000	0.000	-0.000	-0.000	0.000	0.000**	-0.000
	0.5484	0.7621	0.9394	0.7318	0.6741	0.1666	0.0102	0.4251
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]
Female	-0.016	-0.062**	0.056	0.014	0.080	-0.001	-0.008	-0.062
	0.6821	0.0246	0.1034	0.7056	0.1166	0.9674	0.5671	0.1399
	[-0.092,0.061]	[-0.115,-0.008]	[-0.011,0.123]	[-0.057,0.085]	[-0.020,0.180]	[-0.061,0.059]	[-0.037,0.020]	[-0.145,0.021]
Oncological:yes	-0.133***	0.023	0.021	-0.008	-0.132**	-0.019	0.080***	0.168***
	0.0060	0.4965	0.6187	0.8666	0.0374	0.6088	0.0000	0.0014
	[-0.227,-0.038]	[-0.043,0.089]	[-0.062,0.104]	[-0.095,0.080]	[-0.255,-0.008]	[-0.094,0.055]	[0.044,0.115]	[0.065,0.270]
History of ICU:yes	0.054	0.066	0.044	0.013	-0.091	-0.048	-0.037	-0.002
	0.4199	0.1635	0.4497	0.8311	0.3001	0.3646	0.1464	0.9783
	[-0.078,0.186]	[-0.027,0.158]	[-0.071,0.160]	[-0.109,0.136]	[-0.264,0.082]	[-0.152,0.056]	[-0.086,0.013]	[-0.145,0.141]
ICU:yes	-0.122*	-0.058	-0.015	0.077	0.052	0.092*	0.010	-0.035
	0.0818	0.2382	0.8117	0.2384	0.5755	0.0945	0.7109	0.6417
	[-0.260,0.016]	[-0.155,0.039]	[-0.136,0.106]	[-0.051,0.205]	[-0.129,0.232]	[-0.016,0.201]	[-0.042,0.061]	[-0.185,0.114]
Days hospitalised	-0.001	-0.000	-0.000	-0.000	-0.000	0.001***	0.000	0.001***
	0.1405	0.3590	0.2085	0.4794	0.4441	0.0091	0.9681	0.0098
	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.001]	[0.000,0.001]	[-0.000,0.000]	[0.000,0.002]
Constant	0.274***	0.097	0.020	0.088	0.260*	0.128	0.077**	0.055
	0.0089	0.1842	0.8254	0.3638	0.0577	0.1192	0.0489	0.6245
	[0.069,0.479]	[-0.046,0.241]	[-0.159,0.200]	[-0.102,0.278]	[-0.009,0.528]	[-0.033,0.289]	[0.000,0.154]	[-0.167,0.278]
Observations	390	390	390	390	390	390	390	390
R^2	0.105	0.037	0.033	0.061	0.066	0.044	0.097	0.097

Tab. 11: Likelihood Carbapenemases for different Residency – univariate linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Residency									
Arabian Peninsula	0.032	-0.100**	-0.017	0.202***	-0.004	0.101	0.000	-0.008	-0.221***
	0.1507	0.0254	0.7859	0.0001	0.8099	0.2043	1.0000	0.6319	0.0032
	[-0.012,0.075]	[-0.188,-0.012]	[-0.143,0.108]	[0.098,0.305]	[-0.037,0.029]	[-0.055,0.257]	[-0.016,0.016]	[-0.041,0.025]	[-0.368,-0.075]
Other	-0.006	-0.043	-0.022	0.040	0.025**	0.144***	0.010	0.011	-0.183***
	0.6726	0.1688	0.6196	0.2784	0.0346	0.0097	0.1032	0.3431	0.0005
	[-0.037,0.024]	[-0.104,0.018]	[-0.110,0.065]	[-0.032,0.112]	[0.002,0.047]	[0.035,0.252]	[-0.002,0.021]	[-0.012,0.034]	[-0.285,-0.081]
Constant	0.016*	0.100***	0.184***	0.084***	0.004	0.304***	-0.000	0.008	0.364***
	0.0554	0.0000	0.0000	0.0000	0.5259	0.0000	1.0000	0.2070	0.0000
	[-0.000,0.032]	[0.067,0.133]	[0.136,0.232]	[0.045,0.123]	[-0.008,0.016]	[0.245,0.363]	[-0.006,0.006]	[-0.004,0.020]	[0.308,0.420]
Observations	397	397	397	397	397	397	397	397	397
R^2	0.007	0.015	0.001	0.036	0.012	0.018	0.007	0.003	0.043

Notes: Values of b show the estimated coefficients of linear probability models on the likelihood that the respective carbapenemases were detected, together with their p-value and confidence interval (95%). Baseline category for Residency is Germany. Statistical significance level * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

Tab. 12: Likelihood Carbapenemases for different Residency – multivariate linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Residency									
Arabian Peninsula	0.047**	-0.072	-0.013	0.159***	-0.021	0.069	0.000	-0.017	-0.165**
	0.0498	0.1252	0.8445	0.0053	0.2379	0.4186	0.9830	0.3516	0.0352
	[0.000,0.094]	[-0.165,0.020]	[-0.148,0.121]	[0.048,0.271]	[-0.057,0.014]	[-0.098,0.236]	[-0.018,0.018]	[-0.053,0.019]	[-0.319,-0.012]
Other	-0.011	0.008	0.005	-0.021	0.013	0.069	0.009	-0.003	-0.094
	0.5437	0.8135	0.9295	0.6253	0.3517	0.2817	0.2178	0.8122	0.1114
	[-0.046,0.024]	[-0.061,0.078]	[-0.097,0.106]	[-0.105,0.063]	[-0.014,0.039]	[-0.057,0.195]	[-0.005,0.022]	[-0.030,0.024]	[-0.210,0.022]
Control variables									
Age	0.001	0.000	-0.004	-0.001	-0.000	0.001	0.000	0.001	0.003
	0.3234	0.9223	0.3422	0.7710	0.9484	0.9153	0.5152	0.4952	0.5793
	[-0.001,0.004]	[-0.005,0.006]	[-0.012,0.004]	[-0.008,0.006]	[-0.002,0.002]	[-0.010,0.011]	[-0.001,0.002]	[-0.001,0.003]	[-0.007,0.012]
Age × Age	-0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
	0.6617	0.8855	0.2544	0.8578	0.5151	0.7452	0.4806	0.2827	0.8806
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]
Female	0.010	-0.005	0.075*	-0.048	0.013	0.090*	-0.002	0.001	-0.120**
	0.4826	0.8638	0.0780	0.1768	0.2490	0.0906	0.6910	0.9598	0.0143
	[-0.019,0.040]	[-0.063,0.053]	[-0.008,0.159]	[-0.117,0.022]	[-0.009,0.035]	[-0.014,0.194]	[-0.014,0.009]	[-0.022,0.023]	[-0.215,-0.024]
Oncological:yes	0.014	-0.076**	0.140**	-0.109**	-0.012	-0.137**	-0.000	-0.013	0.269***
	0.4583	0.0440	0.0114	0.0174	0.4263	0.0446	0.9516	0.3731	0.0000
	[-0.023,0.052]	[-0.150,-0.002]	[0.032,0.247]	[-0.198,-0.019]	[-0.040,0.017]	[-0.271,-0.003]	[-0.015,0.014]	[-0.042,0.016]	[0.146,0.391]
History of ICU:yes	-0.023	0.203***	0.016	-0.008	-0.005	-0.231**	0.000	-0.013	0.012
	0.3927	0.0002	0.8378	0.8983	0.7992	0.0163	0.9774	0.5361	0.8913
	[-0.076,0.030]	[0.098,0.307]	[-0.136,0.168]	[-0.134,0.118]	[-0.045,0.035]	[-0.420,-0.043]	[-0.020,0.021]	[-0.053,0.028]	[-0.161,0.185]
ICU:yes	0.004	-0.091*	-0.037	-0.038	-0.008	0.115	-0.001	-0.005	0.083
	0.8966	0.0885	0.6317	0.5587	0.6855	0.2314	0.9304	0.8144	0.3483
	[-0.050,0.057]	[-0.195,0.014]	[-0.189,0.115]	[-0.164,0.089]	[-0.048,0.032]	[-0.074,0.304]	[-0.021,0.020]	[-0.045,0.036]	[-0.091,0.256]
Days hospitalised	-0.000	-0.000	-0.000	-0.000	-0.000	0.001*	-0.000	-0.000	0.000
	0.2104	0.9500	0.3338	0.2232	0.7965	0.0662	0.8445	0.5712	0.5725
	[-0.000,0.000]	[-0.001,0.001]	[-0.001,0.000]	[-0.001,0.000]	[-0.000,0.000]	[-0.000,0.002]	[-0.000,0.000]	[-0.000,0.000]	[-0.001,0.001]
Constant	-0.037	0.019	0.205*	0.250**	0.043	0.375**	-0.005	0.023	0.155
	0.3657	0.8144	0.0792	0.0101	0.1627	0.0100	0.7340	0.4615	0.2461
	[-0.117,0.043]	[-0.139,0.177]	[-0.024,0.435]	[0.060,0.440]	[-0.017,0.104]	[0.090,0.659]	[-0.036,0.025]	[-0.038,0.084]	[-0.107,0.416]
Observations	390	390	390	390	390	390	390	390	390
R^2	0.031	0.092	0.035	0.072	0.038	0.052	0.009	0.019	0.113

Tab. 13: Likelihood of colonization with Carbapenemase-encoding Species for different Ethnicity – univariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.aeruginosa b/p-value/CI95
Ethnicity								
Arabic	0.182***	-0.052	0.010	-0.149***	0.134*	-0.025	-0.029	-0.072
	0.0015	0.1868	0.8343	0.0043	0.0697	0.5739	0.1820	0.2562
	[0.070,0.294]	[-0.128,0.025]	[-0.087,0.107]	[-0.251,-0.047]	[-0.011,0.279]	[-0.111,0.062]	[-0.071,0.014]	[-0.197,0.053]
Kashmiri	0.052	-0.074	-0.103	-0.006	0.319*	0.073	-0.029	-0.231
	0.6966	0.4161	0.3715	0.9611	0.0652	0.4815	0.5680	0.1206
	[-0.210,0.314]	[-0.254,0.105]	[-0.330,0.124]	[-0.244,0.232]	[-0.020,0.659]	[-0.130,0.275]	[-0.128,0.071]	[-0.524,0.061]
Punjabi	0.222**	-0.074	-0.103	-0.149*	0.310***	-0.070	-0.029	-0.106
	0.0139	0.2273	0.1846	0.0689	0.0079	0.3119	0.3965	0.2887
	[0.045,0.398]	[-0.195,0.047]	[-0.256,0.050]	[-0.309,0.012]	[0.082,0.539]	[-0.207,0.066]	[-0.096,0.038]	[-0.303,0.091]
Somalian	0.144*	-0.074	0.132*	-0.149*	0.101	0.047	-0.029	-0.173*
	0.0983	0.2143	0.0815	0.0613	0.3728	0.4826	0.3831	0.0771
	[-0.027,0.316]	[-0.192,0.043]	[-0.017,0.281]	[-0.305,0.007]	[-0.121,0.323]	[-0.085,0.180]	[-0.094,0.036]	[-0.364,0.019]
Turkish	0.209**	-0.074	-0.053	-0.149**	0.198*	0.030	-0.029	-0.131
	0.0100	0.1805	0.4472	0.0436	0.0597	0.6345	0.3469	0.1463
	[0.050,0.368]	[-0.183,0.035]	[-0.191,0.084]	[-0.293,-0.004]	[-0.008,0.404]	[-0.093,0.153]	[-0.089,0.031]	[-0.309,0.046]
Other	0.066	0.024	-0.005	0.028	-0.036	0.067	-0.029	-0.114*
	0.2184	0.5197	0.9097	0.5694	0.6005	0.1065	0.1558	0.0578
	[-0.039,0.171]	[-0.049,0.096]	[-0.096,0.086]	[-0.068,0.123]	[-0.173,0.100]	[-0.014,0.148]	[-0.069,0.011]	[-0.231,0.004]
Constant	0.091***	0.074***	0.103***	0.149***	0.252***	0.070***	0.029***	0.231***
	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0007	0.0000
	[0.047,0.135]	[0.044,0.104]	[0.065,0.141]	[0.109,0.189]	[0.195,0.309]	[0.036,0.104]	[0.012,0.046]	[0.182,0.280]
Observations	397	397	397	397	397	397	397	397
R^2	0.050	0.018	0.017	0.043	0.041	0.014	0.011	0.025

Tab. 14: Likelihood of Carbapenemases for different Ethnicity - multivariate linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Ethnicity									
Arabic	0.009	-0.089*	0.057	0.160***	-0.015	0.032	-0.005	0.022	-0.180**
	0.7025	0.0543	0.3913	0.0038	0.3792	0.6973	0.5447	0.2016	0.0178
	[-0.037,0.055]	[-0.179,0.002]	[-0.074,0.187]	[0.052,0.268]	[-0.050,0.019]	[-0.131,0.195]	[-0.023,0.012]	[-0.012,0.057]	[-0.330,-0.031]
Kashmiri	-0.018	-0.104	0.035	0.030	-0.016	0.037	-0.007	-0.009	0.006
	0.7347	0.3017	0.8128	0.8058	0.6715	0.8391	0.7091	0.8131	0.9734
	[-0.119,0.084]	[-0.303,0.094]	[-0.252,0.321]	[-0.208,0.267]	[-0.092,0.060]	[-0.321,0.395]	[-0.046,0.032]	[-0.085,0.067]	[-0.322,0.333]
Punjabi	-0.010	-0.070	0.105	0.117	0.044	0.028	-0.009	-0.009	-0.116
	0.7853	0.3223	0.3041	0.1645	0.1056	0.8271	0.5327	0.7374	0.3192
	[-0.081,0.061]	[-0.209,0.069]	[-0.095,0.305]	[-0.048,0.283]	[-0.009,0.097]	[-0.222,0.278]	[-0.036,0.019]	[-0.062,0.044]	[-0.344,0.113]
Somalian	-0.023	-0.039	0.257***	0.021	0.043*	0.088	-0.007	-0.006	-0.232**
	0.5105	0.5652	0.0091	0.7995	0.0964	0.4703	0.6115	0.8203	0.0389
	[-0.091,0.045]	[-0.172,0.094]	[0.064,0.449]	[-0.139,0.180]	[-0.008,0.094]	[-0.152,0.329]	[-0.033,0.019]	[-0.057,0.045]	[-0.451,-0.012]
Turkish	-0.019	-0.027	-0.011	0.224***	-0.016	0.083	-0.008	-0.009	-0.267**
	0.5517	0.6652	0.9068	0.0030	0.5074	0.4614	0.5079	0.6941	0.0103
	[-0.082,0.044]	[-0.151,0.096]	[-0.189,0.168]	[0.076,0.372]	[-0.063,0.031]	[-0.139,0.306]	[-0.032,0.016]	[-0.057,0.038]	[-0.470,-0.063]
Other	0.002	0.014	0.104*	-0.040	0.005	-0.133*	-0.006	0.056***	-0.063
	0.9182	0.7345	0.0860	0.4282	0.7540	0.0807	0.4621	0.0005	0.3605
	[-0.040,0.045]	[-0.068,0.097]	[-0.015,0.224]	[-0.139,0.059]	[-0.027,0.037]	[-0.281,0.016]	[-0.022,0.010]	[0.024,0.087]	[-0.200,0.073]
Control variables									
Age	0.001	0.001	-0.003	-0.002	0.000	0.000	0.000	0.001	0.002
	0.5553	0.8537	0.5216	0.6336	0.7338	0.9389	0.5710	0.3629	0.6059
	[-0.002,0.004]	[-0.005,0.006]	[-0.011,0.006]	[-0.009,0.005]	[-0.002,0.003]	[-0.010,0.011]	[-0.001,0.001]	[-0.001,0.003]	[-0.007,0.012]
Age × Age	-0.000	0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000
	0.8339	0.9800	0.2904	0.9796	0.3593	0.6980	0.4452	0.2883	0.8452
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]
Female	0.010	-0.007	0.074*	-0.035	0.014	0.096*	-0.003	-0.003	-0.125**
	0.5306	0.8082	0.0866	0.3298	0.2180	0.0736	0.6046	0.7730	0.0109
	[-0.020,0.039]	[-0.066,0.051]	[-0.011,0.158]	[-0.104,0.035]	[-0.008,0.036]	[-0.009,0.201]	[-0.014,0.008]	[-0.026,0.019]	[-0.221,-0.029]
Oncological:yes	0.018	-0.085**	0.133**	-0.090**	-0.016	-0.157**	-0.003	-0.013	0.292***
	0.3406	0.0214	0.0122	0.0405	0.2518	0.0181	0.6320	0.3720	0.0000
	[-0.019,0.055]	[-0.157,-0.013]	[0.029,0.237]	[-0.176,-0.004]	[-0.044,0.011]	[-0.287,-0.027]	[-0.018,0.011]	[-0.040,0.015]	[0.174,0.411]
History of ICU:yes	-0.020	0.192***	0.028	0.016	-0.008	-0.255***	-0.004	-0.011	0.032
	0.4398	0.0002	0.7093	0.7998	0.6980	0.0060	0.6730	0.5765	0.7050
	[-0.072,0.031]	[0.091,0.292]	[-0.118,0.173]	[-0.105,0.136]	[-0.046,0.031]	[-0.436,-0.073]	[-0.024,0.015]	[-0.049,0.027]	[-0.134,0.198]
ICU:yes	0.004	-0.088	-0.031	-0.042	-0.007	0.119	-0.001	-0.007	0.072
	0.8818	0.1011	0.6858	0.5080	0.7214	0.2192	0.9451	0.7316	0.4119
	[-0.050,0.058]	[-0.193,0.017]	[-0.183,0.121]	[-0.168,0.083]	[-0.048,0.033]	[-0.071,0.308]	[-0.021,0.020]	[-0.047,0.033]	[-0.101,0.246]
Days hospitalised	-0.000	-0.000	-0.000	-0.000	-0.000	0.001*	-0.000	-0.000	0.000
	0.2092	0.9199	0.3455	0.3331	0.6905	0.0589	0.8282	0.6377	0.8228
	[-0.000,0.000]	[-0.001,0.001]	[-0.001,0.000]	[-0.001,0.000]	[-0.000,0.000]	[-0.000,0.002]	[-0.000,0.000]	[-0.000,0.000]	[-0.001,0.001]
Constant	-0.013	0.033	0.102	0.224**	0.029	0.434***	0.006	-0.002	0.186
	0.7467	0.6789	0.3761	0.0187	0.3408	0.0026	0.6971	0.9403	0.1575
	[-0.093,0.067]	[-0.123,0.189]	[-0.124,0.327]	[0.038,0.411]	[-0.031,0.089]	[0.152,0.715]	[-0.025,0.037]	[-0.062,0.057]	[-0.072,0.443]
Observations	390	390	390	390	390	390	390	390	390
R^2	0.021	0.100	0.059	0.096	0.050	0.061	0.008	0.055	0.130

Tab. 15: Likelihood of Carbapenemases for different Ethnicity – univariate linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Ethnicity									
Arabic	0.002	-0.103**	0.033	0.176***	-0.004	0.093	-0.004	0.023	-0.231***
	0.9243	0.0189	0.5951	0.0007	0.7999	0.2358	0.6181	0.1609	0.0017
	[-0.041,0.045]	[-0.189,-0.017]	[-0.089,0.155]	[0.075,0.276]	[-0.036,0.028]	[-0.061,0.247]	[-0.020,0.012]	[-0.009,0.055]	[-0.375,-0.088]
Kashmiri	-0.021	-0.103	-0.006	0.068	-0.004	0.090	-0.004	0.000	-0.082
	0.6846	0.3142	0.9676	0.5670	0.9137	0.6245	0.8312	1.0000	0.6319
	[-0.121,0.079]	[-0.305,0.098]	[-0.292,0.280]	[-0.167,0.303]	[-0.079,0.071]	[-0.270,0.450]	[-0.042,0.034]	[-0.074,0.074]	[-0.419,0.254]
Punjabi	-0.021	-0.103	0.039	0.176**	0.058**	0.099	-0.004	0.000	-0.180
	0.5464	0.1352	0.6925	0.0297	0.0235	0.4242	0.7516	1.0000	0.1185
	[-0.088,0.047]	[-0.239,0.032]	[-0.154,0.231]	[0.017,0.334]	[0.008,0.109]	[-0.144,0.341]	[-0.030,0.022]	[-0.050,0.050]	[-0.407,0.046]
Somalian	-0.021	-0.103	0.263***	0.043	0.055**	0.132	-0.004	0.000	-0.250**
	0.5349	0.1244	0.0060	0.5805	0.0289	0.2724	0.7447	1.0000	0.0261
	[-0.086,0.045]	[-0.235,0.029]	[0.076,0.450]	[-0.111,0.197]	[0.006,0.104]	[-0.104,0.367]	[-0.029,0.021]	[-0.049,0.049]	[-0.470,-0.030]
Turkish	-0.021	-0.053	-0.049	0.276***	-0.004	0.111	-0.004	0.000	-0.318***
	0.5034	0.3920	0.5808	0.0002	0.8583	0.3179	0.7254	1.0000	0.0024
	[-0.081,0.040]	[-0.176,0.069]	[-0.222,0.125]	[0.133,0.418]	[-0.050,0.041]	[-0.107,0.330]	[-0.027,0.019]	[-0.045,0.045]	[-0.522,-0.114]
Other	-0.001	-0.005	0.106*	-0.016	0.015	-0.104	-0.004	0.059***	-0.113
	0.9589	0.8983	0.0701	0.7462	0.3130	0.1603	0.5959	0.0001	0.1016
	[-0.041,0.039]	[-0.086,0.076]	[-0.009,0.221]	[-0.110,0.079]	[-0.015,0.046]	[-0.248,0.041]	[-0.019,0.011]	[0.029,0.089]	[-0.248,0.022]
Constant	0.021**	0.103***	0.149***	0.074***	0.004	0.339***	0.004	-0.000	0.368***
	0.0158	0.0000	0.0000	0.0002	0.5183	0.0000	0.2041	1.0000	0.0000
	[0.004,0.037]	[0.070,0.137]	[0.101,0.197]	[0.035,0.114]	[-0.008,0.017]	[0.278,0.399]	[-0.002,0.011]	[-0.012,0.012]	[0.311,0.424]
Observations	397	397	397	397	397	397	397	397	397
R^2	0.003	0.025	0.027	0.068	0.026	0.018	0.002	0.040	0.053

Tab. 16: Likelihood of Carbapenemases for different Residency among patients of Arabic Ethnicity – multivariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.acaruginosa b/p-value/CI95
Residency								
Arabian Peninsula	0.220	-0.005	-0.319**	0.000	-0.138	0.041	0.000	0.202
	0.2973	0.9366	0.0129	.	0.5361	0.6262	.	0.1969
	[-0.203,0.642]	[-0.145,0.134]	[-0.567,-0.072]	[0.000,0.000]	[-0.586,0.311]	[-0.128,0.209]	[0.000,0.000]	[-0.110,0.515]
Other	0.026	0.020	-0.390***	0.000	-0.108	0.078	0.000	0.374**
	0.9150	0.8079	0.0096	.	0.6779	0.4261	.	0.0444
	[-0.466,0.518]	[-0.143,0.182]	[-0.678,-0.101]	[0.000,0.000]	[-0.630,0.415]	[-0.118,0.274]	[0.000,0.000]	[0.010,0.738]
Control variables								
Age	0.004	-0.011**	0.013*	0.000	0.017	-0.014***	0.000	-0.009
	0.7548	0.0131	0.0840	.	0.2281	0.0089	.	0.3586
	[-0.022,0.030]	[-0.020,-0.002]	[-0.002,0.029]	[0.000,0.000]	[-0.011,0.044]	[-0.025,-0.004]	[0.000,0.000]	[-0.028,0.010]
Age × Age	-0.000	0.000**	-0.000**	0.000	-0.000	0.000**	0.000	0.000
	0.8313	0.0292	0.0402	.	0.2974	0.0280	.	0.2216
	[-0.000,0.000]	[0.000,0.000]	[-0.000,-0.000]	[0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[-0.000,0.000]
Female	-0.192	0.008	0.146	0.000	-0.082	-0.049	0.000	0.169
	0.2410	0.8828	0.1310	.	0.6341	0.4491	.	0.1634
	[-0.519,0.135]	[-0.100,0.116]	[-0.046,0.337]	[0.000,0.000]	[-0.429,0.265]	[-0.179,0.081]	[0.000,0.000]	[-0.072,0.411]
Oncological:yes	-0.405	-0.005	-0.215	0.000	0.501*	-0.061	0.000	0.184
	0.1268	0.9557	0.1650	.	0.0769	0.5590	.	0.3421
	[-0.930,0.121]	[-0.178,0.169]	[-0.522,0.093]	[0.000,0.000]	[-0.057,1.059]	[-0.270,0.149]	[0.000,0.000]	[-0.204,0.573]
History of ICU:yes	-0.185	0.016	0.013	0.000	0.551***	-0.040	0.000	-0.356**
	0.3131	0.7867	0.9056	.	0.0069	0.5823	.	0.0118
	[-0.552,0.182]	[-0.105,0.137]	[-0.202,0.227]	[0.000,0.000]	[0.162,0.941]	[-0.186,0.106]	[0.000,0.000]	[-0.627,-0.084]
ICU:yes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	.	.	.	.	.	.	.	.
	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]
Days hospitalised	0.000	0.000	0.001	0.000	-0.005**	0.003***	0.000	0.001
	0.9925	0.8851	0.6700	.	0.0289	0.0006	.	0.4162
	[-0.004,0.004]	[-0.001,0.002]	[-0.002,0.003]	[0.000,0.000]	[-0.010,-0.001]	[0.001,0.005]	[0.000,0.000]	[-0.002,0.004]
Constant	0.266	0.247*	0.158	0.000	-0.059	0.306**	0.000	0.083
	0.4816	0.0532	0.4769	.	0.8819	0.0484	.	0.7669
	[-0.495,1.028]	[-0.004,0.498]	[-0.288,0.603]	[0.000,0.000]	[-0.868,0.749]	[0.002,0.609]	[0.000,0.000]	[-0.480,0.646]
Observations	42	42	42	42	42	42	42	42
R^2	0.212	0.248	0.361	.	0.232	0.437	.	0.367

Tab. 17: Likelihood of Carbapenemase-encoding Species for different Residency among patients of Arabic Ethnicity – multivariate linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Residency									
Arabian Peninsula	0.118	0.000	0.324*	-0.075	0.000	-0.402*	0.000	0.004	0.135
	0.1086	.	0.0526	0.7174	.	0.0669	.	0.9602	0.3859
	[-0.028,0.264]	[0.000,0.000]	[-0.004,0.653]	[-0.491,0.342]	[0.000,0.000]	[-0.834,0.030]	[0.000,0.000]	[-0.144,0.151]	[-0.177,0.447]
Other	0.038	0.000	0.387**	-0.330	0.000	-0.443*	0.000	0.147*	0.183
	0.6480	.	0.0473	0.1762	.	0.0824	.	0.0921	0.3124
	[-0.131,0.208]	[0.000,0.000]	[0.005,0.770]	[-0.815,0.156]	[0.000,0.000]	[-0.947,0.060]	[0.000,0.000]	[-0.025,0.318]	[-0.180,0.547]
Control variables									
Age	-0.003	0.000	0.025**	0.011	0.000	-0.017	0.000	-0.000	-0.008
	0.5366	.	0.0183	0.4011	.	0.2165	.	0.9452	0.4107
	[-0.012,0.006]	[0.000,0.000]	[0.004,0.045]	[-0.015,0.036]	[0.000,0.000]	[-0.043,0.010]	[0.000,0.000]	[-0.009,0.009]	[-0.027,0.011]
Age × Age	0.000	0.000	-0.000*	-0.000	0.000	0.000	0.000	-0.000	0.000
	0.2324	.	0.0513	0.3464	.	0.3875	.	0.9083	0.3440
	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]
Female	-0.005	0.000	0.347***	-0.221	0.000	-0.001	0.000	-0.030	0.065
	0.9285	.	0.0090	0.1715	.	0.9929	.	0.6005	0.5849
	[-0.118,0.108]	[0.000,0.000]	[0.093,0.600]	[-0.543,0.101]	[0.000,0.000]	[-0.336,0.333]	[0.000,0.000]	[-0.144,0.084]	[-0.176,0.307]
Oncological:yes	-0.051	0.000	-0.307	-0.274	0.000	0.306	0.000	-0.029	0.231
	0.5717	.	0.1356	0.2892	.	0.2543	.	0.7514	0.2343
	[-0.232,0.130]	[0.000,0.000]	[-0.715,0.101]	[-0.792,0.244]	[0.000,0.000]	[-0.231,0.844]	[0.000,0.000]	[-0.212,0.155]	[-0.157,0.619]
History of ICU:yes	-0.051	0.000	0.095	0.040	0.000	0.345*	0.000	-0.060	-0.295**
	0.4214	.	0.5014	0.8232	.	0.0705	.	0.3515	0.0340
	[-0.177,0.076]	[0.000,0.000]	[-0.190,0.380]	[-0.322,0.402]	[0.000,0.000]	[-0.031,0.720]	[0.000,0.000]	[-0.188,0.069]	[-0.566,-0.024]
ICU:yes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	.	.	.	.	.	.	.	.	.
	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]
Days hospitalised	0.000	0.000	-0.000	-0.001	0.000	0.000	0.000	0.000	0.001
	0.8431	.	0.7703	0.6085	.	0.9567	.	0.6980	0.5155
	[-0.001,0.002]	[0.000,0.000]	[-0.004,0.003]	[-0.005,0.003]	[0.000,0.000]	[-0.004,0.005]	[0.000,0.000]	[-0.001,0.002]	[-0.002,0.004]
Constant	-0.051	0.000	-0.760**	0.353	0.000	0.951**	0.000	0.063	0.179
	0.6953	.	0.0134	0.3455	.	0.0182	.	0.6314	0.5220
	[-0.313,0.211]	[0.000,0.000]	[-1.351,-0.168]	[-0.397,1.103]	[0.000,0.000]	[0.173,1.730]	[0.000,0.000]	[-0.202,0.329]	[-0.383,0.741]
Observations	42	42	42	42	42	42	42	42	42
R^2	0.179	.	0.371	0.192	.	0.302	.	0.157	0.284

Tab. 18: Likelihood of colonization with Carbapenemase-encoding Species for different Residency - marginal effects after logit regression.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.aeruginosa b/p-value/CI95
Residency								
Arabian Peninsula	0.099*	-0.046	-0.012	-0.194*	0.088	-0.073	0.000	0.002
	0.0501	0.4552	0.8178	0.0693	0.2555	0.2258	.	0.9791
	[-0.000,0.199]	[-0.168,0.075]	[-0.117,0.092]	[-0.404,0.015]	[-0.064,0.239]	[-0.192,0.045]	[0.000,0.000]	[-0.143,0.146]
Other	-0.007	0.024	-0.084*	-0.051	0.111*	0.010	0.000	0.026
	0.8745	0.4713	0.0816	0.2828	0.0506	0.7852	.	0.6145
	[-0.090,0.076]	[-0.042,0.091]	[-0.179,0.011]	[-0.143,0.042]	[-0.000,0.223]	[-0.064,0.084]	[0.000,0.000]	[-0.075,0.127]
Control variables								
Age	-0.003***	0.001	0.001	0.000	-0.001	0.000	0.004	0.001
	0.0010	0.5040	0.3466	0.8521	0.5396	0.9077	0.1284	0.4726
	[-0.005,-0.001]	[-0.001,0.003]	[-0.001,0.003]	[-0.002,0.003]	[-0.004,0.002]	[-0.002,0.002]	[-0.001,0.008]	[-0.002,0.004]
Female	-0.022	-0.068*	0.051*	0.021	0.067	-0.001	-0.001	-0.066
	0.5733	0.0682	0.0975	0.5450	0.1756	0.9698	0.9812	0.1325
	[-0.099,0.055]	[-0.141,0.005]	[-0.009,0.112]	[-0.047,0.088]	[-0.030,0.163]	[-0.060,0.058]	[-0.089,0.087]	[-0.151,0.020]
Oncological:yes	-0.228***	0.034	0.013	-0.018	-0.134*	-0.011	0.131**	0.148***
	0.0076	0.3066	0.7412	0.6973	0.0639	0.7697	0.0402	0.0012
	[-0.396,-0.061]	[-0.031,0.100]	[-0.065,0.092]	[-0.109,0.073]	[-0.275,0.008]	[-0.087,0.064]	[0.006,0.255]	[0.059,0.237]
History of ICU:yes	0.069	0.071*	0.019	0.017	-0.071	-0.036	0.000	0.017
	0.3252	0.0961	0.7287	0.7937	0.4889	0.5490	.	0.8059
	[-0.069,0.207]	[-0.013,0.154]	[-0.090,0.129]	[-0.108,0.141]	[-0.271,0.129]	[-0.155,0.082]	[0.000,0.000]	[-0.117,0.151]
ICU:yes	-0.154**	-0.044	-0.018	0.056	0.056	0.078	0.000	-0.030
	0.0340	0.2811	0.7409	0.3533	0.5811	0.1896	.	0.6642
	[-0.297,-0.012]	[-0.123,0.036]	[-0.128,0.091]	[-0.063,0.175]	[-0.144,0.256]	[-0.038,0.194]	[0.000,0.000]	[-0.166,0.106]
Days hospitalised	-0.001*	-0.000	-0.001	-0.000	-0.000	0.001**	-0.000	0.001**
	0.0567	0.4039	0.2003	0.5667	0.3767	0.0165	0.9711	0.0189
	[-0.002,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.001]	[-0.002,0.001]	[0.000,0.001]	[-0.001,0.001]	[0.000,0.001]
Observations	390	390	390	390	390	390	118	390

Tab. 19: Likelihood of colonization with Carbapenemase-encoding Species for different Ethnicity - marginal effects after logit regression.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.acaruginosa b/p-value/CI95
Ethnicity								
Arabic	0.123**	-0.070	-0.000	0.000	0.096	-0.038	0.000	-0.003
	0.0131	0.3243	0.9965	.	0.1850	0.5218	.	0.9575
	[0.026,0.220]	[-0.209,0.069]	[-0.109,0.108]	[0.000,0.000]	[-0.046,0.237]	[-0.155,0.079]	[0.000,0.000]	[-0.131,0.124]
Kashmiri	-0.004	0.000	0.000	0.007	0.237	0.083	0.000	0.000
	0.9735	.	.	0.9598	0.1167	0.3197	.	.
	[-0.248,0.240]	[0.000,0.000]	[0.000,0.000]	[-0.273,0.288]	[-0.059,0.533]	[-0.080,0.246]	[0.000,0.000]	[0.000,0.000]
Punjabi	0.085	0.000	0.000	0.000	0.247**	0.000	0.000	-0.088
	0.2337	.	.	.	0.0204	.	.	0.4778
	[-0.055,0.224]	[0.000,0.000]	[0.000,0.000]	[0.000,0.000]	[0.038,0.455]	[0.000,0.000]	[0.000,0.000]	[-0.329,0.154]
Somalian	0.104	0.000	0.112*	0.000	0.101	0.062	0.000	-0.201
	0.1730	.	0.0677	.	0.3465	0.3225	.	0.1582
	[-0.045,0.253]	[0.000,0.000]	[-0.008,0.232]	[0.000,0.000]	[-0.109,0.312]	[-0.061,0.184]	[0.000,0.000]	[-0.481,0.078]
Turkish	0.091	0.000	-0.051	0.000	0.153	0.055	0.000	-0.093
	0.1553	.	0.6059	.	0.1088	0.3680	.	0.3814
	[-0.035,0.218]	[0.000,0.000]	[-0.244,0.142]	[0.000,0.000]	[-0.034,0.341]	[-0.064,0.174]	[0.000,0.000]	[-0.302,0.115]
Other	0.026	0.026	0.003	0.032	-0.053	0.070*	0.000	-0.106
	0.6162	0.4908	0.9504	0.5694	0.4792	0.0680	.	0.1330
	[-0.077,0.129]	[-0.048,0.100]	[-0.094,0.100]	[-0.078,0.141]	[-0.200,0.094]	[-0.005,0.146]	[0.000,0.000]	[-0.244,0.032]
Control variables								
Age	-0.003***	0.000	0.001	0.000	-0.000	0.001	0.004*	0.000
	0.0036	0.6878	0.2953	0.9871	0.7357	0.6193	0.0580	0.9593
	[-0.005,-0.001]	[-0.002,0.003]	[-0.001,0.004]	[-0.003,0.003]	[-0.003,0.002]	[-0.002,0.003]	[-0.000,0.008]	[-0.003,0.003]
Female	-0.012	-0.090**	0.050	0.015	0.080	-0.006	0.022	-0.064
	0.7613	0.0443	0.1298	0.7362	0.1001	0.8382	0.5816	0.1427
	[-0.090,0.066]	[-0.177,-0.002]	[-0.015,0.115]	[-0.073,0.104]	[-0.015,0.176]	[-0.068,0.055]	[-0.055,0.099]	[-0.151,0.022]
Oncological:yes	-0.222***	0.022	0.019	-0.015	-0.149**	-0.022	0.173***	0.132***
	0.0088	0.5430	0.6547	0.7934	0.0323	0.5835	0.0045	0.0021
	[-0.388,-0.056]	[-0.049,0.094]	[-0.063,0.101]	[-0.131,0.100]	[-0.285,-0.013]	[-0.099,0.056]	[0.054,0.293]	[0.048,0.216]
History of ICU:yes	0.086	0.059	0.050	0.024	-0.101	-0.048	0.000	0.001
	0.1953	0.1848	0.3873	0.7621	0.2982	0.4303	.	0.9846
	[-0.044,0.216]	[-0.028,0.145]	[-0.064,0.164]	[-0.130,0.177]	[-0.292,0.089]	[-0.166,0.071]	[0.000,0.000]	[-0.128,0.130]
ICU:yes	-0.157**	-0.050	-0.017	0.078	0.063	0.093	0.000	-0.044
	0.0294	0.2890	0.7751	0.3164	0.5351	0.1279	.	0.5329
	[-0.298,-0.016]	[-0.143,0.043]	[-0.134,0.100]	[-0.075,0.232]	[-0.136,0.261]	[-0.027,0.214]	[0.000,0.000]	[-0.182,0.094]
Days hospitalised	-0.001*	-0.000	-0.001	-0.000	-0.000	0.001***	0.000	0.001**
	0.0821	0.3634	0.2071	0.4897	0.4377	0.0083	0.8265	0.0192
	[-0.002,0.000]	[-0.001,0.000]	[-0.001,0.000]	[-0.001,0.001]	[-0.001,0.001]	[0.000,0.001]	[-0.001,0.001]	[0.000,0.001]
Observations	390	330	367	295	390	374	135	383

Tab.20: Likelihood of Carbapenemases for different Residency - marginal effects after logit regression.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Residency									
Arabian Peninsula	0.035*	0.000	-0.011	0.106**	0.000	0.066	0.000	0.000	-0.192**
	0.0742	.	0.8774	0.0172	.	0.4221	.	.	0.0350
	[-0.003,0.074]	[0.000,0.000]	[-0.146,0.125]	[0.019,0.194]	[0.000,0.000]	[-0.094,0.226]	[0.000,0.000]	[0.000,0.000]	[-0.370,-0.014]
Other	-0.010	0.055	0.004	-0.009	0.048*	0.062	0.000	0.001	-0.093
	0.6136	0.3567	0.9335	0.8221	0.0698	0.3007	.	0.9636	0.1267
	[-0.051,0.030]	[-0.062,0.171]	[-0.097,0.106]	[-0.088,0.070]	[-0.004,0.100]	[-0.056,0.181]	[0.000,0.000]	[-0.044,0.046]	[-0.212,0.026]
Control variables									
Age	0.000	0.001	0.001	-0.002*	0.000	-0.001	0.000	-0.001*	0.002
	0.2349	0.4879	0.2965	0.0617	0.8261	0.3928	.	0.0607	0.2781
	[-0.000,0.001]	[-0.002,0.004]	[-0.001,0.004]	[-0.003,0.000]	[-0.002,0.002]	[-0.004,0.002]	[0.000,0.000]	[-0.002,0.000]	[-0.001,0.005]
Female	0.007	-0.002	0.070*	-0.051	0.038	0.089*	0.000	-0.001	-0.120**
	0.5971	0.9497	0.0775	0.1860	0.1143	0.0790	.	0.9803	0.0145
	[-0.019,0.033]	[-0.080,0.075]	[-0.008,0.147]	[-0.125,0.024]	[-0.009,0.085]	[-0.010,0.189]	[0.000,0.000]	[-0.052,0.051]	[-0.217,-0.024]
Oncological:yes	0.020	0.000	0.120**	-0.161**	0.000	-0.146**	0.000	0.000	0.229***
	0.2754	.	0.0125	0.0291	.	0.0393	.	.	0.0000
	[-0.016,0.056]	[0.000,0.000]	[0.026,0.214]	[-0.306,-0.016]	[0.000,0.000]	[-0.284,-0.007]	[0.000,0.000]	[0.000,0.000]	[0.125,0.334]
History of ICU:yes	-0.274***	0.229***	0.019	0.014	0.000	-0.257**	0.000	0.000	0.014
	0.0033	0.0005	0.8041	0.8432	.	0.0171	.	.	0.8613
	[-0.457,-0.091]	[0.099,0.360]	[-0.128,0.165]	[-0.122,0.149]	[0.000,0.000]	[-0.468,-0.046]	[0.000,0.000]	[0.000,0.000]	[-0.146,0.174]
ICU:yes	0.257***	-0.048	-0.039	-0.052	0.000	0.138	0.000	0.000	0.070
	0.0030	0.3067	0.6122	0.4600	.	0.2092	.	.	0.3852
	[0.088,0.427]	[-0.140,0.044]	[-0.188,0.111]	[-0.189,0.086]	[0.000,0.000]	[-0.078,0.354]	[0.000,0.000]	[0.000,0.000]	[-0.088,0.229]
Days hospitalised	-0.000	-0.000	-0.000	-0.001	-0.001	0.001*	0.000	-0.001	0.000
	0.2856	0.8170	0.3542	0.1930	0.2850	0.0538	.	0.3728	0.5372
	[-0.001,0.000]	[-0.001,0.001]	[-0.001,0.000]	[-0.002,0.000]	[-0.003,0.001]	[-0.000,0.002]	[0.000,0.000]	[-0.004,0.001]	[-0.001,0.001]
Observations	390	287	390	390	174	390	76	174	390

Tab.21: Likelihood of Carbapenemases for different Ethnicity - marginal effects after logit regression.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Ethnicity									
Arabic	0.013	0.000	0.061	0.115***	0.000	0.033	0.000	-0.066	-0.196**
	0.5892	.	0.3379	0.0080	.	0.6770	.	0.4746	0.0230
	[-0.034,0.060]	[0.000,0.000]	[-0.064,0.186]	[0.030,0.200]	[0.000,0.000]	[-0.121,0.186]	[0.000,0.000]	[-0.248,0.115]	[-0.365,-0.027]
Kashmiri	0.000	0.000	0.038	0.030	0.000	0.039	0.000	0.000	0.017
	.	.	0.8038	0.7750	.	0.8171	.	.	0.9126
	[0.000,0.000]	[0.000,0.000]	[-0.260,0.335]	[-0.175,0.235]	[0.000,0.000]	[-0.290,0.368]	[0.000,0.000]	[0.000,0.000]	[-0.283,0.317]
Punjabi	0.000	0.000	0.108	0.071	0.302	0.023	0.000	0.000	-0.108
	.	.	0.2653	0.2668	0.6965	0.8457	.	.	0.3835
	[0.000,0.000]	[0.000,0.000]	[-0.082,0.297]	[-0.054,0.196]	[-1.217,1.821]	[-0.210,0.256]	[0.000,0.000]	[0.000,0.000]	[-0.352,0.135]
Somalian	0.000	0.000	0.194**	0.027	0.348	0.083	0.000	0.000	-0.246*
	.	.	0.0119	0.7299	0.6478	0.4665	.	.	0.0795
	[0.000,0.000]	[0.000,0.000]	[0.043,0.345]	[-0.127,0.181]	[-1.145,1.841]	[-0.140,0.305]	[0.000,0.000]	[0.000,0.000]	[-0.521,0.029]
Turkish	0.000	-0.023	-0.022	0.130**	0.000	0.075	0.000	0.000	-0.402**
	.	0.8351	0.8394	0.0131	.	0.4821	.	.	0.0311
	[0.000,0.000]	[-0.238,0.192]	[-0.233,0.189]	[0.027,0.232]	[0.000,0.000]	[-0.134,0.284]	[0.000,0.000]	[0.000,0.000]	[-0.767,-0.037]
Other	0.008	0.018	0.099*	-0.049	0.143*	-0.140*	0.000	0.000	-0.052
	0.7395	0.7509	0.0693	0.4241	0.0871	0.0779	.	.	0.4391
	[-0.038,0.054]	[-0.094,0.130]	[-0.008,0.206]	[-0.170,0.072]	[-0.021,0.306]	[-0.296,0.016]	[0.000,0.000]	[0.000,0.000]	[-0.184,0.080]
Control variables									
Age	0.000	0.000	0.002*	-0.002*	-0.001	-0.002	-0.000	-0.004*	0.001
	0.4629	0.7845	0.0857	0.0755	0.8673	0.2533	.	0.0861	0.3858
	[-0.001,0.001]	[-0.003,0.004]	[-0.000,0.005]	[-0.003,0.000]	[-0.010,0.008]	[-0.005,0.001]	[-0.000,-0.000]	[-0.008,0.001]	[-0.002,0.005]
Female	0.014	-0.008	0.066*	-0.038	0.328	0.095*	0.000	-0.008	-0.120**
	0.4088	0.8519	0.0960	0.3211	0.6684	0.0646	.	0.9354	0.0135
	[-0.019,0.046]	[-0.097,0.080]	[-0.012,0.143]	[-0.114,0.037]	[-1.172,1.828]	[-0.006,0.195]	[0.000,0.000]	[-0.190,0.175]	[-0.214,-0.025]
Oncological:yes	0.027	0.000	0.116**	-0.144**	0.000	-0.164**	0.000	0.000	0.252***
	0.2088	.	0.0119	0.0440	.	0.0156	.	.	0.0000
	[-0.015,0.069]	[0.000,0.000]	[0.026,0.207]	[-0.284,-0.004]	[0.000,0.000]	[-0.298,-0.031]	[0.000,0.000]	[0.000,0.000]	[0.153,0.350]
History of ICU:yes	-0.268	0.212***	0.031	0.035	0.000	-0.279***	0.000	0.000	0.037
	0.9928	0.0004	0.6594	0.5958	.	0.0071	.	.	0.6326
	[-58.668,58.131]	[0.094,0.331]	[-0.108,0.170]	[-0.094,0.164]	[0.000,0.000]	[-0.483,-0.076]	[0.000,0.000]	[0.000,0.000]	[-0.115,0.190]
ICU:yes	0.248	-0.052	-0.034	-0.061	0.000	0.141	0.000	0.000	0.059
	0.9934	0.3191	0.6517	0.3724	.	0.1994	.	.	0.4644
	[-58.151,58.647]	[-0.155,0.051]	[-0.182,0.114]	[-0.197,0.074]	[0.000,0.000]	[-0.075,0.357]	[0.000,0.000]	[0.000,0.000]	[-0.099,0.216]
Days hospitalised	-0.001	-0.000	-0.000	-0.001	-0.006	0.001**	0.000	-0.003	0.000
	0.2310	0.9146	0.3494	0.2845	0.2204	0.0438	.	0.4559	0.8371
	[-0.001,0.000]	[-0.001,0.001]	[-0.001,0.000]	[-0.002,0.000]	[-0.015,0.003]	[0.000,0.002]	[0.000,0.000]	[-0.010,0.005]	[-0.001,0.001]
Observations	330	252	390	390	153	390	70	45	390

Tab.22: Likelihood of colonization with Carbapenemase-encoding Species for different Ethnicity among German residents – multivariate linear regression analysis.

	A.baumannii b/p-value/CI95	Citrobact b/p-value/CI95	E.coli b/p-value/CI95	Enterobact b/p-value/CI95	K.pneumoniae b/p-value/CI95	Other Enterobact b/p-value/CI95	Other Nonfermenter b/p-value/CI95	P.aeruginosa b/p-value/CI95
Ethnicity								
Arabic	0.087	-0.027	0.200*	-0.158	0.059	-0.054	-0.037	-0.070
	0.4216	0.7537	0.0823	0.2005	0.6900	0.5790	0.5118	0.5968
	[-0.125,0.299]	[-0.195,0.141]	[-0.026,0.426]	[-0.401,0.085]	[-0.232,0.349]	[-0.245,0.137]	[-0.147,0.073]	[-0.330,0.190]
Kashmiri	-0.169	0.016	-0.137	0.405	0.216	-0.104	0.040	-0.268
	0.4593	0.9294	0.5724	0.1211	0.4888	0.6137	0.7363	0.3398
	[-0.617,0.280]	[-0.340,0.372]	[-0.615,0.341]	[-0.108,0.919]	[-0.398,0.831]	[-0.509,0.301]	[-0.193,0.273]	[-0.819,0.284]
Punjabi	-0.231	-0.035	-0.091	-0.102	0.161	-0.030	-0.032	0.362
	0.3045	0.8428	0.7037	0.6906	0.6016	0.8821	0.7836	0.1916
	[-0.674,0.212]	[-0.387,0.316]	[-0.563,0.381]	[-0.609,0.404]	[-0.446,0.768]	[-0.430,0.370]	[-0.262,0.198]	[-0.182,0.906]
Somalian	0.092	-0.038	0.118	-0.095	0.200	0.071	-0.096*	-0.251*
	0.4044	0.6665	0.3149	0.4501	0.1848	0.4782	0.0927	0.0639
	[-0.125,0.308]	[-0.210,0.134]	[-0.113,0.349]	[-0.343,0.153]	[-0.096,0.497]	[-0.125,0.266]	[-0.209,0.016]	[-0.517,0.015]
Turkish	0.049	-0.043	-0.109	-0.136	0.145	0.182*	-0.030	-0.056
	0.6543	0.6183	0.3499	0.2786	0.3358	0.0673	0.5932	0.6754
	[-0.167,0.265]	[-0.215,0.128]	[-0.340,0.121]	[-0.384,0.111]	[-0.151,0.441]	[-0.013,0.377]	[-0.143,0.082]	[-0.322,0.209]
Other	0.003	0.130**	-0.057	0.058	-0.092	0.116**	-0.020	-0.137*
	0.9618	0.0111	0.4017	0.4247	0.2920	0.0463	0.5497	0.0811
	[-0.123,0.129]	[0.030,0.230]	[-0.191,0.077]	[-0.086,0.202]	[-0.265,0.080]	[0.002,0.229]	[-0.085,0.045]	[-0.292,0.017]
Control variables								
Age	-0.004	-0.000	0.006	0.002	-0.000	-0.003	-0.007**	0.006
	0.4733	0.9773	0.2714	0.7189	0.9595	0.5382	0.0154	0.3943
	[-0.015,0.007]	[-0.009,0.008]	[-0.005,0.018]	[-0.010,0.014]	[-0.015,0.014]	[-0.013,0.007]	[-0.012,-0.001]	[-0.007,0.019]
Age × Age	0.000	0.000	-0.000	0.000	-0.000	0.000	0.000***	-0.000
	0.8431	0.7694	0.3767	0.9578	0.7712	0.5352	0.0081	0.3725
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]
Female	0.004	-0.080**	0.059	0.028	0.055	-0.016	-0.011	-0.040
	0.9255	0.0227	0.2088	0.5732	0.3594	0.6848	0.6419	0.4597
	[-0.082,0.090]	[-0.148,-0.011]	[-0.033,0.151]	[-0.070,0.127]	[-0.063,0.173]	[-0.094,0.062]	[-0.055,0.034]	[-0.146,0.066]
Oncological:yes	-0.119**	0.053	-0.015	-0.036	-0.113	-0.031	0.092***	0.169***
	0.0198	0.1925	0.7875	0.5355	0.1063	0.5029	0.0006	0.0072
	[-0.220,-0.019]	[-0.027,0.132]	[-0.121,0.092]	[-0.151,0.079]	[-0.250,0.024]	[-0.121,0.060]	[0.040,0.144]	[0.046,0.292]
History of ICU:yes	0.022	0.079	0.039	0.015	-0.050	-0.044	-0.056	-0.006
	0.7420	0.1400	0.5910	0.8431	0.5878	0.4728	0.1113	0.9468
	[-0.110,0.155]	[-0.026,0.184]	[-0.103,0.180]	[-0.136,0.167]	[-0.232,0.132]	[-0.164,0.076]	[-0.125,0.013]	[-0.168,0.157]
ICU:yes	-0.125*	-0.056	-0.018	0.088	-0.005	0.092	0.008	0.016
	0.0593	0.2830	0.7959	0.2475	0.9603	0.1230	0.8155	0.8406
	[-0.256,0.005]	[-0.160,0.047]	[-0.157,0.121]	[-0.061,0.237]	[-0.183,0.174]	[-0.025,0.210]	[-0.060,0.076]	[-0.144,0.176]
Days hospitalised	-0.000	-0.000	-0.001	-0.001**	-0.001	0.001**	0.000	0.001***
	0.6492	0.5267	0.2225	0.0459	0.3139	0.0154	0.7660	0.0039
	[-0.001,0.001]	[-0.001,0.000]	[-0.001,0.000]	[-0.002,-0.000]	[-0.002,0.001]	[0.000,0.002]	[-0.000,0.000]	[0.000,0.002]
Constant	0.373**	0.018	-0.081	0.004	0.393*	0.100	0.182**	0.010
	0.0130	0.8804	0.6128	0.9809	0.0552	0.4564	0.0199	0.9561
	[0.079,0.667]	[-0.215,0.251]	[-0.393,0.232]	[-0.332,0.340]	[-0.009,0.796]	[-0.165,0.366]	[0.029,0.334]	[-0.351,0.371]
Observations	243	243	243	243	243	243	243	243
R^2	0.083	0.072	0.049	0.081	0.064	0.064	0.126	0.124

Tab.23: Likelihood of Carbapenemases for different Ethnicity among German residents-linear regression analysis.

	GES b/p-value/CI95	KPC b/p-value/CI95	NDM b/p-value/CI95	OXA-23 b/p-value/CI95	OXA-24-72 b/p-value/CI95	OXA-48 b/p-value/CI95	OXA-58 b/p-value/CI95	OXA-72 b/p-value/CI95	VIM b/p-value/CI95
Ethnicity									
Arabic	-0.026	-0.069	-0.180	0.245**	-0.014	0.201	0.000	-0.011	-0.221
	0.5638	0.5000	0.1814	0.0114	0.5380	0.1977	.	0.7219	0.1687
	[-0.115,0.063]	[-0.268,0.131]	[-0.445,0.085]	[0.056,0.434]	[-0.058,0.030]	[-0.106,0.507]	[0.000,0.000]	[-0.072,0.050]	[-0.536,0.094]
Kashmiri	0.006	-0.175	-0.124	-0.081	-0.023	-0.429	0.000	-0.007	0.814**
	0.9468	0.4147	0.6621	0.6918	0.6217	0.1935	.	0.9146	0.0170
	[-0.181,0.194]	[-0.598,0.248]	[-0.684,0.436]	[-0.480,0.319]	[-0.117,0.070]	[-1.078,0.219]	[0.000,0.000]	[-0.136,0.122]	[0.147,1.481]
Punjabi	-0.027	-0.029	-0.174	-0.149	-0.023	0.158	0.000	-0.026	0.185
	0.7731	0.8898	0.5346	0.4587	0.6173	0.6270	.	0.6905	0.5802
	[-0.212,0.158]	[-0.447,0.388]	[-0.727,0.378]	[-0.543,0.246]	[-0.116,0.069]	[-0.482,0.798]	[0.000,0.000]	[-0.153,0.101]	[-0.473,0.843]
Somalian	-0.031	-0.008	0.195	0.033	-0.017	0.344**	0.000	-0.013	-0.274*
	0.5061	0.9390	0.1558	0.7341	0.4481	0.0313	.	0.6896	0.0946
	[-0.121,0.060]	[-0.212,0.196]	[-0.075,0.466]	[-0.160,0.226]	[-0.063,0.028]	[0.031,0.657]	[0.000,0.000]	[-0.075,0.050]	[-0.596,0.048]
Turkish	-0.028	-0.060	0.051	0.217**	-0.017	0.129	0.000	-0.016	-0.361**
	0.5470	0.5633	0.7080	0.0276	0.4648	0.4154	.	0.6134	0.0276
	[-0.118,0.063]	[-0.264,0.144]	[-0.218,0.321]	[0.024,0.409]	[-0.062,0.028]	[-0.183,0.442]	[0.000,0.000]	[-0.078,0.046]	[-0.683,-0.040]
Other	-0.021	-0.003	0.077	-0.055	-0.013	-0.068	0.000	0.062***	-0.049
	0.4263	0.9614	0.3339	0.3363	0.3331	0.4600	.	0.0009	0.6091
	[-0.074,0.031]	[-0.122,0.116]	[-0.080,0.234]	[-0.167,0.057]	[-0.039,0.013]	[-0.250,0.114]	[0.000,0.000]	[0.026,0.098]	[-0.236,0.138]
Control variables									
Age	0.001	0.001	-0.001	-0.002	-0.001	0.007	0.000	-0.001	-0.001
	0.5460	0.8246	0.9011	0.6513	0.5956	0.3696	.	0.6048	0.8973
	[-0.003,0.006]	[-0.009,0.011]	[-0.014,0.012]	[-0.012,0.007]	[-0.003,0.002]	[-0.008,0.022]	[0.000,0.000]	[-0.004,0.002]	[-0.017,0.015]
Age × Age	-0.000	0.000	0.000	0.000	0.000	-0.000	0.000	0.000	0.000
	0.6167	0.9397	0.8466	0.8405	0.9767	0.2553	.	0.8472	0.7170
	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]	[0.000,0.000]	[-0.000,0.000]	[-0.000,0.000]
Female	-0.004	-0.013	0.005	-0.031	0.013	0.143**	0.000	0.006	-0.148**
	0.8248	0.7453	0.9328	0.4275	0.1498	0.0246	.	0.6593	0.0234
	[-0.040,0.032]	[-0.095,0.068]	[-0.103,0.112]	[-0.108,0.046]	[-0.005,0.031]	[0.018,0.268]	[0.000,0.000]	[-0.019,0.030]	[-0.277,-0.020]
Oncological:yes	0.024	-0.086*	0.115*	-0.067	-0.009	-0.121	0.000	-0.014	0.242***
	0.2653	0.0745	0.0707	0.1414	0.3891	0.1012	.	0.3485	0.0016
	[-0.018,0.066]	[-0.180,0.009]	[-0.010,0.240]	[-0.156,0.022]	[-0.030,0.012]	[-0.266,0.024]	[0.000,0.000]	[-0.043,0.015]	[0.093,0.391]
History of ICU:yes	-0.019	0.227***	-0.032	0.009	-0.009	-0.184*	0.000	-0.019	-0.032
	0.4935	0.0004	0.7035	0.8822	0.5357	0.0596	.	0.3328	0.7468
	[-0.075,0.036]	[0.102,0.352]	[-0.198,0.134]	[-0.109,0.127]	[-0.036,0.019]	[-0.376,0.008]	[0.000,0.000]	[-0.057,0.019]	[-0.229,0.165]
ICU:yes	0.005	-0.080	-0.058	-0.058	-0.004	0.063	0.000	-0.003	0.148
	0.8428	0.2004	0.4820	0.3233	0.7471	0.5112	.	0.8741	0.1342
	[-0.049,0.060]	[-0.203,0.043]	[-0.221,0.104]	[-0.174,0.058]	[-0.032,0.023]	[-0.125,0.251]	[0.000,0.000]	[-0.040,0.034]	[-0.046,0.341]
Days hospitalised	-0.000	-0.000	0.000	-0.000	-0.000	0.001	0.000	-0.000	-0.000
	0.3775	0.4740	0.8716	0.7356	0.8826	0.1985	.	0.8359	0.7745
	[-0.000,0.000]	[-0.001,0.000]	[-0.001,0.001]	[-0.001,0.001]	[-0.000,0.000]	[-0.000,0.002]	[0.000,0.000]	[-0.000,0.000]	[-0.001,0.001]
Constant	-0.009	-0.024	0.186	0.222*	0.048	0.211	0.000	0.054	0.306
	0.8831	0.8618	0.3190	0.0965	0.1260	0.3288	.	0.2106	0.1691
	[-0.132,0.114]	[-0.301,0.252]	[-0.181,0.552]	[-0.040,0.483]	[-0.014,0.109]	[-0.214,0.635]	[0.000,0.000]	[-0.031,0.138]	[-0.131,0.742]
Observations	243	243	243	243	243	243	243	243	243
R^2	0.022	0.127	0.064	0.088	0.039	0.099	.	0.084	0.134

Notes: Values of b show the estimated coefficients of linear probability models on the likelihood that the respective Species were detected, together with their p-value and confidence interval (95%). Baseline category for Ethnicity is German. Statistical significance level * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

7. List of figures

8. List of tables

9. References

Alice E. Kizny Gordon, Amy J. Mathers, Elaine Y. L. Cheong, Thomas Gottlieb, Shireen Kotay, A. Sarah Walker, Timothy E. A. Peto, Derrick W. Crook, Nicole Stoesser, The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections—A Systematic Review of the Literature, Clinical Infectious Diseases, Volume 64, Issue 10, 15 May 2017, Pages 1435–1444

Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-Resistant *Entero aeruginosa*: Risk Factors and Clini-cal Impact. Antimicrobial Agents and Chemotherapy. 2006 Jan;50(1):43

Aslam B, Wang W, Arshad MI, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018;11:1645-1658. Published 2018 Oct 10

Abraham EP. Cephalosporins 1945-1986. Drugs. 1987;34 Suppl 2:1-14

Bortolaia V, Kaas RF, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AR, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, NykÃ¤senoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy. 2020 Aug 11

Brooks AW, Priya S, Blekhman R, Bordenstein SR. Gut microbiota diversity across ethnicities in the United States. PLoS Biol. 2018;16(12):e2006842. Published 2018 Dec 4

Bush K. Past and Present Perspectives on β-Lactamases. Antimicrob Agents Chemother. 2018;62(10):e01076-18. Published 2018 Sep 24

Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010;54(3):969-976

Cantón R, González-Alba JM, Galán JC. CTX-M Enzymes: Origin and Diffusion. Front Microbiol. 2012;3:110. Published 2012 Apr 2

Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother. 2009;53(6):2227-2238

Cassini A, Högberg LD, Plachouras D, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56-66

Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution?. Pharmacogn Rev. 2017;11(22):57-72

Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med Sci (Basel). 2017;6(1):1. Published 2017 Dec 21

Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe [published correction appears in Euro Surveill. 2008 Nov 27;13(48). pii: 19051]. Euro Surveill. 2008;13(47):19044. Published 2008 Nov 20

Dadgostar P. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 2019;12:3903-3910. Published 2019 Dec 20

El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. Recent updates of carbapenem antibiotics. Eur J Med Chem. 2017;131:185-195

Elander RP. Industrial production of beta-lactam antibiotics. Appl Microbiol Biotechnol. 2003;61(5-6):385-392

Evans BA, Amyes SG. OXA β-lactamases. Clin Microbiol Rev. 2014;27(2):241-263

Frost I, Van Boeckel TP, Pires J, Craig J, Laxminarayan R. Global geographic trends in antimicrobial resistance: the role of international travel. J Travel Med. 2019;26(8):taz036

Gaulke, C.A., Sharpton, T.J. The influence of ethnicity and geography on human gut microbiome composition. Nat Med **24,** 1495–1496 (2018)

Gilbert, J., Blaser, M., Caporaso, J. et al. Current understanding of the human microbiome. Nat Med **24,** 392–400 (2018)

Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. Microb Genom. 2019;5(10):e000306

Hamprecht A, Sommer J, Willmann M, Brender C, Stelzer Y, Krause FF, Tsvetkov T, Wild F, Riedel-Christ S, Kutschenreuter J, Imirzalioglu C, Gonzaga A, Nübel U and Göttig S (2019) Pathogenicity of Clinical OXA-48 Isolates and Impact of the OXA-48 IncL Plasmid on Virulence and Bacterial Fitness. Front. Microbiol. 10:2509

Hawkey PM. The origins and molecular basis of antibiotic resistance. BMJ. 1998;317(7159):657-660

Hopman J, Meijer C, Kenters N, Coolen JPM, Ghamati MR, Mehtar S, et al.Risk Assessment After a Severe Hospital-Acquired Infection Associated WithCarbapenemase-Producing *Pseudomonas aeruginosa*. JAMA network open. 2019 Feb; 2(2):e187665–e187665

Humphreys H, Fitzpatick F, Harvey BJ. Gender Differences in Rates of Carriage and Bloodstream Infection Caused by Methicillin-Resistant Staphylococcus aureus: Are They Real, Do They Matter and Why? Clinical Infectious Diseases. 2015Jul;61(11):1708–1714

Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med. 2005;352(4):380-391

Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev. 2009;22(1):161-182

John Penders, Carel Thijs, Cornelis Vink, Foekje F. Stelma, Bianca Snijders, Ischa Kummeling, Piet A. van den Brandt, Ellen E. Stobberingh, Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy, Pediatrics Aug 2006, 118 (2) 511-521

Kaase M, Schimanski S, Schiller R, Beyreiß B, Thürmer A, Steinmann J, et al. Multicentre investigation of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in German hospitals. Int J Med Microbiol. 306 (2016), pp. 415-420

Kaul TK, Chhina DK. Medical Tourism and New Delhi Metallo β - Lactamase 1- A Concern and Threat. J Anaesthesiol Clin Pharmacol. 2010;26(4):437-438

Koroska F, Göttig S, Kaase M, et al. Comparison of Phenotypic Tests and an Immunochromatographic Assay and Development of a New Algorithm for Detection of OXA-48-like Carbapenemases. J Clin Microbiol. 2017;55(3):877-883

Kracalik I, Ham C, Smith AR, et al. Notes from the Field: Verona Integron-Encoded Metallo-β-Lactamase–Producing Carbapenem-Resistant *Pseudomonas aeruginosa* Infections in U.S. Residents Associated with Invasive Medical Procedures in Mexico, 2015–2018. MMWR Morb Mortal Wkly Rep 2019;68:463–464

Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM and Lund O. Multilocus Sequence Typing of Total Genome Sequenced Bacteria. J. Clin. Micobiol. 2012. 50(4): 1355-1361

Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant *Enterobacteriaceae*: The Impact and Evolution of a Global Menace. J Infect Dis. 2017;215(suppl_1):S28-S36

Lübbert C, Lippmann N, Rodloff AC. Hochresistente Enterobakterien Systematisches Screening ist notwendig. Deutsches Ärzteblatt. 2013; 110(46), 2206-2207

Lynn Bry, Per G. Falk, Tore Midtvedt, Jeffrey I. Gordon, A Model of Host-Microbial Interactions in an Open Mammalian Ecosystem, Science, Vol. 273, Issue 5280, pp. ,06 Sep 1996 : 1380-1383

Markwart R, Willrich N, Haller S, Noll I, Koppe U, Werner G, et al. The rise in vancomycin-resistant Enterococcus faecium in Germany: data from the German Antimicrobial Resistance Surveillance (ARS). Antimicrobial resistance and infection control. 2019 Aug;8:147–147

Marx K, Engels F, Manifest der Kommunistischen Partei, Kommunistischer Arbeiterbildungsverein, Bishopsgate, London, Feb 1848, page 1-23

Matthew M, Harris AM. Identification of beta-lactamases by analytical isoelectric focusing: correlation with bacterial taxonomy. J Gen Microbiol. 1976;94(1):55-67

Michael CA, Dominey-Howes D and Labbate M (2014) The antimicrobial resistance crisis: causes, consequences, and management. Front. Public Health **2**:145

Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Microbiol Spectr. 2016;4(2):10.1128/microbiolspec.VMBF-0016-2015. doi:10.1128/microbiolspec.VMBF-0016-2015

Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252-275

Naylor NR, Pouwels KB, Hope R, et al. The health and cost burden of antibiotic resistant and susceptible *Escherichia coli* bacteraemia in the English hospital setting: A national retrospective cohort study. PLoS One. 2019;14(9):e0221944. Published 2019 Sep 10

Nicolas-Chanoine, M., Vigan, M., Laouénan, C. et al. Risk factors for carbapenem-resistant *Enterobacteriaceae* infections: a French case-control-control study. Eur J Clin Microbiol Infect Dis **38,** 383–393 (2019)

Pal C. Effects of Biocides and Metals on Antibiotic Resistance: a Genomic and Metagenomic Perspective. Ph.D. Thesis. University of Gothenburg. doi: 10.13140/RG.2.2.27592.72967

Palzkill T. Metallo-β-lactamase structure and function. Ann N Y Acad Sci. 2013;1277:91-104

Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother. 2010;54(1):24-38

Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-318

Pulss S, Stolle I, Stamm I, Leidner U, Heydel C, Semmler T, Prenger-Berninghoff E and Ewers C (2018) Multispecies and Clonal Dissemination of OXA-48 Carbapenemase in *Enterobacteriaceae* From Companion Animals in Germany, 2009—2016. Front. Microbiol. 9:1265

Reinheimer C, Abdollahi P, Zacharowski K, et al. Prevalence of multidrug-resistant organisms in refugee patients admitted to a German university hospital depending on

duration of stay in Germany. GMS Hyg Infect Control. 2019;14:Doc07. Published 2019 Jun 28

Rice LB, Bonomo RA. beta -Lactamases: which ones are clinically important?. Drug Resist Updat. 2000;3(3):178-189

Ringbio. Beta lactams antibiotics: The greatest discovery, the biggest danger. 2018, Nov 18. Avaiable from: http://ringbio.com/press-release/introduction-of-beta-lactams-antibiotics

Ron Sender, Shai Fuchs, Ron Milo, Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans, Cell, Volume 164, Issue 3, 2016, Pages 337-340, ISSN 0092-8674

Sawa, T., Kooguchi, K. & Moriyama, K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. j intensive care **8,** 13 (2020)

Segagni Lusignani L, Presterl E, Zatorska B, Van den Nest M, Diab-Elschahawi M. Infection control and risk factors for acquisition of carbapenemase-producing *enterobacteriaceae*. A 5 year (2011-2016) case-control study. Antimicrob Resist Infect Control. 2020;9:18. Published 2020 Jan 17

Sib E, Voigt AM, Wilbring G, Schreiber C, Faerber HA, Skutlarek D, et al. Antibi-otic resistant bacteria and resistance genes in biofilms in clinical wastewater networks.International Journal of Hygiene and Environmental Health. 2019 May;222(4):655–662

Smith JD, Kumarasiri M, Zhang W, et al. Structural analysis of the role of *Pseudomonas aeruginosa* penicillin-binding protein 5 in β-lactam resistance. Antimicrob Agents Chemother. 2013;57(7):3137-3146

So A, Furlong M, Heddini A. Globalisation and antibiotic resistance. BMJ. 2010;341:c5116. Published 2010 Sep 21

Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR. Antibiotics, Resistome and Resistance Mechanisms: A Bacterial Perspective. Front Microbiol. 2018;9:2066. Published 2018 Sep 21

Teare L, Martin N, Elamin W, Pilgrim K, Tredoux T, Swanson J, et al. *Acine-tobacter* – the trojan horse of infection control?Journal of Hospital Infec-tion. 2019 May;102(1):45–53

Toussaint KA, Gallagher JC. β-lactam/β-lactamase inhibitor combinations: from then to now. Ann Pharmacother. 2015;49(1):86-98

Tsao LH, Hsin CY, Liu HY, Chuang HC, Chen LY, Lee YJ. Risk factors forhealthcare-associated infection caused by carbapenem-resistant *Pseudomonas aeruginosa*. Journal of Microbiology, Immunology and Infection. 2018 Jun; 51(3): 359–366

van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant *Enterobacteriaceae*. J Antimicrob Chemother. 2012;67(9):2090-2100

Wei WJ, Yang HF, Ye Y, Li JB. New Delhi Metallo-β-Lactamase-Mediated Carbapenem Resistance: Origin, Diagnosis, Treatment and Public Health Concern. Chin Med J (Engl). 2015;128(14):1969-1976