

Diploma thesis

Linezolid-Resistant Enterococci: Risk Factors and Clinical Impact

submitted by

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Doktorin der gesamten Heilkunde

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Graz, Austria, 11th July 2015

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I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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Abstract

Objectives:

Linezolid is standardly used as part of a second-line treatment for patients with febrile neutropenia at our hospital. Despite the low prevalence of linezolid resistance in enterococci monitored by international surveillance programs, we registered an increase in linezolid resistance at our hospital. The aim of the present study was to identify the mechanism of resistance and risk factors for the acquisition of linezolid-resistant *E. faecium* as well as to evaluate the clinical impact of colonisation or infection.

Methods:

The study was conducted at the Department of Internal Medicine, Division of Hematology, and Section of Infectious Diseases and Tropical Medicine, Medical University of Graz. All patients colonised or infected with *E. faecium* at the hematology ward between January 1st, 2008 and March 31st, 2014 were included.

We compared patients with linezolid-resistant *E. faecium* (LRE; N=57) to patients with linezolid-susceptible *E. faecium* (LSE; N=80) from the same ward during the same period by retrospectively reviewing charts and electronic medical records. The identification of resistance mechanisms was performed at the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Public Health England using PCR methods. To determine the institutional linezolid, vancomycin and daptomycin consumption we reviewed our hospital pharmacy data.

Results:

All tested LRE isolates were positive for heterozygous G2576T mutations in the 23S rRNA; testing for plasmid-mediated resistance via the *cfr*-gene was negative. No statistically relevant differences between LSE and LRE patients concerning age, underlying diseases, receipt of hematopoietic stem cell transplantation, comorbidities, duration of neutropenia, previous hospitalisations, length of stay, in-hospital mortality and one-year mortality could be found.

Statistically significant variables for the acquisition of LRE infections comprise previous linezolid exposure (Chi square $p < 0,0001$) and previous treatment with cefepime ($p = 0,031$) and piperacillin/tazobactam ($p = 0,031$). The administration of trimethoprim/sulfamethoxazole was associated with significantly lower LRE rates ($p = 0,001$).

Risk factors for the acquisition of invasive LRE infections include age (T-test $p=0,018$), duration of neutropenia (T-test $p=0,001$), duration of linezolid therapy (T-test $p=0,016$) and the number of LRE isolations (Mann-Whitney U-test $p=0,007$).

Conclusion:

We confirmed previous linezolid exposure as significant risk factor for the acquisition of LRE, irrespective of the duration of treatment and the number of linezolid exposures. Additional risk factors include previous treatment with cefepime and piperacillin/tazobactam. In terms of LOS and cumulative survival, no significant difference between LSE and LRE patients could be found.

Kurzfassung

Hintergrund:

PatientInnen mit febriler Neutropenie erhalten Linezolid im Rahmen einer Second-Line-Therapie am Universitätsklinikum Graz. Trotz der niedrigen Prävalenz von Linezolid-resistenten Enterokokken in internationalen Resistenz-Surveillance-Programmen, verzeichneten wir einen Anstieg der Linezolid-Resistenz an unserer Klinik. Ziel dieser Studie war die Identifikation des Resistenzmechanismus sowie die Ermittlung von Risikofaktoren für die Akquisition Linezolid-resistenter Enterokokken und des klinischen Impacts.

Methoden:

Die Durchführung der Studie erfolgte im Bereich der Inneren Medizin, Abteilung für Hämatologie und der Sektion für Infektiologie und Tropenmedizin, Medizinische Universität Graz.

Im Zeitraum von 1.Jänner 2008 bis 31.März 2014 wurden alle hämatologischen PatientInnen, welche eine Kolonisation oder Infektion mit *E. faecium* zeigten, in die Studie eingeschlossen.

Eine retrospektive Datenauswertung von elektronischen PatientInnendaten und Krankengeschichten ermöglichte einen Vergleich von PatientInnen mit Linezolid-resistentem *E. faecium* (LRE; N=57) und PatientInnen mit Linezolid-empfindlichem *E. faecium* derselben Station und desselben Zeitraums. Die Bestimmung von Resistenzmechanismen erfolgte mittels PCR an der Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Public Health England. Daten zum institutionellen Antibiotikaverbrauch erhielten wir von unserer Krankenhausapotheke.

Resultate:

Alle getesteten LRE-Isolate zeigten heterozygote G2576T Mutationen in der 23S rRNA; eine plasmid-medierte Resistenz über das *cfr*-Gen konnte ausgeschlossen werden. PatientInnen mit LSE und LRE wiesen keine statistisch signifikanten Unterschiede in Bezug auf Alter, Grunderkrankungen, Erhalt einer hämatopoetischen Stammzelltransplantation, Komorbiditäten, Dauer der Neutropenie, vorangegangene Hospitalisierungen, Dauer des stationären Aufenthalts, Hospitalsterblichkeit und 1-Jahres-Überlebensrate auf.

Statistisch signifikante Faktoren für LRE-Infektionen umfassen vorangegangene Linezolid- (Chi square $p < 0,0001$), Cefepim- ($p = 0,031$) und Piperacillin/Tazobactam-Therapien ($p = 0,031$).

Die Verabreichung von Trimethoprim/Sulfamethoxazol zeigte eine signifikante Risikoreduktion für LRE ($p=0,001$).

Risikofaktoren für die Akquisition invasiver LRE-Infektionen inkludieren Alter (T-test $p=0,018$), Dauer der Neutropenie (T-test $p=0,001$), Dauer der Linezolid-Therapie (T-test $p=0,016$) und die Anzahl der LRE-Nachweise (Mann-Whitney U-test $p=0,007$).

Zusammenfassung:

Wir identifizierten vorangegangene Linezolid-Therapie als signifikanten Risikofaktor für die Akquisition Linezolid-resistenter Enterokokken, unabhängig von Behandlungsdauer und Anzahl an Linezolidexpositionen. Weitere Risikofaktoren inkludieren vorangegangene Cefepim- und Piperacillin/Tazobactam-Therapie. In Bezug auf stationäre Aufenthaltsdauer und kumulatives Überleben konnte kein statistischer Unterschied zwischen PatientInnen mit LSE und LRE gefunden werden.

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Abbreviation

GIT	=	gastrointestinal tract
VRE	=	vancomycin-resistant enterococci
ICU	=	intensive care unit
UTI	=	urinary tract infection
BSI	=	bloodstream infection
CRP	=	C-reactive protein
MIC	=	minimum inhibitory concentration
transposons	=	transposable genetic elements
HLR	=	high-level resistance
PBP	=	penicillin-binding proteins
LOS	=	length of stay
GVHD	=	graft-versus-host disease
VSE	=	vancomycin-susceptible enterococci
CCI	=	Charlson Comorbidity Index
FDA	=	Food and Drug Administration
MDR	=	multi-drug resistance
Q/D	=	quinupristin/dalfopristin
ZAAPS	=	Zyvox® Annual Appraisal of Potency and Spectrum
HSCT	=	hematopoietic stem cell transplantation
ECIL	=	European Conference on Infections in Leukemia
ANC	=	absolute neutrophil count
IDSA	=	Infectious Diseases Society of America
AHA	=	American Heart Association
MRSA	=	methicillin-resistant <i>Staphylococcus aureus</i>
VREF	=	vancomycin-resistant <i>E. faecium</i>
LRE	=	linezolid-resistant enterococci
LSE	=	linezolid-susceptible enterococci
BMT	=	bone marrow transplantation
CLSI	=	Clinical and Laboratory Standards Institute
EUCAST	=	European Committee of Antimicrobial Susceptibility Testing
ALL	=	acute lymphocytic leukemia

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1 Introduction

1.1 Enterococcus Species

1.1.1 Taxonomy

In 1933 Rebecca Lancefield first classified enterococci as group D streptococci (1).

Group D streptococci included both enterococcal as well as nonenterococcal species, e.g. *Streptococcus bovis* or *Streptococcus equinus*. About fifty years later it was demonstrated that enterococci belong to a separate genus, *Enterococcus*, as they vary significantly from streptococci (2). Enterococci distinguish themselves by their ability to grow in the presence of 6,5% NaCl, 40% bile salts and at temperatures ranging from 10°C to 45°C (1). To definitively prove that former *Streptococcus faecalis* and *Streptococcus faecium* belong to the genus *Enterococcus*, molecular techniques have been used (3).

Apart from molecular approaches, phenotypic tests are applied for identification. On the basis of a positive/negative acid formation from mannitol or sorbose, and a positive/negative hydrolysis of arginine, enterococci are assigned to one of five different groups of species, which are shown in **Table 1** (3).

Group 1	Group 2	Group 3	Group 4	Group 5
<i>E. avium</i>	<i>E. faecalis</i>	<i>E. dispar</i>	<i>E. asini</i>	<i>E. canis</i>
<i>E. gilvus</i>	<i>E. faecium</i>	<i>E. durans</i>	<i>E. caccae</i>	<i>E. columbae</i>
" <i>E. hawaiiensis</i> "	<i>E. casseliflavus</i>	<i>E. hirae</i>	<i>E. cecorum</i>	<i>E. hermanniense</i>
<i>E. malodoratus</i>	<i>E. gallinarum</i>	<i>E. ratti</i>	<i>E. phoeniculicola</i>	<i>E. italicus</i>
<i>E. pallens</i>	<i>E. haemoperoxidus</i>	<i>E. villorum</i>	<i>E. sulfureus</i>	<i>E. moraviensis</i>
<i>E. pseudoavium</i>	<i>E. mundtii</i>			
<i>E. raffinosus</i>	" <i>E. sanguinicola</i> "			
<i>E. saccharolyticus</i>				

Table 1 Enterococcus Species (adapted from (3))

1.1.2 Microbiology

Enterococci are described as catalase-negative gram-positive cocci that occur in singles, pairs or as short chains. They are facultative anaerobes and have multiple intrinsic characteristics, which allow them to persist almost everywhere (3). This environmental ruggedness is the reason for enterococcal survival of inadequate cleaning processes leading to their persistence in hospitals (1). As a consequence, contaminated medical equipment, ward surfaces or the hands of medical personnel may lead to enterococcal spread via cross infection (4,5).

Enterococci can be detected in human feces, as they are natural inhabitants of the gastrointestinal tract (GIT) (2,6,7). They are an essential part of the normal enteric flora and can also be found in oropharyngeal and vaginal secretions as well as on the skin of the perineal region (2).

1.1.3 Clinical Aspects of Enterococcal Infections

1.1.3.1 Epidemiology

Although the genus *Enterococcus* includes more than 40 species, *E. faecalis* and *E. faecium* represent the two most important species of *Enterococcus*, which are significant pathogens for humans (7,8). Until recently, *E. faecalis* was responsible for 80% to 90% of enterococcal infections. In about 5% to 10% of the analysed clinical isolates *E. faecium* was found as causative pathogen (2,9).

Recent literature reports that the prevalence of enterococcal infections caused by *E. faecium* increased in the last years leading to a substitution of *E. faecalis* as the dominant enterococcal species (2,10). Seedat et al (9) presumes that the reason for this increase could be intrinsic and acquired resistance to many antimicrobial agents.

Concerning distribution in hospitals, nosocomial enterococcal bacteremia is ranked second after staphylococcal bacteremia regarding hospital-acquired infections in the United States (11,12).

1.1.3.2 Colonisation

One important factor that facilitates the dissemination of antibiotic resistance is the ability of enterococci to colonize the GIT for a long period of time (11). In order to determine how long carriage of e.g. vancomycin-resistant enterococci (VRE) lasts, Karki et al (13) conducted a 12-year retrospective cohort study. Patients with known VRE-colonisation and/or infection were included. After one year the percentage of colonised patients amounted to 40%. In year four the colonisation rate decreased to 23,3%. The maximum duration of carriage was 46,5 months (13).

Statistically relevant factors influencing the duration of colonisation included recent hospital admission as well as recent exposure to any antibiotics (oral and intravenous), yet amoxicillin-clavulanic acid, piperacillin/tazobactam, glycopeptides, meropenem, flouoroquinolones and aminoglycosides in particular (13).

As most enterococcal infections occur in a clinical setting, a reason for the dissemination in hospitals and between institutions has to be found (2).

In fact, colonized medical personnel may function as a reservoir of resistant enterococci, spreading them to patients under their care (2,5). It appears that colonised patients carry the resistant organisms in their gastrointestinal tract, before acquiring an infection (2).

1.1.3.3 Infection

1.1.3.3.1 Risk Factors

Colonisation with resistant strains is a well-known predisposing factor for consecutive infection (1,14).

Risk factors for a subsequent infection further involve patient age, debilitation, disruption of mucosal or epithelial barriers or an unbalance of the normal gastrointestinal flora by antibiotic treatment (8).

Accordingly the risk profile for developing a nosocomial enterococcal infection includes serious underlying disease, hospitalization for prolonged periods, prior surgery, renal insufficiency, immunosuppression (e.g. transplantation), neutropenia, invasive devices, as well as stay in an intensive care unit (ICU) (2).

Zhou et al (10) conducted a study to identify independent risk factors for *E. faecium* bloodstream infections (BSI) in hematology patients. Since patients with hematologic malignancies are debilitated by severe disease, immunosuppression and intensive treatment, mortality rates in patients with BSI are high ranging from 25% to 51%. In order to determine the risk of an *E. faecium* BSI and to consider pre-emptive treatment, a prediction model has been developed (10).

The prediction model is based on following risk factors: colonization with *E. faecium* 30 days prior to blood culture, combination of neutropenia and abdominal focus, age > 58 years, hospital stay prior to blood culture > 14 days and C-reactive protein (CRP) level >125 mg/l. Patients with five out of five variables at the moment of blood culture withdrawal have a risk of 47,5% for an *E. faecium* BSI. In patients without any of the described factors, the risk is almost zero (10).

1.1.3.3.2 Clinical Spectrum

Enterococci can cause a wide spectrum of infections ranging from urinary tract infections (UTI) as most common type of enterococcal infections to severe illness such as endocarditis or meningitis (3,15). In the United States enterococci rank as second or third leading cause of nosocomial UTIs, wound infections and bacteremia (3).

UTIs are primarily seen in hospitalized women and they are correlated with urologic manipulation by urinary catheterization or instrumentation (2).

In the majority of cases of enterococcal bacteremia there is no association with endocarditis. Endocarditis is less common than bacteremia, mainly occurring community acquired in older patients with structural abnormalities like valvular heart disease or prosthetic valves. Many cases of hospital acquired enterococcal bacteremia are associated with polymicrobial infections, which make the development of an endocarditis even less likely. Patients presenting with monomicrobial enterococcal bacteremia usually have severe underlying diseases and/or immunosuppression (2).

1.1.3.3.3 Enterococcal Pathogenicity and Virulence

In monomicrobial enterococcal bacteremia complications such as septic shock or disseminated intravascular coagulation occur rarely. However, the mortality rates in patients with enterococcal bacteremia are high ranging from 42% to 68%. Especially in polymicrobial bacteremia it is difficult to assess the involvement of enterococci concerning morbidity and mortality. Another problem in determining the pathogenicity of enterococci results in patient characteristics. Most of the patients with enterococcal bacteremia are severely debilitated by underlying illness, often also advanced in age (2). For that reason the determination of an attributable mortality of enterococcal bacteremia is rather complicated, as risk factors for mortality include severity of illness, patient age and use of broad-spectrum antibiotics (1).

Notwithstanding the above, various studies have described an attributable mortality of 31% to 37% (2).

As enterococci do not have typical virulence factors such as secretion of exotoxins or production of superantigens, they are less virulent than e.g. organisms like *Staphylococcus aureus* or *Streptococcus pyogenes* (2).

Enterococci are not highly toxigenic, invasive or infectious, however they are responsible for a considerable number of infections (1).

Relating to increasing enterococcal resistance to many standardly used antimicrobial agents, serious enterococcal infections have evolved into one of the leading therapeutic challenges (3).

1.1.3.4 Treatment

Antimicrobial therapy of enterococcal infections is complicated by inadequate activity of many classes of antimicrobial agents due to intrinsic resistance (**Table 2**) (2,16).

Zhou et al. (10) reports that in *E. faecium* infections clinicians are faced with intrinsic resistance to cephalosporins, aminoglycosides, clindamycin and trimethoprim/sulfamethoxazole. Consequently, the spread of antimicrobial resistance represents a substantive threat to public health (17).

Antimicrobial	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>
Fusidic Acid	R	R
Cephalosporins	R	R
Aminoglycosides	R	R
Erythromycin	R	R
Clindamycin	R	-
Quinupristin/dalfopristin	R	-
Vancomycin	-	-
Teicoplanin	-	-
Fosfomycin	-	-
Sulphonamides	R	R

Table 2 Intrinsic Resistance in *Enterococcus faecalis* and *Enterococcus faecium* (adapted from (16))
R = resistant.

1.1.3.4.1 Antimicrobial Susceptibility

As enterococci often present unusual patterns of susceptibility, specialized techniques for detecting their true susceptibility are needed in order to predict antimicrobial resistance. Standard methods of antimicrobial susceptibility testing may produce misleading results especially in β -lactamase-producing strains (2). The reason is that the quantity of β -lactamase produced by enterococci may be insufficient for detection by routine antimicrobial susceptibility testing (8).

In fact, β -lactamase producing enterococci do not exhibit minimum inhibitory concentrations (MICs) significantly different from those of non- β -lactamase-producing organisms (2).

Enterococcal susceptibility to standardly used antimicrobial agents is shown in **Table 3** (2).

Antimicrobial	<i>Enterococcus faecalis</i>		<i>Enterococcus faecium</i>	
	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
Ampicillin	1	1	8	32
Penicillin	2	4	16	64
Piperacillin	2	4	16	64
Imipenem	2	2	16	32
Vancomycin	2	2	1	2
Teicoplanin	0,5	1	0,5	1
Tetracycline	>16	>16	>16	>16
Choramphenicol	8	>16	4	16
Erythromycin	>256	>256	>256	>256
Ciprofloxacin	1	2	4	16
Linezolid	2	4	2	4
Quinupristin/dalfopristin	4	16	0,5	4
Daptomycin	0,5	1	2	4

Table 3 Antimicrobial Susceptibility of *Enterococcus faecalis* and *Enterococcus faecium*

(adapted from (2))

MIC = minimal inhibitory concentration.

The results presented in **Table 3** underlie temporal and geographic variation. Especially in the United States there are much higher resistance rates of *E. faecium* to ampicillin, penicillin and vancomycin than described in the table (2). The rate of vancomycin-resistant isolates of enterococci in the United States ranges from 19,4% in West North Central to 38,5% in the Middle Atlantic (18).

Latest data of enterococcal susceptibility results from the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) program for 2011 are presented in **Table 4** (19). Some of the results shown in **Table 4** differ from those reported by Moellering et al (2) in 2005, though it also includes strains of *E. avium* (14 strains), *E. casseliflavus* (3 strains), *E. durans* (3 strains), *E. gallinarum* (12 strains), *E. hirae* (4 strains), *E. raffinosus* (4 strains) and two unspciated *Enterococcus* spp. beside *E. faecalis* (452 strains) and *E. faecium* (266 strains) (19).

Antimicrobial agents	MIC ₅₀	MIC ₉₀
Linezolid	1	2
Ampicillin	1	>8
Ciprofloxacin	>4	>4
Daptomycin	1	2
Erythromycin	>16	>16
Levofloxacin	>4	>4
Teicoplanin	≤2	≤2
Vancomycin	1	4

Table 4 Antimicrobial Susceptibility of *Enterococcus* Species (adapted from (19))

MIC = minimal inhibitory concentration.

1.1.3.4.2 Antimicrobial Resistance

Intrinsic and Acquired Antibiotic Resistance

The striking attribute of most enterococcal species is their resistance to many antibiotic classes. There are two forms of antibiotic resistance, intrinsic and acquired (3).

Intrinsic resistance is characterized by inherent features occurring in all or most enterococci. Particular mechanisms of intrinsic resistance are typically related to certain enterococcal species. Intrinsic resistance affects two elementary groups of antimicrobial agents, aminoglycosides and β -lactams (3).

In addition, enterococci may acquire new mechanisms of resistance (2). Acquired enterococcal resistance is caused by mutations in existing DNA or by acquisition of new genetic determinants encoded on plasmids or transposable genetic elements (transposons) (3,10).

Enterococci are able to transfer resistance genes via three distinct transfer systems (2).

The first mechanism is defined by narrow-host-range plasmids, which are responsible for transferring resistance genes only among enterococci (2).

The second mechanism, however, involves broad-host-range plasmids contributing to a conjugal exchange between *Enterococcus* species, various species of streptococci, *S. aureus*, lactobacilli, and others (2).

Thirdly, enterococci can use conjugative transposons for transferring resistance genes (2).

Notably, the exchange of conjugative plasmids and transposons among enterococci contributed to a worldwide increase in multiple antimicrobial resistance mechanisms, particularly in high-level resistance (HLR) to aminoglycosides, β -lactams and glycopeptides (mainly vancomycin) (2,3).

Arias et al (11) reports that the majority of *E. faecium* isolates are meanwhile resistant to ampicillin and vancomycin, additionally they frequently express HLR to aminoglycosides. Since these antibiotics represent three of the standardly used antienterococcal antibiotics, therapeutic options are massively restricted (11).

Intrinsic and acquired resistance to a variety of antimicrobial agents is depicted in **Table 5** (2).

<i>Intrinsic resistance</i>	<i>Acquired resistance</i>
Aminoglycosides	Aminoglycosides
β -Lactams (relatively high MICs)	β -Lactams (altered PBPs)
Lincosamides	Cell wall-active agents
Trimethoprim/sulfamethoxazole (in vivo only)	Fluoroquinolones
Quinupristin/dalfopristin (<i>E.faecalis</i>)	Lincosamides
	Macrolides
	Penicillin, Ampicillin
	Rifampin
	Tetracyclines
	Vancomycin
	Quinupristin/dalfopristin
	Oxazolidinones (e.g. Linezolid)

Table 5 Antimicrobial Resistance in Enterococci: Intrinsic and Acquired Resistance (adapted from (2))

MIC = minimal inhibitory concentration. PBP = penicillin-binding protein.

1.1.3.4.2.1 β -lactam Antibiotics

As shown in **Table 5** all enterococci share an intrinsic, relative resistance to β -lactam antibiotics (2).

Relative resistance signifies that the enterococci present an increase in minimum inhibitory concentrations (MICs), yet they are susceptible at antibiotic concentrations higher than generally used (20).

Enterococci with absolute (high level) resistance show a sudden MIC-increase, which can't be overcome by higher doses of the antimicrobial agent (20).

Enterococci are resistant to all cephalosporins, furthermore they are able to exhibit resistance to penicillins via two different mechanisms (8).

The first mechanism to describe is the enterococcal β -lactamase-production, which causes resistance to penicillin and ampicillin (8).

β -lactamase-producing *E. faecium* strains were initially detected in eastern Texas in the early 1980s and subsequently disseminated to a number of other cities in the United States and Argentina (2). Isolates of β -lactamase-producing *E. faecalis* currently have been found in diverse locations in the United States and other geographic locations (8).

However, enterococci exhibiting the resistance mechanism of β -lactamase-production have been identified rarely compared to those presenting the second mechanism of penicillin resistance (3).

The second mechanism is characterized by an alteration of the penicillin-binding proteins (PBP), particularly PBP5, leading to a decreased affinity of *E. faecalis* and *E. faecium* for penicillin, ampicillin and other β -lactam antibiotics such as cephalosporins (2,8).

This mechanism is common among *E. faecium* strains, which are typically more resistant to β -lactams than isolates of *E. faecalis* (8).

Actually, recent data suggests a remarkable increase in intrinsic resistance to penicillins among *E. faecium* strains. Continuing alterations in PBP5 have led to the fact that many enterococcal isolates in the United States and other countries currently show a relative and absolute resistance to penicillin and ampicillin (2).

1.1.3.4.2.2 Aminoglycosides

Enterococcal resistance to aminoglycosides results from the diminished ability of these antibiotics to penetrate through the outer cell wall envelope of enterococci (2).

To hinder enterococci from blocking the uptake of aminoglycosides at the cell wall, the addition of a cell wall-active agent is needed (1).

As mentioned earlier, there is an increase in acquired antimicrobial resistance, transferred by resistance genes on plasmids and transposons. Another mechanism of acquired resistance, which isn't plasmid or transposon mediated, is the tolerance of enterococci to cell wall-active agents, especially if briefly exposed (2). Additionally to the quick acquisition of tolerance to cell wall-active agents, there is a rapid dissemination of HLR to aminoglycosides, making the treatment of serious enterococcal infections even more challenging (1,2).

Most enterococci exhibit resistance to streptomycin, while HLR to gentamicin is less widespread, yet steadily increasing since the 1980s (2,8).

Causes for the acquisition of aminoglycosidic HLR include either ribosomal mutation (only streptomycin) or the production of aminoglycoside-modifying enzymes, mediated by plasmids (2). In fact, ribosomal mutations occur rarely, while aminoglycoside-modifying enzymes present the most common resistance mechanism concerning aminoglycosides (16).

The enzyme responsible for high-level gentamicin resistance also confers resistance to all other therapeutically used aminoglycosides except streptomycin. If that certain enzyme co-occurs with the enzyme 6-adenylyltransferase, which affects susceptibility to streptomycin, enterococci are resistant to all available combinations of cell wall-active agents and aminoglycosides. In order to prevent clinical failures and relapse in seriously ill patients, high-level aminoglycoside testing should be performed (2).

1.1.3.4.2.3 Trimethoprim/Sulfamethoxazole

Enterococcal susceptibility to trimethoprim/sulfamethoxazole is only given in vitro. In vivo enterococci circumvent the block in folate synthesis induced by trimethoprim/sulfamethoxazole by using exogenous folinic acid, dihydrofolate and tetrahydrofolate (2).

1.1.3.4.2.4 Vancomycin

The first detection of VRE dates back to 1988 (21). Initially, resistant isolates could be found in western Europe followed by a dissemination to the United States as well as other geographic locations (3).

Over the next years the resistance rate has increased significantly from 0,3% around the early 1990s up to 47% (22). DiazGranados et al (23) report a prevalence of vancomycin resistance among enterococcal infections of 14% to 25% in the United States, whereas Mave et al (24) refer to 60% of vancomycin-resistant *E. faecium* isolates causing nosocomial BSIs. Further to 2011 ZAAPS Program results from 79 medical centers on five continents, the enterococcal vancomycin resistance rate (59,5% *E. faecalis*, 35% *E. faecium*) increased from 8,2% in 2007 to 11,7-12,9% in 2008-2009 following a decrease to 8% in 2010 and to 8,2% in 2011 (19).

Regarding the emergence of VRE in Europe, a correlation could be found with the agricultural use of the glycopeptide avoparcin (11,25). Subsequently the use of avoparcin has been banned, which led to a decrease of VRE rates in animals (26), yet the dissemination of VRE didn't terminate (11).

Data suggest that specific gene clusters are responsible for transferring glycopeptide resistance in *E. faecium* (11).

Consequently, vancomycin has become obsolete as antimicrobial agent in patients with *E. faecium* infections (at least in the US) (11).

In 1997 the Surveillance Network Database-USA collected data from more than 100 laboratories. Out of 1482 obtained isolates of *E. faecium* 52% exhibited resistance to vancomycin, 83% were resistant to ampicillin as well. Multidrug resistance profiles like these complicate therapeutic options and are therefore of great concern. The vancomycin resistance rates in isolates of *E. faecalis* greatly differ from those of *E. faecium* exemplified by an incidence of only 1,9% of 4364 isolates (2).

In a more recent study conducted in 2006/2007 by the Center of Disease Control the vancomycin resistance rate of *E. faecium* strains amounted to 80% among 983 tested isolates. As well as in previous studies, the resistance rate of *E. faecalis* was much lower with only 6,9% among 1542 analysed clinical isolates (11).

In 2013 the highest vancomycin resistance rates in Europe have been reported in Latvia, Luxembourg and Greece (for isolates of *E. faecalis*), as well as Ireland, the United Kingdom and Cyprus (for isolates of *E. faecium*) (27).

1.1.3.4.2.4.1 Resistance Phenotypes

The classification of VRE strains is based on phenotypic and genotypic characteristics. By now six types of VRE have been identified, including three major vancomycin resistance phenotypes (3).

While enterococci presenting the VanA phenotype (encoded by the *vanA* gene) exhibit HLR to vancomycin and teicoplanin, strains with the VanB phenotype (encoded by *vanB1* and *vanB2* genes) show moderate to high level resistance to vancomycin, yet remain susceptible to teicoplanin (2,3,15). Reportedly, there are some cases of emerging teicoplanin resistance during therapy in VanB strains (8).

The VanA and VanB phenotypes appear to be the most clinically relevant phenotypes occurring mainly in *E. faecium* and *E. faecalis* (3).

By cloning and sequencing it could be demonstrated that both the *vanA* gene and the *vanB* genes are transferable. Additionally it was shown that the VanA phenotype is associated with the

production of a ligase with altered specificity resulting in the inability of vancomycin to bind to the altered cell wall of affected enterococci (2).

The third of the three most common phenotypes, VanC, has been described in strains of *E. gallinarum* (vanC1 genotype) and *E. casseliflavus* (vanC2 and vanC3 genotypes) conferring low-level resistance to vancomycin without teicoplanin resistance (3). VanC genes are generally not transferable (2).

Further glycopeptide resistance phenotypes include VanD, VanE and VanG, though occurring less frequently (3). VanD has been found in isolates of *E. faecium* showing moderate level resistance to both vancomycin and teicoplanin. VanE has been described in *E. faecalis* and appears to exhibit similarities to the VanC phenotype (2).

Concerning VanA, VanB and VanD phenotypes, the encoding genes are located on transposons, which do not seem to have originated in enterococci. Up to now the original source has not been identified, however gastrointestinal anaerobes have been discussed as potential origin (2).

1.1.3.4.2.4.2 Predisposing Factors

In fact, an infection with VRE is a characteristic of patients with a complex medical condition illustrating the severity of underlying disease (6).

Risk factors for a colonisation or infection with VRE involve the duration of antimicrobial treatment, intravenous administration of vancomycin, invasive devices, severe underlying disease, length of stay (LOS), immunosuppression and abdominal surgery (2,5).

Bradley et al (4) conducted a study to examine the acquisition of glycopeptide-resistant enterococci in a hematology unit. Data indicates that patients already colonised are the most significant risk factor for colonisation of new patients, being more important than antibiotic exposure and absence of infection control measures (4).

The results from a study by Vydra et al (6) determine colonization with VRE, delay in engraftment, as well as severe acute graft-versus-host disease (GVHD) as significant risk factors for acquisition of VRE bacteremia.

Another study by Bradley et al (28) examined the clinical impact of a formulary switch from empirical ceftazidime to piperacillin/tazobactam in hematologic patients with neutropenic fever. The altered antimicrobial regimen in combination with reinforced infection control measures lead

to a decrease in colonization and infection by VRE. To confirm these results, VRE subsequently reemerged as soon as returning to empirical ceftazidime (**Table 6**) (28).

<i>Phase*</i>	<i>Empirical Therapy</i>	<i>Colonization with VRE</i>	<i>No. of Infections with VRE/Total No. of Patients</i>
1	Ceftazidime	57%	5/75
2a	Piperacillin/tazobactam + infection control reinforced	29%	0/70
2b	Piperacillin/tazobactam + infection control reinforced	8%	0/59
3	Ceftazidime + infection control reinforced	36%	3/58

Table 6 Incidence of Colonization and Infection in Hematologic Patients in Relation to Preferred Empirical Antibiotic Treatment in Neutropenic Fever (adapted from (28))

* each phase was defined as a 4-month-period; identical regimes were used in phases 2a and 2b

Up to recently an enterococcal bacteremia was listed as a rare complication in patients with malignancies. Besides it was uncomplicated in treatment for the case it did occur as it either subsided spontaneously or responded well to therapy. With the dissemination of VRE the situation changed dramatically. Currently the number of bacteremias and infections with VRE in bone marrow transplant patients is rising, resulting in an increased risk of VRE infections for other patients with malignancies. Extensive vancomycin use for therapeutic or pre-emptive reasons acts as a substantive predisposing factor for colonisation and bacteremia with VRE (2).

1.1.3.4.2.4.3 Clinical Impact

The emergence of VRE poses enormous challenges for clinicians since there are only a few clinical trials regarding therapy options. Additionally only a few antibiotics have an FDA approval for the treatment of severe VRE-BSIs (12,24). Possible antibiotic agents in the treatment of VRE include daptomycin, linezolid, quinupristin/dalfopristin and tigecycline (12).

To determine the impact – in terms of outcome, LOS and hospitalization costs – of vancomycin resistance in patients with enterococcal bacteremia, Cheah et al (29) collected data from 116 patients with VRE (VanB phenotype) and matched these 1:1 with data from patients with vancomycin susceptible enterococci (VSE).

It was shown that vanB VRE bacteremia is not significantly associated with increased mortality. The results suggest that VRE bacteremia is linked to prolonged LOS and increased hospitalization costs without affecting mortality. Risk factors for increased mortality include prior ICU admission, Charlson Comorbidity Index (CCI) ≥ 4 as well as longer time to appropriate antibiotics. Antimicrobial treatment with linezolid was proven to decrease mortality in patients with VRE bacteremia (29).

In patients with VSE bacteremia, a longer duration to appropriate antibiotic treatment independently increased mortality rates, LOS and cost (29).

To summarize, the study highlights that there is no association between vanB VRE bacteremia and increased mortality. On the contrary, the meta-analysis of previous study findings in terms of vanA VRE bacteremia showed more than doubled odds of mortality compared to a VSE bacteremia (12,24,29). Regarding this, a potential bias has been discussed as the effect of vancomycin resistance may have been distorted by antibiotics ineffective against VRE (29).

In contrast, Christiansen et al (30) report that studies with a small number of patients could not find a statistically relevant increase in mortality rates in patients with vancomycin resistance, whereas larger studies determined increased mortality. In common with Cheah et al (29), prolonged LOS in patients with VRE bacteremia was demonstrated (30).

Vydra et al (6) conducted a study to analyse outcomes in VRE and VSE BSIs among allogeneic hematopoietic stem cell transplantation (HSCT) recipients. A comparison of mortality rates and overall survival of pediatric and adult patients, respectively, is shown in **Table 7** (6).

<i>Outcome</i>	<i>VRE BSI</i>	<i>VSE BSI</i>	<i>No enterococcal BSI</i>
Pediatric patients, no.	10	22	229
1-y nonrelapse mortality	30%	9%	15%
1-y overall survival	70%	86%	80%
Adult patients, no.	40	21	430
1-y nonrelapse mortality	53%	33%	22%
1-y overall survival	23%	48%	63%

Table 7 Comparison of Overall Survival and Nonrelapse Mortality in Patients with Vancomycin-Resistant or Vancomycin-Susceptible Bloodstream Infections (adapted from (6))

VRE = vancomycin-resistant enterococci. VSE = vancomycin-susceptible enterococci.
BSE = bloodstream infection.

According to DiazGranados et al (23) the impact of vancomycin resistance on outcomes in enterococcal BSI is difficult to determine. As a consequence, various studies have provided conflicting results. These differences may result from distinct patient populations. Studies that have reported increased mortality in VRE bacteremia often enrolled high-risk patient populations, whereas studies with diverse patient groups could not determine a statistically significant association (except for multicenter studies) (23).

Especially patients with hematologic malignancies are at high risk, because of long-lasting neutropenia. A comparison of mortality of VRE vs. VSE BSI among patients with neutropenia showed that VRE BSI was significantly associated with increased mortality rates, yet this effect only became apparent 10 days after the onset of bacteremia (23).

Apart from that it is important to know that the enrollment of patients happened before the approval of linezolid and quinupristin/dalfopristin with the result that the availability of these agents for initial therapy (within 48h of the onset of bacteremia) was limited (23).

McKinnell et al (12) have used the fact that the majority of previous studies were conducted prior to routinely use of novel antimicrobials with activity against VRE as an opportunity for further investigation. The results from this study suggest that improved survival linked to treatment with antibiotics active against VRE only reaches statistical significance in univariate analysis, while those findings are not significant in multivariate analysis (12).

1.1.3.4.3 Treatment Options

As mentioned earlier, treatment of serious enterococcal infections necessitates routinely performed antimicrobial susceptibility testing in order to determine antibiotic resistance and to prevent relapse after therapy (2).

1.1.3.4.3.1 Uncomplicated Enterococcal Infections

In terms of enterococcal infections not requiring bactericidal treatment (e.g. uncomplicated UTIs, wound infections, intra-abdominal infections) the standard antimicrobial therapy is penicillin or ampicillin (2).

However, tolerance to penicillin and other β -lactams is widespread in enterococci, differentiating

them from most streptococci, which are commonly susceptible at lower β -lactam concentrations (11). For patients with penicillin allergy or isolates with penicillin resistance, other agents such as vancomycin or teicoplanin can be used (2).

Further agents used to treat lower uncomplicated UTIs attributable to enterococci include nitrofurantoin and fosfomycin. Fosfomycin has an Food and Drug Administration (FDA) approval for the treatment of UTIs caused by vancomycin-susceptible *E. faecalis* (11).

Fluoroquinolones do not belong to the antibiotics of choice because of their diminished activity for enterococcal infections in general (ciprofloxacin, ofloxacin) and their increasing resistance. The enterococcal resistance rates against erythromycin and related macrolides amounts to 80% to 90% in the United States (2).

Referring to significance of enterococci in UTIs, Hooton et al (31) showed that, although frequently isolated from midstream urine, enterococci rarely cause acute uncomplicated cystitis by themselves. Among 202 paired specimens of midstream urine and catheter urine, enterococci were isolated from 20 cultures of midstream urine, but only from two cultures of catheter urine. The positive predictive values for enterococci causing bladder bacteriuria are low, regardless of colony counts (31).

1.1.3.4.3.2 Severe Enterococcal Infections

Currently the standard treatment for severe enterococcal infections (e.g. endocarditis, meningitis) is based on a combination therapy including an aminoglycoside (usually gentamicin or streptomycin) plus a cell wall-active agent (usually penicillin, ampicillin or vancomycin) (2).

As most enterococci are resistant to streptomycin, the use of gentamicin should be preferred, unless susceptibility testing indicates otherwise (8).

Concerning limitations in the use of aminoglycosides, the nephrotoxic potential, primarily in high risk patients, has to be mentioned. Alternatively, a combination of ampicillin and ceftriaxone/cefotaxime could be used, although not FDA approved and only in *E. faecalis* strains (11).

Patients with endovascular infections do not respond appropriately to β -lactam monotherapy. An adequate bactericidal therapy cannot be achieved, since enterococci express a lack of killing to ampicillin or penicillin alone (11).

Another important mechanism in penicillin monotherapy is called the “Eagle effect”. The “Eagle effect” describes a decreased killing effect of penicillin if exceeding the concentrations for optimal effectiveness (paradoxical bactericidal effect) (32).

Before the detection of the penicillin-aminoglycoside synergism in 1947, there have been relapse rates between 30% and 60% in patients with enterococcal endocarditis and penicillin monotherapy (2).

In **Table 8** the standard antibiotic therapy regimen for the treatment of enterococcal endocarditis is depicted (33).

<i>Antibiotic</i>	<i>Dosage</i>	<i>Route of Administration</i>	<i>Duration (weeks)</i>
β-lactam and gentamicin susceptible strain (for resistant isolates see^{a,b,c})			
Amoxicillin <i>with</i>	200 mg/kg/day in 4-6 doses	IV	4-6
Gentamicin ^d	3 mg/kg/day in 2 or 3 doses	IV or IM	4-6
OR			
Ampicillin <i>with</i>	200 mg/kg/day in 4-6 doses	IV	4-6
Gentamicin ^d	3 mg/kg/day in 2 or 3 doses	IV or IM	4-6
OR			
Vancomycin ^e <i>with</i>	30 mg/kg/day in 2 doses	IV	6
Gentamicin ^d	3 mg/kg/day in 2 or 3 doses	IV or IM	6

Table 8 Antimicrobial Therapy of Infective Enterococcal Endocarditis (adapted from (33))

IV = intravenous. IM = intramuscular.

^aHLR to gentamicin (MIC >500mg/L): if susceptible to streptomycin, replace gentamicin with streptomycin 15 mg/kg/day in two equally divided doses. Otherwise, use more prolonged course of β-lactam therapy. The combination of ampicillin with ceftriaxone was recently suggested for gentamicin-resistant *E. faecalis*.

^bβ-Lactam resistance: (1) if due to β-lactamase production, replace ampicillin with ampicillin-sulbactam or amoxicillin with amoxicillin-clavulanate; (2) if due to PBP5 alteration, use vancomycin-based regimens.

^cMultiresistance to aminoglycosides, β-lactams, and vancomycin: suggested alternatives are

(1) linezolid 2 x 600 mg/day IV or orally for ≥8 weeks (monitor hematological toxicity),

(2) quinupristin/dalfopristin 3 x 7.5 mg/kg/day for ≥8 weeks,

(3) β-lactam combinations including imipenem plus ampicillin or ceftriaxone plus ampicillin for ≥8 weeks

^dMonitor serum levels of aminoglycosides and renal function.

^eIn β-lactam allergic patients. Monitor serum vancomycin concentrations.

In cases of uncomplicated enterococcal bacteremia data does not consistently recommend a combination therapy since numerous studies could not provide an advantage over monotherapy. However, particularly in immunocompromised and critically ill patients, infectious disease specialists often prefer combination therapy as it appears to be more effective in these clinical scenarios (2).

With the emergence of multi-drug resistant (MDR) enterococci, choosing the appropriate antimicrobial regimen became very challenging, often requiring the backup of an accomplished infectious disease specialist (2).

Different options for antimicrobial treatment in relation to specific resistance patterns are shown in **Table 9** (8).

Resistance Pattern	Recommended Therapy
β-Lactamase production	ampicillin/sulbactam gentamicin + amoxicillin/clavulanate imipenem <i>or</i> vancomycin
β-Lactam resistance, no β-Lactamase production	gentamicin + vancomycin
High-level gentamicin resistance	<u>Streptomycin-sensitive isolate:</u> streptomycin + ampicillin <i>or</i> vancomycin <u>Streptomycin-resistant isolate:</u> no proven therapy (continuous-infusion ampicillin, prolonged treatment)
Vancomycin resistance	ampicillin + gentamicin
Vancomycin and β-lactam resistance	No established therapies providing uniformly bactericidal activity; <u>experimentally:</u> ciprofloxacin + rifampin + gentamicin <i>or</i> ampicillin + vancomycin <i>or</i> chloramphenicol <i>or</i> tetracycline <u>newer agents:</u> linezolid (all enterococci) <i>or</i> quinupristin/dalfopristin (<i>E. faecium</i> only)

Table 9 Treatment Options in Relation to Antibiotic Resistance Patterns (adapted from (8))

Another alternative for the treatment of endovascular enterococcal infections are higher doses of daptomycin and a combination of daptomycin with other antimicrobial agents, respectively (11). Daptomycin is a lipopeptide antibiotic and FDA approved for the treatment of complicated skin and skin structure infections as well as bacteremia caused by gram-positive organisms (11,34). In vitro, daptomycin exhibits rapid concentration-dependent bactericidal activity against enterococci, synergism with rifampin against *E. faecium* and with fosfomicin and gentamicin against *E. faecalis* (11,24). For that reason, possible alternative therapeutic options in the treatment of vancomycin-resistant *E. faecium* endocarditis include high-dose daptomycin plus ampicillin plus gentamicin, daptomycin plus gentamicin plus rifampin or daptomycin plus tigecycline (11).

However, the FDA approval of daptomycin only includes treatment of vancomycin-susceptible isolates of *E. faecalis*, not VRE or *E. faecium* (24).

Additionally, daptomycin is not licensed for the treatment of left-sided infective endocarditis (34). According to Mave et al (24), daptomycin is the only FDA approved antimicrobial agent showing bactericidal activity against VRE, yet clinical data are still lacking except for a few case reports. Because of that, Mave et al (24) conducted a study to compare the efficacy of daptomycin with that of linezolid in treatment of VRE BSI. It was shown that daptomycin is as effective as linezolid, since microbiological cure, relapse and mortality rates appeared to be equivalent (24).

Clinical breakpoints for daptomycin and enterococci have not yet been established by EUCAST though (35).

Quinupristin/dalfopristin (Q/D) was the first antibiotic agent approved for the therapy of VRE-BSIs (24). Q/D is a streptogramin antibiotic and has an FDA approval for the treatment of infections with vancomycin-resistant *E. faecium*. Strains of *E. faecalis* are not included because of their high resistance rate caused by the *lsa* gene (11,24). According to the recommendations of the American Heart Association, Q/D is a possible option for the treatment of MDR *E. faecium* endocarditis (11).

By an interaction with the 50S ribosomal subunit, Q/D leads to an inhibition of bacterial protein synthesis, although multiple enterococcal resistance patterns have already been reported. There are recommendations of using Q/D as part of a combination regimen (e.g. in the treatment of *E. faecium* endocarditis), preferably with doxycycline, gentamicin, rifampin, high-dose ampicillin, imipenem or levofloxacin. Potential adverse events of Q/D-therapy include arthralgia and myalgia, leading to a discontinuation of antibiotic treatment in some patients (11).

Tigecycline as broad-spectrum antibiotic and derivate of minocycline is FDA approved for the treatment of skin and soft tissue infections, exhibiting eradication rates in vancomycin-susceptible *E. faecalis* infections comparable to vancomycin plus aztreonam (91,7%). The mechanism of action includes an interaction with the bacterial 30S ribosomal subunit leading to an inhibition of protein synthesis. The resistance rate is low with only one reported case of tigecycline-resistant *E. faecalis*. The combination of tigecycline with bactericidal agents may result in a synergistic effect (11).

A further treatment option in VRE infections is chloramphenicol. The results from a recent study with 51 patients have shown a microbiological eradication rate of 79%, also no major side effects have been reported. A combination therapy of chloramphenicol plus minocycline has been used in the treatment of prosthetic valve endocarditis caused by MDR *E. faecium* (11).

Additionally, tetracycline antibiotics are components of combination therapies in the treatment of severe MDR enterococcal infections (11).

Furthermore doxycycline had a preventive effect for the development of linezolid resistance (11).

Table 10 gives a brief summary about the treatment options discussed concerning MDR (in this case: ampicillin plus vancomycin) enterococcal infections (11).

<i>FDA approved</i>	<i>Not approved but potential clinical use</i>	<i>Investigational</i>
Linezolid	Daptomycin	Oritavancin
Quinupristin/dalfopristin (<i>E. faecium</i> only)	Tigecycline (not recommended as monotherapy)	Ceftobiprole (only <i>E. faecalis</i>)
	Nitrofurantoin*	Ceftaroline (only <i>E. faecalis</i>)
	Fosfoymcin*	
	Doxycycline, Minocycline [§]	
	Fluoroquinolone [§]	
	Rifampin [§]	
	Chloramphenicol	

Table 10 Antibiotic Treatment of Ampicillin- and Vancomycin-Resistant Enterococcal Infections (adapted from (11))

* only for uncomplicated UTIs

§ only if susceptible and as part of a combination therapy

1.1.3.4.3.3 Escalation and De-escalation Strategies in Febrile Neutropenic Patients

Treatment of malignancies, especially in patients with hematologic malignancy and/or HSCT, is associated with intensive immunosuppression and/or myelosuppression leading to an increased risk of serious infections (10,14).

The prevalence of BSI in HSCT recipients amounts to 13-60% with mortality rates of 12-42% (14).

The risk of infection increases with severity (absolute neutrophil count (ANC)) and duration of neutropenia (36). Risk groups are defined as following:

- low-risk at a duration of neutropenia >5 days,
- standard risk at a duration of 6-7 days, and
- high risk at a duration of ≥ 10 days (37).

The Infectious Diseases Society of America (IDSA) definition of febrile neutropenia includes:

- a) *neutropenia*: ANC < 500 cells/mm³ or an expected ANC decrease to < 500 cells/mm³ during the following 48 h
- b) *fever*: a single oral temperature measurement of $\geq 38,3^{\circ}\text{C}$ or a temperature of $\geq 38^{\circ}\text{C}$ sustained for the duration of at least 1 h (38)

In view of growing antibiotic resistance and a restricted contingent of new antibiotic agents, the Expert Group of the 4th European Conference on Infections in Leukemia (ECIL) has presented guidelines for initial empirical therapy in patients with febrile neutropenia (14).

The ECIL-4 guidelines differentiate between an escalation and a de-escalation approach and consider the local resistance epidemiology, as well as the patient's risk factors for resistant bacteria and for a complicated clinical course (14).

The principle of the escalation strategy is described as an initial empirical monotherapy (e.g. ceftazidime, cefepime or piperacillin/tazobactam) covering typical pathogens as *Enterobacteriaceae* and *P. aeruginosa* excluding bacteria producing ESBL, carbapenemases or other MDR bacteria (14).

In case of clinical deterioration or isolation of a resistant pathogen, therapy is "escalated" to an antibiotic with a broader spectrum or a combination therapy (14).

The de-escalation approach is based on a very broad initial empirical therapy with coverage of even MDR pathogens. The de-escalation strategy recommends the early use of “reserved” broad-spectrum antibiotics like carbapenems (e.g. imipenem or meropenem), or combinations of colistin plus a β -lactam, or an aminoglycoside plus a β -lactam, if necessary plus a further anti-Gram-positive agent. If no resistant pathogen could be identified, the antibiotic regimen is de-escalated to a narrower spectrum (14).

Criteria for choosing either the escalation or the de-escalation approach are listed in

Table 11 (14).

	<i>Escalation</i>	<i>De-escalation</i>
INDICATION	<ul style="list-style-type: none"> a) uncomplicated presentation b) no known colonisation with resistant bacteria c) no previous infection with resistant bacteria d) in centers where infections due to resistant pathogens are rarely seen at the onset of febrile neutropenia 	<ul style="list-style-type: none"> a) complicated presentation b) known colonisation with resistant bacteria c) previous infection with resistant bacteria d) in centers where resistant pathogens are regularly seen at the onset of febrile neutropenia

Table 11 ECIL-4 Criteria for Choosing Escalation or De-Escalation Approach (adapted from (14))

1.2 Linezolid

Linezolid is a synthetic antibiotic that inhibits early stages of bacterial ribosomal protein synthesis by interaction with domain V of the 23S rRNA. The mechanism of action involves binding to the 50S ribosomal subunit and therefore preventing the translation of mRNA. It exhibits bacteriostatic activity against staphylococci and enterococci, furthermore a bactericidal effect has been shown against certain strains of streptococci. Linezolid is an oxazolidinone antibiotic and the first member of the oxazolidinone class to obtain an FDA approval. Further, the oxazolidinone class was the first new class of antibiotics to reach the market since 1980 (7,11,19,22,39–44).

The efficacy of linezolid comprises the majority of clinically relevant gram-positive cocci, a few gram-negative aerobes, as well as some mycobacteria (39,41). Linezolid is currently approved for the treatment of uncomplicated and complicated skin and skin structure infections and nosocomial pneumonia due to gram-positive susceptible bacteria (40,44).

Besides, linezolid is commonly used to treat serious infections caused by VRE and methicillin-resistant *Staphylococcus aureus* (MRSA) and shows activity against penicillin-resistant streptococci. Additionally it has an American Heart Association (AHA) recommendation for the treatment of infective endocarditis caused by MDR enterococci (11,40,41,45).

Notably, linezolid represents the first antibiotic approved for the treatment of MRSA infections in more than 40 years and the first oral antibiotic for the treatment of VRE infections (43).

The pharmacokinetic profile involves 100% oral bioavailability, an elimination half-life of 5-7 h, a distribution volume of >0,8 l/kg, a good tissue penetration, and a clearance of 140 ml/min (39).

Dosage for adult patients and administration of linezolid are depicted in **Table 12** (46).

Infection	Dosage, Route and Frequency of Administration	Duration (days)
Nosocomial pneumonia	600 mg intravenous or oral every 12 hours	10 to 14
Community-acquired pneumonia, including concurrent bacteremia		
Complicated skin and skin structure infections		
Vancomycin-resistant <i>Enterococcus faecium</i> infections, including concurrent bacteremia		14 to 28
Uncomplicated skin and skin structure infections	400 mg oral every 12 hours	10 to 14

Table 12 Dosage Guidelines for Linezolid (adapted from (46))

The maximum duration of treatment should not exceed 28 days (46).

Linezolid is metabolised by non-enzymatic oxidation of the morpholine ring, hence no dosing adjustments for patients with renal or hepatic dysfunction are needed. Additionally, no testing for adequate serum concentrations is needed (7,39).

Before the drug's FDA approval in 2000 (18,40,41,47), Birmingham et al (39) conducted an open-label, non-comparative, non-randomized compassionate-use program to evaluate safety, tolerance and efficacy of linezolid. Linezolid was administered to patients requiring effective treatment of infections due to MDR gram-positive organisms, yet not meeting the criteria to be enrolled in phase 3 trials for several reasons (e.g. severity of illness, MDR pathogens, numerous comorbidities). Between 1997 and 2000, 796 patients were enrolled, most of them presenting with severe comorbid conditions such as malignancy, neutropenia, receipt of organ transplant and end-stage renal disease. Isolated causative organisms mainly included vancomycin-resistant *E. faecium* (VREF) (59,2%) and MRSA (19,4%). About 25% of the patients didn't respond to previous vancomycin therapy, 19% didn't respond to QD therapy either, yet only 11,8% of these non-responders failed to respond to linezolid (39).

Study findings from Birmingham et al (39) reported a clinical cure rate of 81,4% in patients with VREF infections (5,8% of patients failed to respond; 12,8% had indeterminate outcomes) and a microbiological cure rate of 86,4% (12,7% failed to respond; 0,9% indeterminate outcomes).

No patient exhibited linezolid-resistant pathogens at the time of enrollment, ten of 828 organisms developed linezolid resistance during treatment (nine *E. faecium*, one *E. faecalis*) (39).

In all patients but one the development of linezolid resistance was associated with a length of therapy >28 days. Regarding adverse events, linezolid was well tolerated at large. Common adverse events comprised gastrointestinal disturbances (e.g. nausea, vomiting, diarrhea) and dermatologic events (e.g. rashes, itching). Especially when linezolid therapy lasted >14 days, hematologic events (decreased platelet counts, hemoglobin/hematocrit levels, and white blood cell counts) were reported, though presenting only mild to moderate, transient and reversible upon termination of treatment (39).

To monitor the activity, spectrum and resistance rate of linezolid in the United States, the LEADER surveillance program was established in 2004. In its eighth successive year (2011) susceptibility tests were performed for a total of 7303 gram-positive pathogens from 60 medical centers. The susceptibility testing of 1160 enterococcal isolates (66% *E. faecalis*, 30,6% *E. faecium*) yielded an ampicillin susceptibility rate of 71,9%, the overall vancomycin resistance rate amounted to 26,6%

(93,1% VanA phenotype). The overall MIC₉₀ value for enterococci was 1 µg/ml with only four enterococcal isolates (three *E. faecium*, one *E. faecalis*) presenting MIC values ≥4 µg/ml. Each of these four isolates was associated with G2576T mutations (18).

Mendes et al (48) summarized linezolid activity over nine consecutive years (2004-12). Concerning linezolid potency against enterococci, stable MIC results were shown irrespective of species or vancomycin resistance phenotype. Over 98,2% of examined *E. faecalis* isolates showed susceptibility to linezolid, ampicillin and glycopeptides, whereas isolates of *E. faecium* presented MDR phenotypes (48).

Linezolid non-susceptibility rates when tested against a total of 6718 isolates of enterococci are shown in Table 13 (48).

Organism (no. tested)	Percentage linezolid non-susceptibility by year (number of isolates tested)								
	2004	2005	2006	2007	2008	2009	2010	2011	2012
Enterococci (6718)	0,0 (719)	0,0 (718)	0,9 (423)	0,7 (906)	0,7 (864)	0,5 (744)	0,5 (787)	0,4 (760)	0,8 (797)

Table 13 Linezolid Non-Susceptibility Rates of Enterococci – Summary of the ZAAPS Program Results (2004-12) (adapted from (48))

1.2.1 Resistance to Linezolid

Linezolid-resistant isolates of enterococci and staphylococci were initially detected in 2001, soon after the clinical implementation of linezolid (41,49). Halle et al (50) reported the first case of septic multiorgan failure (pancreatitis, peritonitis, septic shock) in Germany in 2002 due to a mixed culture of linezolid-resistant *E. faecium* and *E. faecalis*.

However, reported clinical outbreaks due to linezolid-resistant enterococci (LRE) remain uncommon, despite the widespread use of linezolid (41,43,49). With reference to Ntokou et al (49) linezolid resistance in enterococci has mainly been described in case studies or case series in the literature thus far. Irrespective of the low prevalence of linezolid resistance, the mechanisms of resistance have been accurately described (44).

Enterococcal and staphylococcal resistance to linezolid both occurs in patients with prior exposure and in patients without any administration of linezolid (42,47).

Because of its synthetic derivation, the pre-existence of naturally occurring resistance mechanisms is rather unlikely (43). Further, the mechanism of action differs from that of other protein synthesis inhibitors leading to a hampered development of de novo resistance (19). Additionally, the binding site of linezolid (domain V of the 23S rRNA) is encoded by genes (rDNA), which exist in multiple copies in bacteria. *E. faecalis* exhibits four copies, *E. faecium* and *S. aureus* present five to six copies. Development of linezolid resistance requires mutations in several copies of these genes (43,51). Marshall et al (52) found a correlation between the number of mutant 23S rRNA genes and the level of resistance.

The comparison of two different isolates of *E. faecium*, one isolate with a mutation in one of six 23S rRNA genes, the other with mutations in five of six 23S rRNA genes, revealed a MIC of 8 µg/ml and a MIC of 64 µg/ml, respectively (43,52).

1.2.1.1 Mechanisms of Resistance

Antimicrobial resistance to linezolid is associated with single-nucleotide changes of domain V of 23S rDNA (42,43). Concretely, a nucleotide change from guanine (G) to uracil (U) at position 2576 in the 23S rRNA (G2576T) expresses the most frequent mutation leading to linezolid resistance among enterococci (9,40–42,44). Further mutations comprise T2500A, C2192T, G2447T, A2503G, T2504C, G2505A, G2766T, and C2461T. Besides, alterations of the ribosomal proteins L3 and L4 may lead to linezolid resistance (44).

Potential transmission of linezolid resistance is dependent on the type of resistance: mutational or plasmid-mediated resistance (53).

Mutational resistance is believed to occur sporadically as a spontaneous mutation, therefore it is not transferable and adequate infection control measures will prevent the transmission of resistant isolates (40,53). Further, there's a link between mutational resistance and previous exposure to linezolid (44).

In contrast, non-mutational, plasmid-mediated resistance is transferable among strains of enterococci and other species via the *cfr* (chloramphenicol-florfenicol)-gene (horizontal transfer). The *cfr*-gene was first isolated in 2000 from *Staphylococcus* spp. of animal origin and has been

associated with the use of chloramphenicol and florphenicol. It leads to an alteration of RNA at A2503 mediated by the Cfr rRNA methyltransferase. Cfr-mediated posttranscriptional methylation of position A2503 induces MIC elevation to a variety of drug classes: *Phenicols*, *Lincosamides*, *Oxazolidinones*, *Pleuromutilins*, and *Streptogramin A* antibiotics. Referring to involved antibiotic classes it is named PhLOPS_A phenotype. In common with linezolid, all those affected drug classes bind to the peptidyl-transferase center on the 50S ribosomal subunit. The MIC increases in strains expressing the *cfr*-gene amount from four- through to ≥ 4 096-fold. The *cfr*-gene represents the first gene to confer resistance to five different classes of antibiotics, also it is the first gene to confer plasmid-mediated resistance to pleuromutilins and oxazolidinones (40,44,53,54).

Patel et al (40) conducted a study to investigate linezolid-resistance in enterococci. Susceptibility testing of 2829 isolates of enterococci was performed between 2010 and 2012 and revealed 12 isolates of linezolid-resistant *E. faecium* (0,4%), most of them submitted in 2010 (75%). All linezolid-resistant isolates additionally exhibited resistance to ampicillin, ciprofloxacin and vancomycin (*vanA* gene), 33% were non-susceptible to daptomycin and 41% were resistant to Q/D (40).

Of the 12 linezolid-resistant isolates of *E. faecium*, 8 (66,7%) presented the most frequent mutational resistance, G2576T. None of the isolates exhibited one of the less common mutations as for instance A2503G, T2504C or G2505A. Also no mutations in ribosomal proteins L3 and L4 could be found in any of the isolates. 1/12 isolates contained the *cfr*-gene (40).

1.2.1.2 Risk Factors

In order to determine risk factors for the acquisition of linezolid resistance in patients previously colonised or infected with linezolid-susceptible enterococci (LSE), Kainer et al (7) examined 15 LRE cases (seven of them additionally vancomycin-resistant) and compared those case-patients with a LSE control group. It was found that case-patients had a longer duration of previous antimicrobial treatment as well as prolonged linezolid therapy in particular and a higher frequency of linezolid exposure. The results from this study determined prior clinical isolation of MRSA, duration of hospitalisation before index culture as well as duration of previous linezolid therapy as significant risk factors for LRE infection. Overall, patients with LRE infection had increased in-hospital mortality rates and LOS. Prior MRSA infection as strong risk factor for the acquisition of LRE may act as a surrogate marker for possible patient-to-patient transmission through contaminated medical equipment or the hands of medical personnel (7).

On the contrary, Seedat et al (9) disproved the hypothesis that linezolid resistance only emerges after prolonged treatment. This case report accounted the detection of linezolid-resistant *E. faecium* isolates from an ICU patient after 12 days of linezolid therapy (9).

Also Spiliopoulou et al (42) demonstrated that the duration of linezolid therapy does not act as main risk factor for the acquisition of linezolid resistance. Only 6 out of 14 tested isolates of LRE (6 *E. faecium*, 8 *E. faecalis*) had previous linezolid exposure, indicating a possible patient-to-patient transmission (42).

1.2.1.3 Linezolid Resistance in Vancomycin-Resistant Enterococci

Linezolid- and vancomycin-resistant isolates of enterococci are found rarely. For the case they do occur, a correlation with a prolonged exposure to linezolid was assumed by now. Though, more recent studies have detected linezolid-resistant isolates of VRE from patients without prior linezolid exposure (55).

To determine independent risk factors associated with the emergence of linezolid-resistant, vancomycin-resistant enterococci, Pogue et al (55) conducted a retrospective case-case-control study. Receipt of solid organ transplantation, receipt of total parenteral nutrition, peripheral vascular disease and administration of piperacillin/tazobactam or cefepime were identified as risk factors for the development of linezolid- and vancomycin-resistant enterococci. Previous linezolid exposure was not found to be a statistically significant risk factor, supporting the presumption of a person-to-person transmission (55).

A case-control study by Pai et al (22) investigated the impact of prior linezolid exposure and duration of treatment on the development of linezolid resistance in VREF.

They found that none of the patients without previously administration of linezolid exhibited resistance to linezolid. These findings disagree with the theory of de novo resistance. The most important risk factors identified in this study include exposure to multiple antibiotic agents, prior linezolid therapy, longer duration of linezolid treatment and steroid use (22).

McGregor et al (47) conducted a case-control study to identify risk factors for linezolid non-susceptible *Enterococcus* infections in patients with VRE. The small sample size of 15 case patients (positive clinical culture for LRE) and 60 control patients (negative clinical culture for LRE) didn't allow multivariate regression analysis, yet following risk factors could be found: prior linezolid and sulfonamide therapy, Chronic Disease Score for VRE, prior hospitalisation and admission to a medical service (47).

1.2.1.4 Clinical Impact

Especially in hematology wards, where patients stay for prolonged periods and antibiotic consumption is high, glycopeptide resistance in enterococci is rising (4).

In such cases linezolid is used as last-resort antimicrobial agent for the control of MDR infections (54). In parallel with increased numbers of MRSA and VRE infections, an increase in linezolid consumption was noted (7).

Treatment of MRSA, VRE and other MDR infections pose enormous challenges for clinicians since there are only a few antibiotics available (40). Owing to limited treatment options, transferable mechanisms of linezolid resistance are especially concerning (54).

Relating to transferable linezolid resistance via the *cfr*-gene, the development of a novel oxazolidinone, tedizolid, may dam the spread as it shows activity against staphylococci containing the *cfr*-gene. Additionally, tedizolid comprises improved in vitro activity compared to linezolid, besides it is less toxic (51).

In fact, the drug profile of tedizolid suggests the assumption that it may serve as an important therapeutic option in the treatment of MDR, gram-positive infections in future (56).

2 Materials and Methods

2.1 Study Population and Design

The study was conducted at the Department of Internal Medicine, Division of Hematology, and Section of Infectious Diseases and Tropical Medicine, Medical University of Graz with the approval of the local ethics committee (No. 1141/2014).

Patients with febrile neutropenia at our hospital receive empirical first-line treatment with cefepime (14). For patients, who do not respond to first line treatment within four days, an escalation approach with a combination of linezolid and meropenem is standardly used, unless microbiology culture results suggest otherwise (14,57).

All patients colonised or infected with *E. faecium* from the Division of Hematology between January 1st, 2008 and December 31st, 2013 were included.

2.2 Study Objectives

The aim of the present study was to identify risk factors for the acquisition of linezolid-resistant enterococci and to evaluate the clinical impact of colonisation or infection with linezolid-resistant enterococci. For that purpose, we compared patients with linezolid-resistant enterococci to patients with linezolid-susceptible enterococci from the same ward during the same period.

Our null hypothesis was, that there are risk factors associated with colonisation and/or infection with linezolid-resistant enterococci in a study population of hematologic patients and this leads to increased mortality and length of hospital stay.

Our alternative hypothesis states that there are no associated risk factors; therefore there is no influence on mortality rates and length of stay.

Further study objectives were the mechanism leading to linezolid resistance and the clonal relationship of linezolid-resistant enterococcal isolates.

2.3 Methods

Patients were identified using our microbiology laboratory database. All patients admitted to the Division of Hematology between January 1st, 2008 and December 31st, 2013 with a positive culture result of *Enterococcus faecium* were included with the exception of a positive stool culture for *E. faecium* alone. We identified 137 patients during the study period, 80 patients with LSE (58,4%) and 57 patients with LRE (41,6%). Patients who had culture results of both LSE and LRE (n=13) were counted as LRE cases.

Each group was further divided into two subgroups:

1.) Cases (LRE):

- (a) patients colonised with LRE: ≥ 1 LRE isolate from a non-sterile site (skin, urine, faeces or respiratory tract)
- (b) patients with invasive LRE infection: ≥ 1 LRE isolate from a normally sterile site (blood, cerebrospinal fluid, peritoneal fluid)

2.) Controls (LSE):

- (a) patients colonised with LSE: ≥ 1 LSE isolate from a non-sterile site (skin, urine, faeces or respiratory tract)
- (b) patients with invasive LSE infection: ≥ 1 LSE isolate from a normally sterile site (blood, cerebrospinal fluid, peritoneal fluid)

The primary interest was the comparison of risk factors and mortality rates between group 1 (cases) and group 2 (controls). Our secondary objective was the comparison of group 1b (LRE infection) and group 2b (LSE infection) concerning risk factors for the acquisition of an invasive infection with LRE and related mortality rates. The third aim of the study was to compare group 1a (LRE colonisation) and group 1b (LRE infection) in order to identify risk factors for the evolution of an invasive LRE infection.

The identification of our mechanism of resistance was performed at the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Public Health England using PCR methods.

Linezolid susceptibility was determined using the disk diffusion method, which has been demonstrated to be similarly specific as PCR methods but slightly less sensitive (58). Susceptibility Testing was performed according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST) guidelines (59).

From patients in the bone marrow transplantation (BMT) unit, routine surveillance swabs (throat/nasal/umbilical/genital/rectal) and stool cultures were obtained once a week. Blood cultures and other microbiological cultures were obtained as clinically indicated (new onset of fever, other clinical signs of infection) at the discretion of the attending physician.

Patient data were gathered by reviewing charts and electronic medical records with the use of MEDOCS, the electronic medical documentation system at the University Hospital of Graz.

The following parameters were obtained:

- demographic information: gender, age
- first record/last record of LSE/LRE, number of LSE/LRE isolates, switch from LSE to LRE – if yes: linezolid therapy in the intervening time, invasive/non-invasive isolates (blood, cerebrospinal, peritoneal, pleural fluid, swab, sputum, urine) – if invasive: antibiotic therapy and specific outcome
- number of linezolid exposures, duration of linezolid therapy, active linezolid therapy yes/no while first isolation of LSE/LRE, p.o. administration yes/no
- underlying disease, presence of comorbidities (CCI)
- receipt of HSCT - if yes: allogeneic/autogeneic, GvHD
- neutropenia (days) at the time of detection of LSE/LRE
- hospital course (number and duration of hospitalisations 24 months prior to index isolate, duration of current hospitalisation, ICU stay, duration of ICU stay)
- preceding antimicrobial treatment (antibiotics, antifungals) during current hospital stay

To determine the institutional linezolid consumption during the study time frame, we reviewed our hospital pharmacy data. We additionally analysed the institutional vancomycin and daptomycin consumption during the study period.

According to the WHO Collaborating Centre for Drug Statistics Methodology (60), the defined daily dose (DDD) of linezolid was 1200 mg, the DDD of vancomycin was 2000 mg and the DDD of daptomycin was 280 mg.

Criteria of including patients comprised age ≥ 18 years and ≥ 1 isolation of LSE and LRE, respectively. Patients were excluded from the study if they were < 18 years of age or if the LSE isolate was only detected in a stool culture. Reason for this exclusion criterion is the fact that the existence of LSE in fecal specimen expresses a physiologic condition.

2.4 Definitions

A case was defined as one patient with at least one isolation of LRE from any site. Most of the case patients had more than one LRE and LSE isolation, nevertheless they were counted as one patient respectively.

If a patient had LSE isolated following isolation of LRE, the patient was defined as case patient.

The index hospitalisation was defined as the hospital admission during which LRE or LSE was detected for the first time.

Invasive clinical isolates were defined as isolates from any of the numerated sources: blood, cerebrospinal, peritoneal and pleural fluid (bronchoalveolar lavage) or any other normally sterile site. Non-invasive isolates included isolates from swabs, sputum, urine and faeces. The growth of *E. faecium* in a blood culture defined the presence of an *E. faecium* blood stream infection.

In order to measure the influence of comorbid conditions on the overall survival, the Charlson Comorbidity Index was calculated for each patient (61). The CCI (age adjusted or unadjusted) considers i.a. cardiovascular or cerebrovascular diseases, malignancies, HIV/AIDS, diabetes, organ damage or hemiplegia (a total of 22 conditions). Each condition is linked with a score from 1 to 6. The sum of the scores amounts to the likelihood of death (12).

The Defined Daily Dose is used for antibiotic measurement and is defined as assumed average maintenance dose per day for a drug. If the recommended dose is dependent on body weight, the average adult is considered to weigh 70 kg (60).

Outcome was measured by mortality until the end of the study period.

2.5 Statistical Analysis

Potential risk factors for the isolation of LRE and the clinical impact of colonisation and/or infection with LRE compared to LSE were evaluated with the IBM SPSS software for Windows, version 22.

For descriptive tables, frequencies, means, medians and standard deviations were calculated.

Univariate analysis was done by use of the Fisher's exact test or Chi-square methods including Phi and Cramer's V for categorical variables. For statistical analyses of continuous variables, the student's t-test or Mann-Whitney U-test were used.

Statistical significance was defined by a 2-tailed p -value $\leq 0,05$.

3 Results

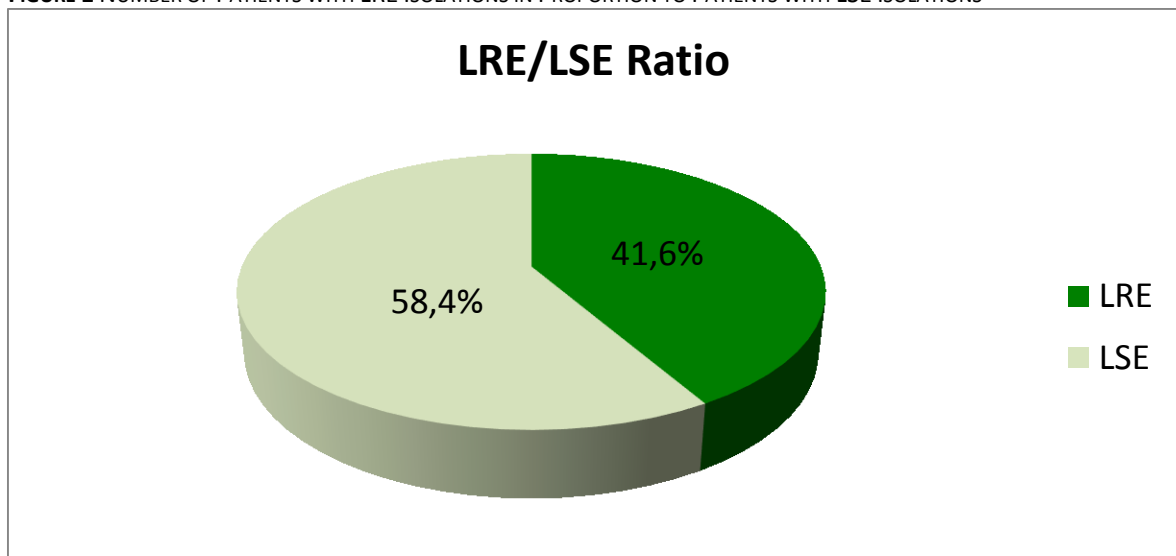
3.1 Molecular Mechanism of Resistance

The identification of our mechanism of resistance was performed by the Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit, Health Protection Agency in London (62). All tested linezolid-resistant *E. faecium* isolates were positive for heterozygous G2576T mutations in the 23S rRNA. Testing for plasmid-mediated resistance via the *cfpA*-gene was negative in all isolates.

3.2 Patients' Characteristics

From January 2008 to March 2014, a total of 137 patients were included, 57 (41,6%) patients with LRE and 80 (58,4%) patients with LSE (**Figure 1**). The mean age was 57,5 years (SD 13,3, range 21,9-81,0) for LRE cases and 55,7 years (SD 14,1, range 20,0-83,9) for LSE controls, respectively.

FIGURE 1 NUMBER OF PATIENTS WITH LRE ISOLATIONS IN PROPORTION TO PATIENTS WITH LSE ISOLATIONS



3.2.1 Microbiological Characteristics

9/57 (16%) of the case patients had an invasive LRE infection compared to 15/80 (19%) of the control patients presenting with invasive LSE infection. All other patients were colonised (**Table 14**).

Sources	Number of first isolates	
	LRE	LSE
Blood	9	15
Cerebrospinal fluid*	1	0
Swab	25	73
Peritoneal fluid	0	1
Sputum	1	2
Bronchoalveolar lavage	3	0
Urine	3	10
Tip of central venous catheter*	0	1
Stool	34	n.d.**
INVASIVE	9	15

Table 14 Number and Sources of First Isolates of LRE and LSE

*The patients with positive cultures from cerebrospinal fluid and central venous catheter tip also had positive blood cultures.

** Linezolid-susceptible *E. faecium* from stool samples was considered physiologic.

The number of LRE and LSE isolations per patient are depicted in **Table 15**.

	LRE	LSE
Mean	3,07	2,71
Standard deviation	3,104	2,904
Minimum	1	1
Maximum	16	14
Median	2	1,5

Table 15 Number of LRE and LSE Detections per Patient

The number of patients with linezolid-resistant or linezolid-susceptible *E. faecium* isolates from superficial swab cultures and blood cultures from 2008 to 2014 are shown in **Figures 2** and **3**. Linezolid-resistant isolates of *E. faecium* were first detected in 2010. The ratio of LRE/LSE-colonised patients was 2 (LRE)/22 (LSE) in 2010 and increased to 12/14 in 2011. There was a decrease in 2012 (5/17) and 2013 (7/25) and an increase again in 2014 (12/19).

Similarly, the number of patients with invasive LRE infections increased over the study period beginning with 2010. In 2012, 3/4 *E. faecium* blood culture isolates were linezolid-resistant. The absolute number of LRE bacteremias remained stable from 2011 to 2014.

FIGURE 2 PATIENTS WITH LINEZOLID-RESISTANT AND LINEZOLID-SUSCEPTIBLE *E. FAECIUM* FROM SWAB CULTURES

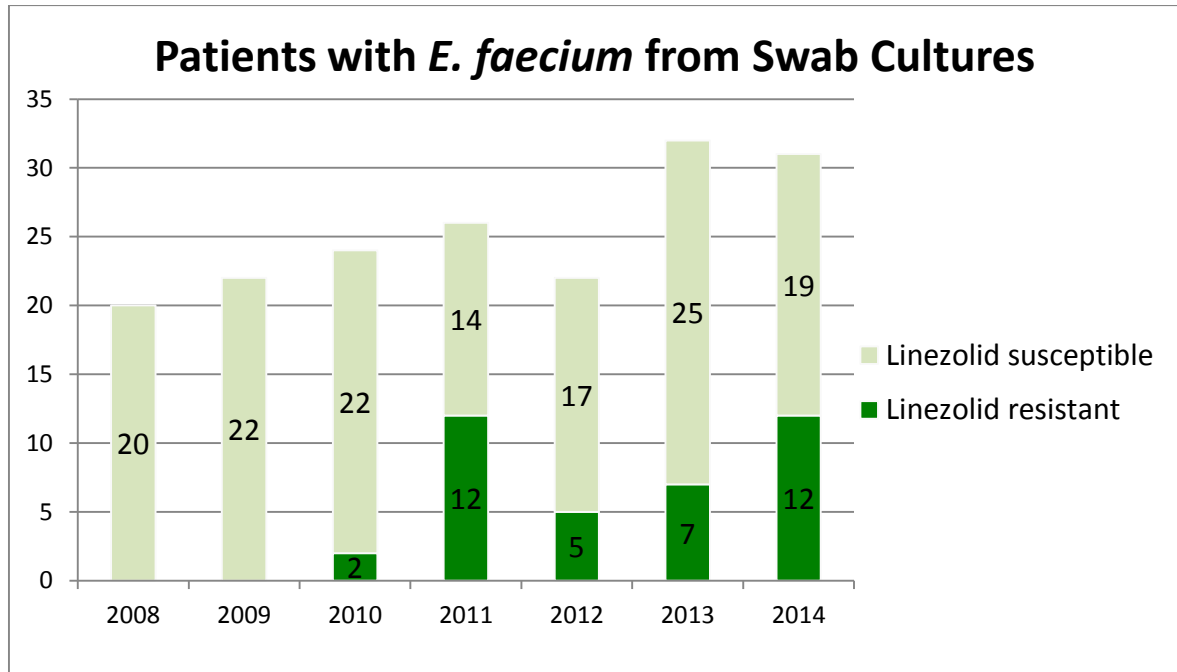
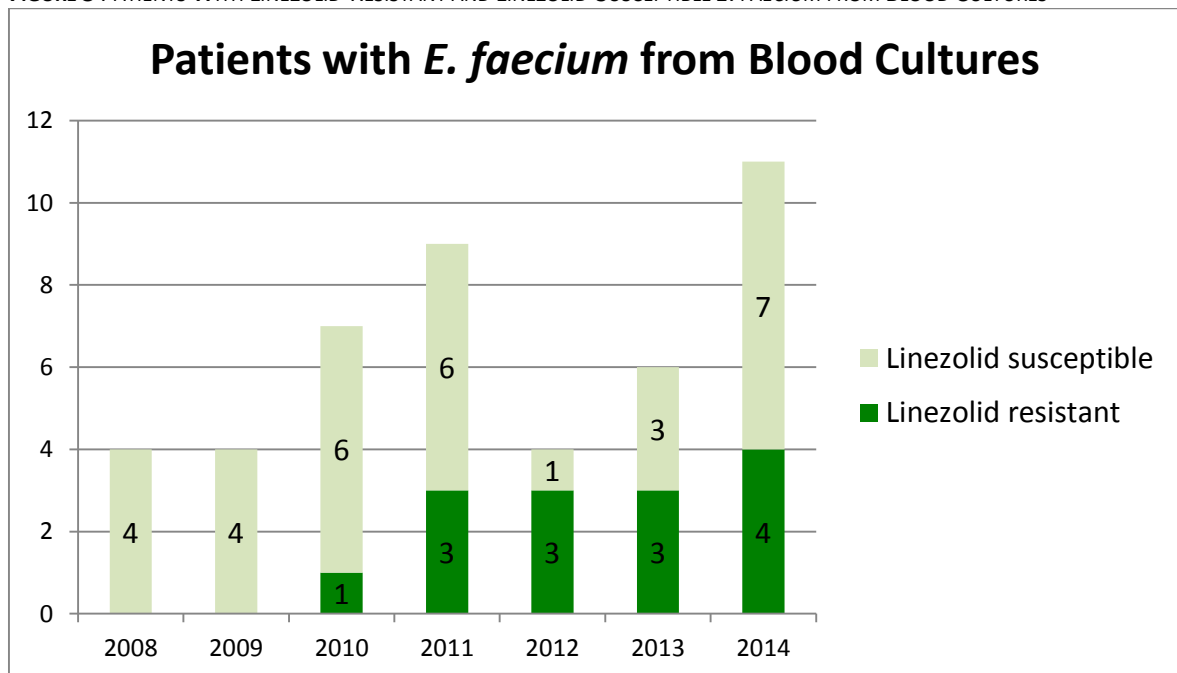


FIGURE 3 PATIENTS WITH LINEZOLID-RESISTANT AND LINEZOLID-SUSCEPTIBLE *E. FAECIUM* FROM BLOOD CULTURES



3.2.2 Risk Factors Associated with Linezolid Resistance of *E. faecium*

Analysed data for the evaluation of patients' condition in relation to the acquisition of LRE included following variables: age, underlying diseases, receipt of a HSCT, presence of GvHD (in HSCT recipients only), CCI age-adjusted, as well as the duration of neutropenia. The statistical analysis did not yield any statistically relevant differences between LSE and LRE patients as presented in **Table 16**.

Variables	LRE (n=57)	LSE (n=80)	p-value
	No. (%)		
Age (mean)	57,5	55,7	0,440 (T-test)
Underlying diseases			0,7 (Phi & Cramer's V)
Multiple Myeloma	4 (7)	5 (6,3)	
Myeloid Neoplasia	31 (54,4)	42 (52,5)	
Acute Lymphocytic Leukemia (ALL)	4 (7)	6 (7,5)	
Lymphoma	18 (31,6)	23 (28,8)	
Other Neoplasia	1 (1,8)	0	
Non-neoplastic	0	3 (3,8)	
HSCT			0,155 (Phi & Cramer's V)
Allogeneic	17	33	
Autogeneic	11	8	
GvHD			0,720 (Chi square)
yes	11	23	
no	6	10	
CCI age-adjusted	median 4, IQR 3, min 2, max 9	median 4, IQR 3, min 2, max 10	0,504 (Phi & Cramer's V)
Duration of neutropenia [days]	8,6 (SD 12, min 0, max 52, median 4)	18,9 (SD 98,2, min 0, max 876, median 2,5)	0,432 (T-test)

Table 16 Patient Characteristics

IQR = interquartile range. SD = standard deviation.

Patients' hospitalisations comparing cases and controls are shown in **Table 17**. The number and duration of current and preceding hospitalisations did not allow any statistically significant association.

Hospitalisations	LRE (n=57)	LSE (n=80)	p-value
Mean duration of current hospitalisation prior to index isolate [days]	25,9 (SD 20,1, min 0, max 52, median 4)	26,0 (SD 32,3, min 2, max 196, median 16)	0,986 (T-test)
Mean duration of current hospitalisation after isolation of the index isolate [days]	23,0 (SD 16,7; min 0, max 62, median 18)	23,8 (SD 21,7; min 1, max 138, median 20)	0,835 (T-test)
Mean total duration of current hospitalisation [days]	48,8 (SD 27,2; min 8, max 126, median 43)	49,7 (SD 39,2; min 7, max 207, median 38)	0,874 (T-test)
Mean number of hospitalisations prior to index isolate within 24 months	5,16	5,04	0,841 (T-test)
Mean total duration of hospitalisations 24 months prior to index isolate [days]	84,1 (SD 55,8; min 19, max 273, median 65)	89,0 (SD 48,3; min 11, max 216, median 91)	0,582 (T-test)
Patients admitted to ICU prior to first detection	7	8	0,489 (Chi square)

Table 17 Hospital Course
SD = standard deviation.

The influence of antimicrobial treatment on the acquisition of LRE is depicted in **Table 18**.

Statistical analysis determined linezolid exposure as statistically significant independent risk factor for the presence of LRE ($p < 0,0001$) (**Figure 4**). Also the duration of linezolid therapy in LRE cases compared to LSE controls showed statistically significant differences. However, this difference was no longer present, if patients were excluded who never had received linezolid. The same effect could be found concerning administration of oral linezolid; There was a statistically significant difference between LRE and LSE patients but only, if the entire study population was included, the effect disappeared if only patients who had received linezolid were included. Concerning antibiotic therapy during the index hospitalisation, treatment with cefepime ($p = 0,031$), meropenem ($p = 0,002$) and piperacillin/tazobactam ($p = 0,031$), as well as carbapenem therapy in general ($p = 0,002$) was significantly associated with the presence of LRE. To analyse a potential dependence between carbapenem therapy and administration of linezolid, a contingency table was compiled (**Table 19**). Patients who received meropenem were significantly associated with concurrent linezolid administration prior to the index isolate (Chi square $p = 0,000$). Only one patient was administered meropenem without concomitant linezolid therapy.

Carbapenem treatment could therefore not be considered a risk factor for LRE in our study population.

The administration of trimethoprim/sulfamethoxazole was associated with significantly lower LRE rates ($p=0,001$).

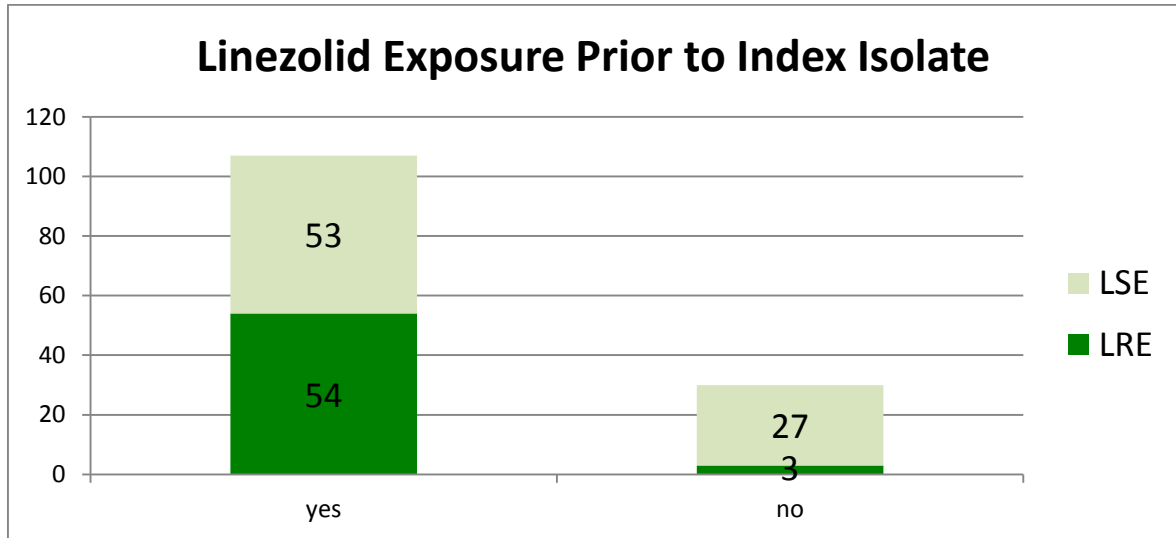
13 patients had LSE detected before isolation of LRE; nine out of them had received linezolid therapy in the intervening time.

Antibiotics	LRE (n=57)	LSE (n=80)	p-value
Linezolid exposure prior to index isolate	54	53	<0,0001 (Chi square)
Number of linezolid exposures	median 1, IQR 2, min 0, max 6	median 1, IQR 2, min 0, max 7	0,036 (Mann-Whitney U-test)
Mean total days of linezolid treatment until first detection of LSE/LRE (incl. non-exposed patients)	20,02 (SD 15,7)	12,1 (SD 16,26)	0,005 (T-test)
Mean total days of linezolid treatment until first detection of LSE/LRE (excl. non-exposed patients)	21,02 (SD 15,3*)	18,3 (SD 16,9 [§])	0,380 (T-test)
Linezolid therapy at the time of first LRE/LSE isolation			0,115 (Chi square)
yes	20	37	
no	18	61	
p.o. administration of linezolid (including the whole study population)			0,042 (Chi square)
yes	14	9	
no	42	69	
p.o. administration of linezolid (excl. patients without linezolid therapy)			0,282 (Chi square)
yes	14	9	
no	40	43	
Antibiotic Therapy during index hospitalisation			
Cefepime	41	43	0,031
Meropenem	36	29	0,002
Trimethoprim/Sulfamethoxazole	18	48	0,001
Piperacillin/Tazobactam	20	15	0,031
Carbapenem Therapy	36	29	0,002
Median number of different antibiotics during index hospitalisation	3	3	

Table 18 Antimicrobial Treatment

IQR = interquartile range. SD = standard deviation. p.o. = peroral. * n=54 § n=53

FIGURE 4 LINEZOLID EXPOSURE PRIOR TO INDEX ISOLATE



	Administration of meropenem		Total
	no	yes	
Linezolid exposure prior to index isolate no	29	1	30
Linezolid exposure prior to index isolate yes	43	64	107
Total	72	65	137

Table 19 Administration of Meropenem * Linezolid Exposure Prior to Index Isolate

3.2.3 Risk Factors for The Acquisition of Invasive *E. faecium* Infections

3.2.3.1 Invasive LRE Infection

To determine risk factors for the acquisition of an invasive LRE infection compared to LRE colonisation, group 1a (LRE colonisation) and group 1b (LRE infection) were compared. Statistically significant variables comprise age, duration of neutropenia, duration of linezolid therapy and the number of LRE isolations (**Table 20**).

Risk factors	LRE		<i>p-value</i>
	invasive (n=9)	non-invasive (n=48)	
Mean age	47,9 (SD 12,2)	59,3 (SD 12,9)	0,018 (T-test)
Duration of neutropenia [days]	20,3 (SD 18,4)	6,37 (SD 9,2)	0,001 (T-test)
Duration of linezolid therapy [days]	31,33 (SD 23,1)	17,7 (SD 13,1)	0,016 (T-test)
Number of LRE isolations	5,78 (SD 4,14)	2,56 (SD 2,62)	0,007 (Mann-Whitney U-test)

Table 20 Risk Factors for the Acquisition of an Invasive LRE Infection
SD = standard deviation.

3.2.3.2 Invasive LSE Infections

The evaluation of potential risk factors for the acquisition of an invasive LSE infection by comparison of group 2a (LSE colonisation) and group 2b (LSE infection) identified following statistically significant differences: administration of carbapenems (chi square $p=0,034$), mainly meropenem (fisher's exact test $p=0,042$). Trimethoprim/Sulfamethoxazole was found to act protective against LSE bacteremia (4/15 LSE infection vs. 44/65 LSE colonisation, $p=0,007$).

3.3 Antimicrobial Consumption

The antibiotic consumption at the Department of Hematology was evaluated from January 2008 to December 2014 using data from the hospital pharmacy.

The annual linezolid consumption amounted to 1655 DDDs in 2008, increased to a maximum of 2245 DDDs in 2010 and was steadily reduced to 1385 DDDs in 2014. Accordingly, the vancomycin consumption was highest in 2011 (296,25 DDDs), whereas the fewest vancomycin prescriptions were recorded in 2010 (67,75 DDDs). Antibiotic treatment with daptomycin was first registered in 2009 (8 DDDs) and steadily increased up to 42,2 DDDs in 2014.

The numbers of DDDs of linezolid, vancomycin and daptomycin per year are listed in **Figures 5, 6 and 7**.

FIGURE 5 LINEZOLID CONSUMPTION

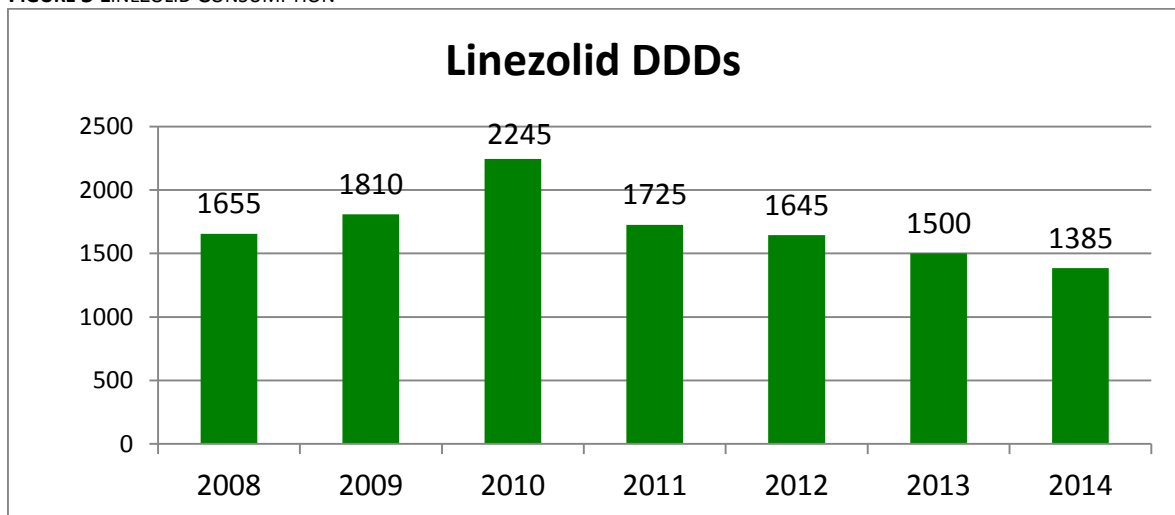


FIGURE 6 VANCOMYCIN CONSUMPTION

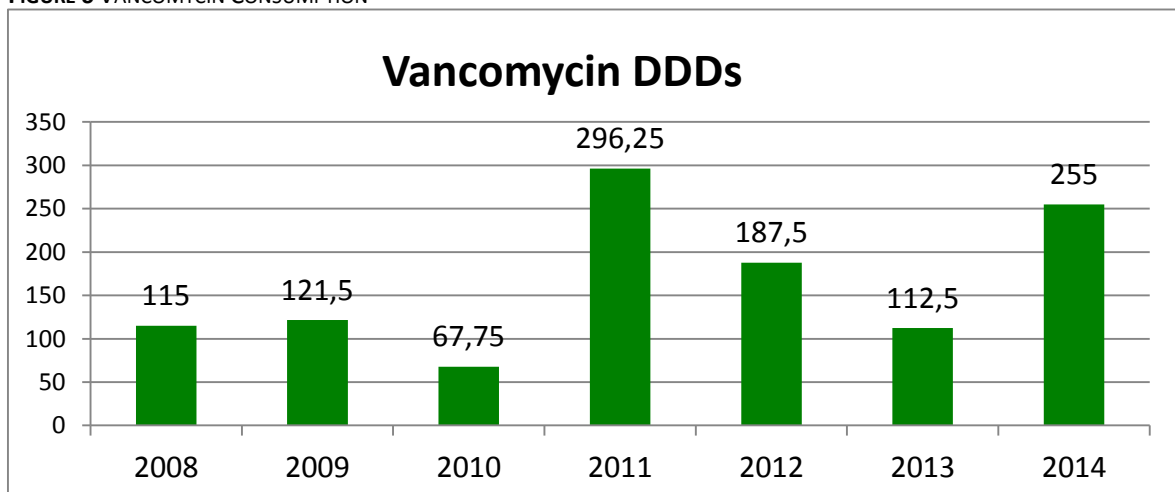
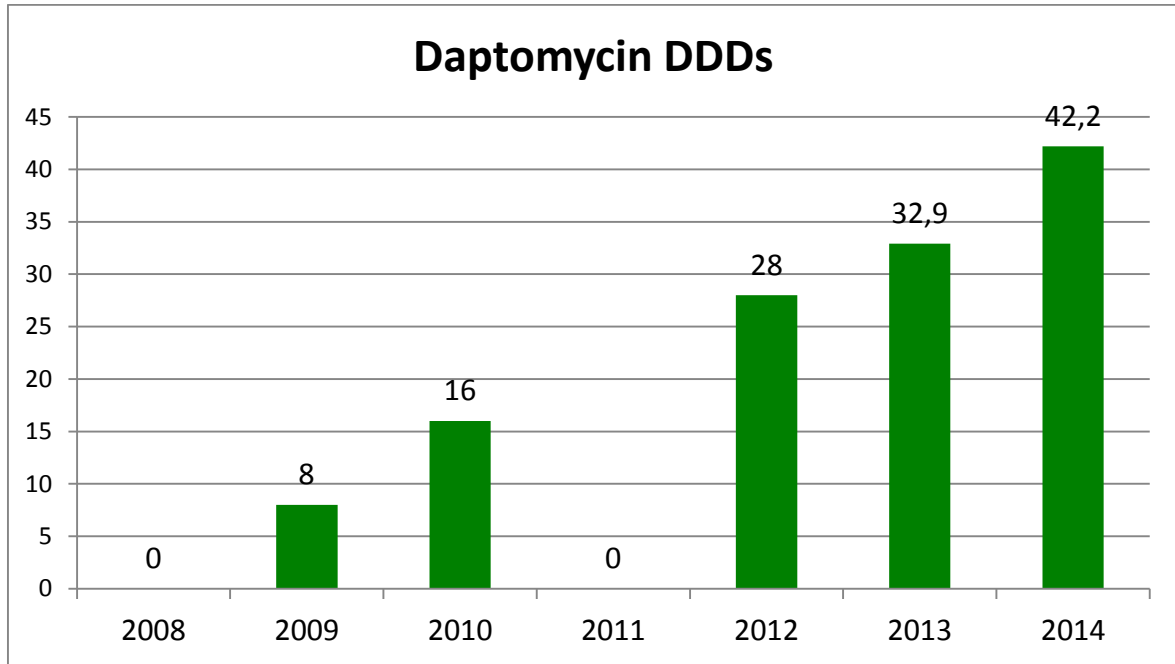


FIGURE 7 DAPTOMYCIN CONSUMPTION



3.4 Mortality Rates

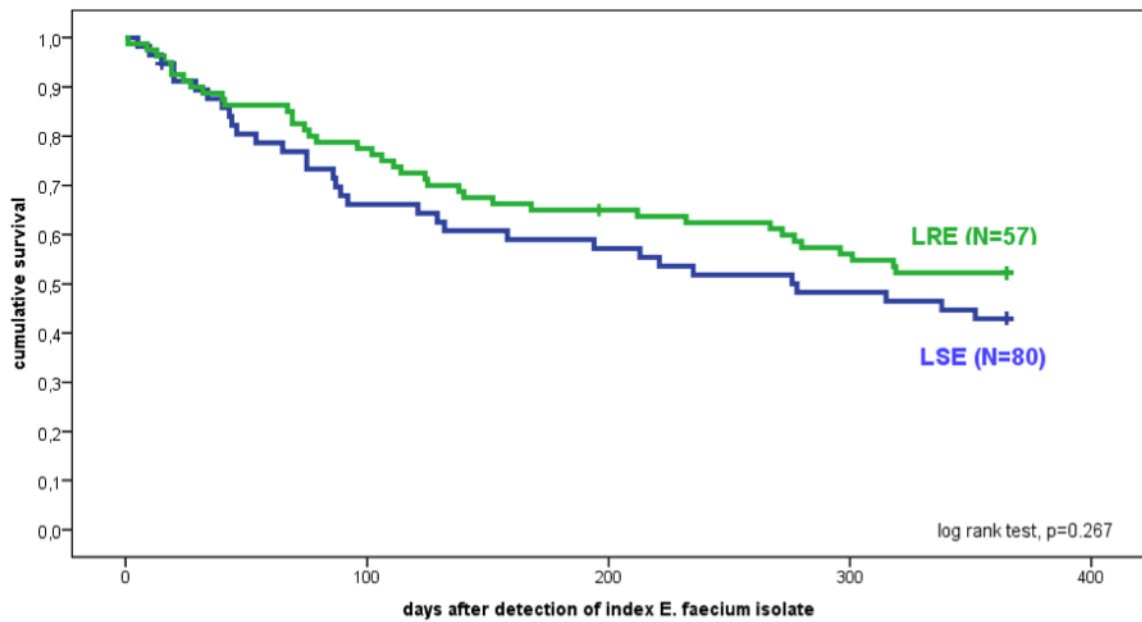
The in-hospital mortality rate during the index hospitalisation in patients with LRE compared to patients with LSE did not show any statistically significant differences (chi square, $p=0,543$).

For the evaluation of the one-year mortality rate, two patients were lost to follow-up. 32/56 (57,1%) of the remaining LRE patients died within one year compared to 38/79 (49,4%) of the controls (chi square, $p=0,30$) (**Table 21**). Cumulative survival was plotted and survival functions calculated and also did not show a statistical significant difference between LRE and LSE patients (**Figure 8**).

		LZD S/R		Total
		R	S	
Alive after 1 year	no	32	38	70
	yes	24	41	65
Total		56	79	135

Table 21 One-Year Mortality Rate

FIGURE 8 COMPARISON OF CUMULATIVE SURVIVAL IN PATIENTS WITH LINEZOLID-RESISTANT OR LINEZOLID-SUSCEPTIBLE ENTEROCOCCI



Patients with invasive LRE infections showed comparable mortality rates to those with invasive LSE infections, therefore no statistically significant differences in definite attributable mortality could be found. Of the nine patients with invasive LRE infections one patient died, compared to 2/13 of the patients with invasive LSE infections (fisher's exact test $p=1.0$).

4 Discussion

The present study assessed risk factors for the acquisition of linezolid-resistant *Enterococcus faecium* in hematologic patients and evaluated the clinical impact of colonisation or infection with LRE.

Despite the widespread use of linezolid, international surveillance data shows a very limited number of linezolid-resistant isolates among enterococci (18,19,48,55). Nonetheless, the linezolid resistance rate in our hospital was found to be much higher than monitored by diverse surveillance programs (e.g. LEADER surveillance program (18)).

Linezolid-resistant isolates of *E. faecium* were first detected in 2010 at the Department of Hematology at the University Hospital of Graz. Simultaneously the institutional linezolid consumption reached its maximum in 2010. The number of patients with LRE isolates from swab cultures or blood cultures increased significantly following the increase of linezolid consumption. In response to that, the amount of linezolid prescribed per year was steadily decreased, accompanied by an increase in vancomycin and daptomycin consumption as therapeutic alternatives.

Our outbreak was polyclonal, therefore it does not support a possible patient-to-patient transmission, rather indicating institutional linezolid-usage as risk factor for the acquisition of linezolid resistance.

Consistently with the results of previous studies, we confirmed previous linezolid exposure as statistically significant independent risk factor for the acquisition of linezolid resistance in enterococci (7,22,47). The main difference is accounted for by the fact that other studies mostly determined a protracted course of linezolid therapy as significant risk factor (7,22,63), whereas our results identified prior linezolid exposure as major risk factor for the acquisition of linezolid resistance, irrespective of the duration of treatment. Also the number of previous linezolid exposures as well as a current linezolid therapy at the time of the first LRE isolation did not have an effect on the acquisition of linezolid resistance in our study population. Further, there was no significant difference between intravenous or oral administration of linezolid.

Antibiotic exposure, in particular the receipt of cefepime, piperacillin/tazobactam and carbapenems, was significantly associated with acquisition of LRE. Since patients usually receive carbapenems (mainly meropenem) simultaneously with linezolid, their significance as risk factor is not valid due to a dependence on linezolid administration. Treatment with

trimethoprim/sulfamethoxazole (cotrimoxazol) was found to act as a protective factor concerning presence of linezolid resistant *E. faecium*.

By comparison of the invasive and the non-invasive LRE group we found that patients with LRE colonisation were of higher age than patients in the invasive LRE group. A possible explanation for this statistically significant difference in age may be due to the dominant underlying disease. Whereas patients in the invasive LRE group predominantly presented with myeloid neoplasia, the patient group with LRE colonisation showed a high rate of lymphoma.

Our data determined the duration of neutropenia as independent risk factor for the acquisition of invasive linezolid-resistant *E. faecium* infections. The number of neutropenic days is only pertinent to LRE infections; there is no statistically significant difference in LSE infections.

Second, the duration of linezolid therapy was significantly higher in patients with invasive LRE infections, again without pertaining to the LSE group. The maximum number of cumulative linezolid days exceeded more than 3-fold the maximum permissible duration for a single exposition of linezolid. No patient was found to have an invasive LRE infection without prior linezolid exposition. Third, the number of LRE isolations was significantly associated with invasive LRE infection.

To conclude, patients with prolonged neutropenia, prolonged linezolid therapy, as well as multicolonisation with LRE, are at significantly higher risk for the acquisition of invasive LRE infections.

In terms of outcome and length of stay the literature provides conflicting results. Generally, there are many studies evaluating the clinical impact of vancomycin resistance, yet there is a lack in studies determining the impact of linezolid resistance.

Clinical data comparing mortality rates and LOS of patients with VRE and VSE bacteremia are often inconsistent. Whereas previous studies consistently described prolonged LOS in patients with VRE infections, they do not coincide regarding mortality (6,12,23,24,29,30).

The results of our present study yielded no significant differences in the duration of hospitalisation and mortality rates in patients with LRE infections compared to patients with LSE infections.

Limitations

In comparison to previous published studies, which examined potential risk factors for the acquisition of LRE infections (42,47,49), our number of cases identified represented a comparatively large sample size. Over a study period of six years, 57 case patients with LRE colonisation or infection were matched with 80 LSE controls. For the comparison of cases and controls the statistical analysis yielded applicable results due to an adequate number of patients. Further study objectives required the division into subgroups (e.g. the comparison of group 1b and group 2b or group 1a and group 1b). This led to a distribution of the total study population in subgroups containing only a small number of patients. For that reason, some results did not exhibit statistical significance, which perhaps would have been shown with a major cohort. As an example, the comparison of group 1a (LRE colonisation) and group 1b (LRE infection) did not allow a statistically significant association between myeloid neoplasia and invasiveness of LRE, though 8/9 (89%) of patients with invasive LRE infection had myeloid neoplasia compared to 23/48 (48%) of the patients with LRE colonisation (ϕ and Cramer's V $p=0,081$).

Another possible limitation of our study is due to the study design. We have chosen a case-control study with a control group consisting of patients with positive cultures for linezolid-susceptible enterococci. According to a systematic review by Harris et al (64), patients with clinical LSE isolates do not represent the study base. A LSE-positive control group only accounts for a small proportion of the cohort of hospitalised patients, which make up the base population. This leads to a selection bias, potentially overestimating the association between prior linezolid exposure and resistance to linezolid (7,17,47,64).

Conclusion and Outlook

Linezolid resistance, especially in concomitant vancomycin-resistant enterococci is of particular concern due to extremely limited treatment options. In fact, various therapeutic strategies are often not FDA approved for the treatment of MDR infections.

As an example, the use of televancin, a derivative of vancomycin, is limited to the treatment of complicated skin and soft tissue infections caused by vancomycin-susceptible *E. faecalis*. It is not approved for the treatment of infections due to VRE, because effective killing is not proven at the doses recommended (11). The second-generation, semisynthetic glycopeptide oritavancin may turn out to be a promising antibiotic agent for the treatment of infections caused by vancomycin-resistant gram-positive bacteria. To date, oritavancin has presented comparable efficacy to vancomycin with additional activity against vancomycin-resistant staphylococci and enterococci. Reported adverse events have been mild and limited (11,65).

The previously mentioned novel oxazolidinone tedizolid comprises enhanced efficacy and less toxicity compared with linezolid. Additionally it shows activity against staphylococci containing the *cfr*-gene (51).

To conclude, we confirmed previous treatment with cefepime, piperacillin/tazobactam and linezolid as statistically significant risk factors for the acquisition of linezolid resistance.

Previous linezolid exposure was independently associated with the acquisition of linezolid resistance, irrespective of the duration of treatment and the number of expositions. Therefore we recommend prudent use of linezolid in combination with continuous surveillance to estimate resistance rates in order to guarantee further efficacy of linezolid for the treatment of serious gram-positive infections.

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EDUCATION

Charité Universitätsmedizin Berlin Semester abroad	2015
Medical University of Graz Medicine (6-year MD-program)	since 2010
University of Graz, Graz University of Technology Molecular biology (2 terms)	2009 - 2010
BORG Ried im Innkreis High School	2005 - 2009

ADDITIONAL MEDICAL EDUCATION AND TRAINING

AGN Arbeitsgemeinschaft für Notfallmedizin: 6th Conference for Emergency Medicine , Graz	2012
AGN Arbeitsgemeinschaft für Notfallmedizin: 7th Conference for Emergency Medicine , Graz	2014

CLINICAL ELECTIVES

University Hospital Hamburg-Eppendorf, Department for Psychiatry Psychiatry – Geriatric psychiatry (2 weeks)	08 2014
University Hospital Hamburg-Eppendorf, Department for Pediatrics Pediatrics (4 weeks)	07/08 2014
Victoria Hospital, English River Health Centre Internal Medicine (3 weeks)	02 2014
University Hospital Hamburg Eppendorf, Department for Anesthesiology and Critical Care Medicine Anesthesiology (4 weeks)	07/08 2013
General Hospital St. Josef Braunau, Department for Anesthesiology and Critical Care Medicine Anesthesiology (2 weeks)	02 2013
Charité Campus Benjamin Franklin, Department for Cardiology, Pulmonology and Angiology Cardiology – Rhythmology (4 weeks)	07/08 2012
Central Hospital Ried/Innkreis, Department for Trauma Surgery Trauma surgery (3 weeks)	07/08 2011

EXTRACURRICULAR ACTIVITIES AND ENGAGEMENT

Red Cross Austria, Ambulance Service Graz	2012
Executive Committees, Medical University of Graz	
Curriculum Committee Medicine , full member	2013 - 2015
Task Force for Equal Opportunities , full member and secretary	2013 - 2015
Austrian National Union of Students - Medical University of Graz	
Responsible official for Women and Equal Treatment	2012 - 2014
Appointments Committee for Anesthesiology , full member	2012 - 2014
University Student Parliament , representative	2013 - 2015
Speaker for Women and Equal Treatment	2014 - 2015
Teddybear-Hospital, Austrian Medical Student's Association (AMSA)	
Teddy-Doc (volunteer)	2010, 2011, 2014