Introduction

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INTRODUCTION

Enterococci are facultative anaerobic gram-positive cocci which can be found as commensals in the gastrointestinal tract of humans, other mammals, birds, insects and reptiles [1]. The genus *Enterococcus* has originated around 425-500 million years ago. Around this time of animal terrestrialization, enterococci emerged from their ancestor *Vagococcus*. Vagococci diverged from *Carnobacteriaceae*, which resided in marine environments [2, 3]. Vagococci were thereby adapted to salty habitats. These environmental conditions predisposed this genus to colonize the gastro-intestinal tracts of animals, in which the bacteria are exposed to bile salts. Vagococci were already able to colonize ecologies with high levels of bile, a characteristic feature in enterococci [4]. As a consequence of the migration of animals from water to land, the environmental conditions for enterococci changed. When the bacteria were outside the host in the environment on land, they were exposed to dry conditions and starvation, in contrast to the humid coastal conditions of the previous habitat. These conditions selected for species with highest tenacity. Compared to their ancestor, enterococci are significantly enriched in the cell wall modification and de novo purine biosynthesis, forming cell wall components that increases its integrity [5, 6]. These functions are related to environmental stress responses. The thickened cell wall protects the enterococci against desiccation and starvation. The thick and impermeable cell wall also resulted in non-permeability for many antibiotic classes. Thereby, enterococci are intrinsically resistant to cephalosporins, low-level aminoglycosides and clindamycin [1]. In addition to their intrinsic antibiotic resistances, they can easily acquire antibiotic resistance genes [7] of which vancomycin resistance is clinically most relevant.

Subsequently, the evolution of the animal hosts had a great influence on the evolution of enterococci. Utilization of carbohydrates provided by the host has been, and still is a major driver in enterococcal speciation. Large gains of genes for carbohydrate metabolic pathways are seen in the emergence and proliferation of enterococci which parallels the radiation of hosts [4]. The availability of uric acid in the hosts' gut, and the ability of enterococci to metabolize this carbon source, is of particular interest. Biofilm formation can be triggered by the metabolites formed in uric acid degradation [8]. This biofilm formation is suggested to increase the virulence of enterococci in uricotelic hosts [4].

Enterococci are generally considered as non- or low-pathogenic micro-organisms and mainly being clinically relevant in case of hospital associated (HA) infections. Around the 1970s and 1980s, enterococci emerged as a leading cause of HA infections mainly due to *E. faecalis* and *E. faecium*. Especially *E. faecium* seemed to rapidly emerge as a nosocomial

pathogen worldwide. Indeed, the worldwide emergence of vancomycin resistant enterococci (VRE) is largely caused by the rise of vancomycin resistant *E. faecium* (VREfm) [9, 10]. The successful *E. faecium* and VREfm lineages that are circulating in hospitals are characterized by ampicillin resistance, pathogenicity islands and are associated with hospital outbreaks [11]. Studies have shown that these HA *E. faecium* isolates acquired a number of traits making them successful in the hospital environment. These strains contain more antibiotic resistance and virulence genes enhancing biofilm formation and colonisation [12].

Within a short period of time, *E. faecium* has rapidly evolved as a successful nosocomial pathogen. By ease they have withstand and adapted to environmental changes in life, such as human urbanization, antibiotic pressure and the modern hospital environment. Further insight in the successful evolution of *E. faecium* is reviewed in Chapter 2 of this thesis.

Scope and outline of this thesis

The first chapters of this thesis aim to gain insight in the evolution and epidemiology of *E. faecium* (Chapters 2, 3 and 6). From these insights, this thesis proceeds to innovations that have value for patient care. The rapid emergence of hospital lineages imposes challenges for controlling, detecting and typing of VRE. To overcome these challenges, antibiotic stewardship strategies and diagnostic innovations using molecular techniques are required. This thesis describes such innovations, including model-based antibiotic prescription guidance, tailor made diagnostic tools for (vancomycin resistant) *E. faecium*, targeted VREfm infection prevention measures and highly discriminating typing methods in VREfm outbreak investigations (Chapters 2 and 4-7).

Chapter 2 provides an overview of the background and historical evolution of *E. faecium*. We aimed to describe which successful traits and conditions have had a high impact on *E. faecium*, becoming a successful nosocomial pathogen. The increase of *E. faecium* infections in hospitals worldwide as well as the subsequent emergence and epidemiological background of vancomycin resistant *E. faecium* (VREfm) will be reviewed. Additionally, the role of current modern laboratory diagnostics and infection prevention measures in the emergence of VREfm will be discussed. Finally, we aim to translate the insights based on evolutionary research of how *E. faecium* has become such a successful nosocomial pathogen to practical infection control guidances.

The prevalence and molecular epidemiology of extended-spectrum β-lactamase-producing (ESBL)/plasmid AmpC (pAmpC) bacteria and HA *E. faecium* (including VRE) in the Northern Dutch-German cross-border region is described in **Chapter 3**. For this

purpose, a point-prevalence study was performed in hospitalized patients in the Northern Netherlands and North-West Germany. Also, healthy individuals from the Dutch community were screened. A genome-wide gene-by-gene typing approach was applied to study the molecular epidemiology of ESBL-*Escherichia coli* and VRE.

In **Chapter 4** of this thesis we aimed to identify certain risk factors for the development of an *E. faecium* bloodstream infection in patients with haematologic malignancies. Haematology patients have a high risk of an *E. faecium* bloodstream infection, but empirical therapy usually does not cover this bacterium. Antibiotic treatment of *E. faecium* includes glycopeptides such as vancomycin. However, prudent use of vancomycin is needed for the control of VRE. Therefore, we aimed to design a prediction model based on identified risk factors for *E. faecium* infections to corroborate the clinical decision to start glycopeptides pre-emptively in haematology patients.

Chapter 5 describes the evaluation of a PCR-based diagnostic method, the Xpert *vanA/vanB* assay, for the detection of *vanB* VRE carriage. This assay runs on a Cepheid GeneXpert system which is, after adding the clinical sample to a cartridge, fully automated combining DNA extraction, real-time PCR amplification and detection. Direct detection of *vanB* VRE on faecal samples is complicated due to the presence of non-enterococcal *vanB* genes from anaerobic gut bacteria. This could lead to many false-positive results. The assay was used on enriched broth, containing antibiotics selective for enterococci but suppressing anaerobes. Additionally, an adjusted cycle threshold (C_t) cut-off value was determined to optimize the accurate and rapid detection of *vanB* VRE.

In **Chapter 6** the diagnostic evasion of highly-resistant microorganism (HRMOs) as a critical factor in outbreaks is described. Various examples of resistance mechanisms in carbapenemase-producing Enterobacteriaceae (CPE), VRE, methicillin resistant *Staphylococcus aureus* (MRSA) and ESBL are given that result in evasion of detection by routine diagnostic approaches. For each HRMO, mechanisms and examples of national and international outbreaks are described. Next, we aimed to provide practical laboratory detection advices to overcome the diagnostic evasion for these HRMOs.

Chapter 7 shows the application of whole genome sequencing (WGS) in VREfm outbreak diagnostics. The dissemination of VREfm is due to both clonal spread and spread of mobile genetic elements (MGEs) such as transposons. We analysed VREfm outbreaks that occurred in the University Medical Center Groningen (UMCG) in 2014. For this purpose, all epidemiological data of patients carrying these VREfm, including patients' transfer data, were gathered. Representative isolates with WGS data available were typed by core-genome

multi-locus sequence typing (cgMLST). Additionally, *vanB*-carrying transposons of all sequenced isolates were characterised. By combining cgMLST, transposon characterization and epidemiological data, we aimed to elucidate the pathways of transmission of VREfm outbreaks.

Finally, a summary of the results of this thesis is given in **Chapter 8**. This chapter also gives the overall conclusion and discussion, pointing towards some future perspectives.

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