Antibiotic-Induced Damage on the Intestinal Microbiota and Treatment of Urinary Tract Infections Caused by ESBL- and Non-ESBL-Producing Bacteria

HANNA MONTELIN
Therapeutic options for urinary tract infection (UTI) caused by *Escherichia coli* and *Klebsiella pneumoniae* are limited due to resistance against cephalosporins and carbapenems, which is typically mediated by the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases. ESBL-producing bacteria are frequently co-resistant to other antibiotic classes, resulting in a shortage of treatment options. While all systemic antibiotic treatments are likely to disturb the microbiota and increase selection of resistance, evidence on the extent and persistence of such effects for different antibiotics is limited. The primary objective of this thesis was to investigate the therapeutic effect of carbapenem-sparing and narrow-spectrum oral antibiotics in the treatment of UTI, and to evaluate the impact of commonly used antibiotics on the intestinal microbiota.

The first study investigated the efficacy of nitrofurantoin and pivmecillinam for lower UTI in men (n=171), with trimethoprim as a comparator. We concluded that nitrofurantoin and pivmecillinam are suitable for empirical treatment of lower UTIs in men, considering their high activity against *Escherichia coli* and limited impact on the microbiota.

In a prospective multi-center study conducted at 15 infectious diseases hospital departments, patients (n=235) with UTI caused by ESBL-producing Enterobacterales were recruited. We aimed to evaluate clinical and microbiological treatment outcomes and relapse rates. The results indicate that carbapenem-sparing antibiotics were effective for UTI caused by ESBL-producing Enterobacterales and can be recommended for non-critically ill patients. Moreover, we noted that certain bacterial genetic features (e.g., ST131 in *Escherichia coli* and haemolysin) were associated with microbiological failure and relapse.

In a randomized, controlled trial with healthy adults (n=86), we investigated the impact on the microbiota of five antibiotics (ceftibuten, ciprofloxacin, nitrofurantoin, pivmecillinam, trimethoprim-sulfamethoxazole) that are commonly used for UTI. Fecal samples were collected before and up to one year after five days of antibiotic treatment. Ciprofloxacin demonstrated significant immediate and long-term disruption of the intestinal microbiota in terms of diversity and taxonomy and stands out in comparison with the other antibiotics included in the study.

In a prospective study, we investigated the intestinal microbiota in patients with hematological diseases undergoing hematopoietic stem cell transplantation (HSCT, n=88). Changes over time and during antibiotic treatment and potential associations between the intestinal microbiota at baseline and patient outcomes were explored. Oral ciprofloxacin demonstrated a significant impact on the intestinal microbiota, which was greater than the impact of intravenous broad-spectrum antibiotics. A low microbiome diversity at baseline was associated with neutropenic fever and antibiotic treatment following HSCT.

**Keywords:** Intestinal microbiota, *Escherichia coli*, *Klebsiella pneumoniae*, extended-spectrum β-lactamases, urinary tract infection, antibiotic resistance genes

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To Markus, August and Märtha
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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# Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>ARG</td>
<td>Antibiotic resistance genes</td>
</tr>
<tr>
<td>BLBLI</td>
<td>β-lactam/β-lactamase inhibitors</td>
</tr>
<tr>
<td>BMD</td>
<td>Broth microdilution</td>
</tr>
<tr>
<td>BSI</td>
<td>Bloodstream infections</td>
</tr>
<tr>
<td>EN</td>
<td>Enteral nutrition</td>
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<tr>
<td>ESBL</td>
<td>Extended spectrum β-lactamase</td>
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<tr>
<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
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<tr>
<td>HGT</td>
<td>Horizontal gene transfer</td>
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<td>HSCT</td>
<td>Hematopoietic stem cell transplantation</td>
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<tr>
<td>KPC</td>
<td><em>Klebsiella pneumoniae</em> carbapenemase</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharides</td>
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<tr>
<td>MBL</td>
<td>Metallo-β-lactamase</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MLST</td>
<td>Multilocus sequence typing</td>
</tr>
<tr>
<td>PBP</td>
<td>Penicillin-binding proteins</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>TPN</td>
<td>Total parenteral nutrition</td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
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<tr>
<td>WHO</td>
<td>The World Health Organization</td>
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</table>
Introduction

Antibiotic resistance increases the risk of treatment-related complications and mortality, leading to extended hospitalization and increased healthcare-associated costs, which pose a serious threat to human health and modern healthcare.\textsuperscript{1–4} The widespread use and misuse of antibiotics, together with the slow development of new antibiotic agents,\textsuperscript{5} have accelerated the emergence of resistance, especially among Gram-negative bacteria.\textsuperscript{6} Urinary tract infections (UTI) caused by Gram-negative bacteria such as \textit{Escherichia coli} and \textit{Klebsiella pneumoniae}, are major contributors to global antibiotic use as well as mortality.\textsuperscript{4,7} Resistance in \textit{E. coli} and \textit{K. pneumoniae} against the commonly used broad-spectrum β-lactam antibiotics is often mediated by the production of extended-spectrum β-lactamase (ESBL)- and carbapenemase enzymes. ESBL transmission is predominantly plasmid-mediated, which facilitates the rapid spread of this resistance. Moreover, treatment options for UTI caused by ESBL-producing bacteria are limited due to frequent multidrug resistance (MDR), defined as co-resistant to three or more relevant antibiotic classes, resulting in a shortage of oral treatment options, in particular.\textsuperscript{8,9}

Optimal use of available antibiotics is important to preserve their therapeutic efficacy.\textsuperscript{10} More knowledge regarding the efficacy of narrow-spectrum and carbapenem-sparing antibiotics, as well as oral therapeutic alternatives for the treatment of UTI caused by ESBL- and non-ESBL-producing bacteria is needed to prevent further resistance development (paper I, II). The commensal bacteria in our intestine, the microbiota, is a reservoir of antibiotic resistance genes (ARGs) and a hub for their horizontal transfer to more pathogenic bacteria such as invasive \textit{E. coli} and \textit{K. pneumoniae}.\textsuperscript{11} While all systemic antibiotic treatments are likely to disturb the microbiota and increase selection of resistance, evidence on the extent and persistence of such effects with different antibiotics is limited.\textsuperscript{12} Novel sequencing methods have significantly improved our ability to characterize the composition of the intestinal microbiome and its associated resistome with far greater precision compared to traditional culture-based approaches.\textsuperscript{12–14} Combining sequencing and culture-based methods can enhance our understanding of how different antibiotics impact the microbiome (paper III, IV). This knowledge facilitates the prediction of antibiotic-induced microbiome changes in treatment guidelines and antimicrobial stewardship programs.
Antibiotic resistance

Bacterial resistance is a natural evolutionary process that enables bacteria to adapt and survive in the presence of other substances such as antibiotics, in their environment. Antibiotic resistance can be acquired through different evolutionary pathways and is accelerated by exposure to antibiotics. The use of antibiotics exerts a selection pressure on bacterial populations by eliminating susceptible bacteria, thereby facilitating the proliferation of resistant bacterial strains. In a clinical setting, this selection of resistance may cause treatment failure and colonization with resistant strains, potentially contributing to future infections caused by resistant bacteria as well as community transmission of the resistance. The level of antibiotic consumption within a country or population is closely associated with the prevalence of antibiotic resistance. After the clinical introduction of a new antibiotic class, the effectiveness of the antibiotic declines over time due to the emergence of antibiotic resistance in bacteria, a phenomenon that typically occurs within a few years (Figure 1).

![Timeline of antibiotic deployment and the identification of antibiotic resistance. Only a selection of antibiotic classes is shown. Information retrieved from Clatworthy et al., Rice et al., Papp-Wallace et al. Created with BioRender.com.](image)

The latest antibiotic class was discovered in 1987, leading to the period from 1987 until today being referred to as the era of discovery void. Linezolid and daptomycin were introduced in 2000 and 2003, respectively. However, their chemical classes were first reported or patented in 1978 and 1987, respectively. The research for novel antibiotics has been halted by pharmaceutical companies, primarily due to low profitability resulting from short treatment duration, limited use of novel antibiotics (necessary to extend their
lifespan), the high risk of rapid resistance development, and the complexity of discovery and development. The development of novel antibiotics for Gram-negative bacteria is particularly challenging due to their double membrane cell wall, as it acts as a barrier, making it difficult for antibiotics to reach their target inside the cell. There are currently no novel oral antibiotic classes targeting Gram-negative bacteria undergoing clinical trials. Most current candidates are modifications of existing drugs aimed to avoid specific resistance mechanisms. While the development of novel antibiotics is crucial, it alone cannot address the issue of antibiotic resistance. The key priority lies in restricting the use of available antibiotic treatments, reviving of existing antibiotics, and opting for narrow-spectrum treatments whenever possible to mitigate the selection pressure leading to antibiotic resistance.

The clinically most important mechanism causing antibiotic resistance in Gram-negative bacteria within the order Enterobacterales (explained below) is the enzymatic production of ESBL. In 2017, the World Health Organization presented a priority list of antibiotic-resistant bacteria based on their threat to human health, considering factors such as resistance to current treatments and the potential for community transmission, demanding urgent further research and development of novel antibiotics targeting these pathogens. The highest priority was given to ESBL-producing Enterobacterales.

Gram-negative bacteria

This thesis explores treatment options for UTI and genetic characteristics of the two most prominent Gram-negative bacterial species in terms of morbidity and mortality: *E. coli* and *K. pneumoniae*. These bacteria exhibit significant diversity, ranging from non-pathogenic beneficial commensal strains within the intestinal microbiota, which mostly lacks virulence factors, to highly virulent strains responsible for severe infections. *E. coli* and *K. pneumoniae* are facultative aerobic bacterial species belonging to the Gram-negative bacterial family *Enterobacteriaceae* within the order Enterobacterales. *E. coli* is the leading cause of both community- and hospital-acquired UTI and among the most prevalent pathogens in bloodstream infections (BSI), including nosocomial bacteremia. *K. pneumoniae* can cause a wide range of infections such as UTI, BSI and pneumonia. Patients with *K. pneumoniae* infections often have underlying conditions such as malignancies with immunosuppression. This makes patients susceptible to nosocomial infections such as ventilator-associated pneumoniae, catheter-associated UTI, BSI and postsurgical wound infections. The severity of the infection varies depending on factors such as infection site, bacterial genetic characteristics, and host-related factors.
The Gram-negative cell wall

The Gram-negative cell wall consists of a double membrane separated by a thin peptidoglycan layer. The outer membrane contains phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet, while the inner membrane is a phospholipid bilayer. LPS comprises three structural components: O-antigen, oligosaccharide, and Lipid A (Figure 2). The O-antigen, the most diverse segment of the LPS molecule, exhibits variability in composition among strains within the same species. Modification of the O-antigen plays a role in the evasion of the host's immune system. Lipid A, a potent endotoxin, can lead to the overstimulation of the host's immune system and induces septic shock when released during cell lysis. Another important component in the cell wall is the presence of porins - small proteins that generate hydrophilic channels spanning across the outer membrane, facilitating the entry of metabolites and other hydrophilic compounds, including antibiotics (Figure 2). The peptidoglycan layer is a vital component of the bacterial cell wall, providing structural support, protection against osmotic pressure, and facilitating cell division.

Figure 2. Structure of the cell wall in Gram-negative bacteria and common virulence factors in *E. coli*: fimbrial adhesins, exotoxins (hemolysin), iron-acquisition systems (siderophore), proteases, polysaccharide capsule, modification of the O-antigen. Abbreviations: PBP; penicillin-binding protein. Created with BioRender.com.
**β-lactam antibiotics**

**Mechanism of action**

The β-lactam antibiotic group is the most widely used antibiotic class for treating acute infections and is of great importance for the treatment of Gram-negative bacterial infections.\(^ {33,34} \) This class comprises penicillins, cephalosporins, carbapenems and monobactam antibiotics. The main advantages of β-lactams compared to other antibiotic classes are their high efficacy, low toxicity, and a diverse range of antibacterial spectra.\(^ {34,35} \) β-lactam antibiotics contain a four-atom ring (β-lactam ring) in their molecular structure. These antibiotics target the peptidoglycan layer in bacterial cell walls through inhibitory binding to penicillin-binding proteins (PBPs), which are crucial for the synthesis of the peptidoglycan layer (Figure 2). This interaction leads to disruption of the cell wall and bacterial cell death.\(^ {34,36–38} \) There are many different PBPs. Different bacterial species possess unique sets of PBPs, of which one or two are essential. Each β-lactam group exhibits distinct affinities for specific PBPs. This is the reason for the differences in the antimicrobial spectrum between the β-lactam groups.\(^ {35} \)

**β-lactam resistance**

Resistance to β-lactams is mainly mediated by the **production of β-lactamases.** These enzymes, located in the periplasm, hydrolyze the β-lactam ring, thereby preventing the binding of the β-lactam to PBPs, rendering the β-lactam antibiotic ineffective (Figure 3).\(^ {39,40} \) Additional acquired resistance mechanisms against β-lactam antibiotics involve **modification of the drug target** (PBPs) and **reduced membrane permeability and efflux.**\(^ {41} \) Reduced membrane permeability and efflux are often due to factors such as porin loss and increased exportation of antibiotics through efflux pumps. This reduction of porins limits the entry of antibiotics into the periplasm. However, this impermeability frequently correlates with reduced fitness, as essential nutrients are unable to penetrate the cell wall.\(^ {42} \) The presence of multiple resistance mechanisms within the same bacterium can lead to increased resistance against antibiotics.
Fitness cost
Carriage of resistance genes undermines bacterial fitness and reduces the ability of bacteria to obtain nutrients or survive in certain environments. However, compensatory mutations may occur in some bacteria, which restores or improves bacterial fitness. In the presence of antibiotics, resistance genes provide the bacteria with a competitive advantage. However, without the selective pressure exerted by antibiotics, these genes may be lost due to fitness costs, while the bacteria may acquire alternative advantageous traits.43

Plasmid-mediated horizontal gene transfer
Antibiotic resistance can be acquired through mutations in chromosomal genes or through horizontal gene transfer (HGT).41 HGT can occur through three different mechanisms: (i) transformation, uptake of extracellular DNA that is then incorporated in the genome of the recipient; (ii) transduction, bacteriophage mediated transfer of genetic material and (iii) conjugation: genetic transfer of mobile genetic elements (e.g., plasmids) through cell-to-cell contact mediated by pili (Figure 4).44 Plasmids carry genes that are beneficial for the bacteria such as virulence genes, metabolic functions and antimicrobial resistance genes.45-48 The HGT of plasmids carrying resistance genes significantly contributes to the spread of antibiotic resistance because plasmids provide the resistant bacteria with an increased capacity to spread to other bacteria.
of the same or other bacterial species (Figure 4). The β-lactamases are usually plasmid-encoded and can be produced by all Enterobacterales.

![Diagram showing horizontal gene transfer via conjugative plasmids](https://BioRender.com)

**Figure 4.** Horizontal gene transfer via conjugative plasmids. Created with BioRender.com.

Extended-spectrum β-lactamases (ESBLs) and carbapenemases

The Ambler classification system is commonly employed to categorize β-lactamases, dividing them into four classes (A, B, C, and D) according to their amino acid sequences and hydrolysis mechanisms (serine- versus metalloenzymes). ESBL\textsubscript{A} and ESBL\textsubscript{M} can be categorized into Ambler classes A, C, and D, exerting hydrolytic activity on penicillins and cephalosporins (e.g., penicillinase, cephalosporinase), whereas ESBL\textsubscript{CARBA} includes enzymes from classes A, B, and D, exhibiting varying degrees of hydrolysis of carbapenems and of most β-lactams (Table 1). Thousands of unique β-lactamases have been discovered, encoding enzymes that exhibit diverse hydrolytic activities against different β-lactam antibiotics. Various classifications exist for β-lactamases. In Sweden, the classification includes ESBL\textsubscript{A} (classic ESBL, inhibited by clavulanic acid), ESBL\textsubscript{M} (plasmid-mediated AmpC-cephalosporinase, inhibited by cloxacillin), and ESBL\textsubscript{CARBA} (carbapenemase). This thesis primarily focuses on ESBL\textsubscript{A} and ESBL\textsubscript{M}, collectively referred to as ESBL.
Table 1. Classification of ESBL enzymes as sorted by Ambler molecular classes A-D.

<table>
<thead>
<tr>
<th>Class of ESBL</th>
<th>Ambler class</th>
<th>Catalytic site</th>
<th>Enzymes</th>
<th>Diagnostic inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL\textsubscript{A}</td>
<td>A</td>
<td>Serine</td>
<td>CTX-M, TEM, SHV</td>
<td>Clavulanic acid</td>
</tr>
<tr>
<td>ESBL\textsubscript{M}</td>
<td>C</td>
<td>Serine</td>
<td>Plasmid AmpC, CMY</td>
<td>Cloxacillin</td>
</tr>
<tr>
<td>ESBL\textsubscript{A}</td>
<td>D</td>
<td>Serine</td>
<td>OXA</td>
<td></td>
</tr>
<tr>
<td>ESBL\textsubscript{CARBA}</td>
<td>A</td>
<td>Serine</td>
<td>KPC</td>
<td>Boronic acid</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Zinc</td>
<td>VIM, NDM, IMP</td>
<td>EDTA</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>Serine</td>
<td>OXA-48</td>
<td>No inhibitor*</td>
</tr>
</tbody>
</table>

* Inhibited by β-lactamase-inhibitor avibactam, no commercial assays (phenotypic methods) available. Resistant to temocillin.

Class A serine β-lactamases

Class A encompasses a broad category of enzymes that exhibit diverse activities. Some of the most frequently encountered class A β-lactamases in Enterobacterales are CTX-M, TEM, SHV, and KPC enzymes (Table 1).\textsuperscript{35} Historically, variants of TEM and SHV were the predominant ESBL in Gram-negative bacteria. Today, CTX-M-15 is one of the most frequently encountered ESBL in clinical isolates of \textit{E. coli} and \textit{K. pneumoniae}, which is carried by the prevalent \textit{E. coli} clone ST131.\textsuperscript{45} Typically, CTX-M enzymes demonstrate higher efficacy in hydrolyzing cefotaxime and ceftriaxone compared to ceftazidime. However, the magnitude of this activity varies among different CTX-M variants.\textsuperscript{50} These enzymes are commonly susceptible to inhibition by β-lactamase inhibitors such as clavulanic acid and tazobactam, although to varying degrees (Table 2).\textsuperscript{51}

\textit{Klebsiella pneumoniae} carbapenemase (KPC) is the most prevalent carbapenemase globally. KPC carbapenemases are predominantly identified in \textit{K. pneumoniae} but can also be present in other Enterobacterales. The widespread dissemination of KPC is mainly attributed to the spread of \textit{K. pneumoniae} isolates from the highly successful ST258 clone. In general, KPC enzymes demonstrate effective hydrolysis of penicillins, cephalosporins (weak hydrolytic activity against ceftazidime), monobactams, carbapenems, and older β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. However, KPC activity can be inhibited by avibactam, relebactam, and vaborbactam (Table 2).\textsuperscript{51-53} Nevertheless, resistance to ceftazidime-avibactam has been documented and may emerge during treatment.\textsuperscript{54}
**Class B metallo-β-lactamases**

Class B metallo-β-lactamases (MBLs) exhibit structural and mechanistic differences from the serine β-lactamases found in classes A, C, and D. NDM, VIM, and IMP are the most frequently identified MBLs in clinical strains. They confer high-level resistance to most β-lactams, including carbapenems. MBLs consistently demonstrate resistance to ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam (Table 2). Nevertheless, in most cases, they remain susceptible to cefiderocol and monobactams (aztreonam).

Aztreonam-avibactam is a BLBLI that was recently approved by the European Medicines Agency (EMA). This is an attractive combination since the majority of strains producing MBLs often co-produce other β-lactamases capable of hydrolyzing aztreonam. The addition of Avibactam will then serve as a co-administered drug to protect aztreonam from enzymatic hydrolysis. For severe infections caused by MBL-producing Enterobacterales, treatment recommendations include aztreonam-avibactam or cefiderocol in combination with aztreonam.

**Class C serine β-lactamases**

Most AmpC β-lactamases are chromosomally encoded in many Enterobacteriales, such as *Enterobacter cloacae*, and can be induced following treatment with extended-spectrum cephalosporins. However, AmpC can be transferred to transmissible plasmids and appear in bacteria such as *E. coli* and *K. pneumoniae*. CMY-2 is the most prevalent plasmid-encoded AmpC enzyme in Gram-negative bacteria, including *E. coli* and *K. pneumoniae*. AmpC β-lactamases hydrolyze penicillins, cephalosporins, and to a lesser extent, monobactams. Tazobactam has generally low but measurable activity against AmpC enzymes and avibactam, relebactam and vaborbactam exhibit activity against all AmpC (Table 2).

**Class D serine β-lactamases**

The majority of the class D β-lactamases belong to the OXA family. OXA enzymes are not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and vaborbactam. Avibactam and relebactam have variable inhibitory activity against OXA enzymes (Table 2). Tazobactam can inhibit only certain OXA enzymes (e.g., OXA-2 and OXA-32). Avibactam can inhibit some variants (e.g., OXA-23 and OXA-24), in addition to OXA-48. OXA-1 is capable of hydrolyzing some cephalosporins, including cefepime. Notably, OXA-1 continues to be widespread among CTX-M-15-producing strains, making them resistant to all cephalosporins due to CTX-M-15 cephalosporinase activity.

OXA-48 is the most prevalent OXA carbapenemase in Enterobacterales. OXA-carbapenemases typically exhibit a weaker hydrolyzing effect on carbapenems compared to other carbapenemases. Due to their relatively low
carbapenemase activity, OXA-48 enzymes pose challenges in identification and frequently demonstrate only a modest increase in carbapenem minimum inhibitory concentrations (MICs).\textsuperscript{64} As a result, they are often classified as susceptible to carbapenems using standard susceptibility testing. However, it is crucial to identify them from an infection control perspective, as co-production of other β-lactamases (e.g., ESBLs), minor mutations, porin loss, or alteration in expression can rapidly cause significant high-level resistance to carbapenems.\textsuperscript{63} OXA-48 demonstrates limited hydrolysis of ceftazidime. Avibactam is the only inhibitor with effective inhibition of OXA-48 (Table 2).\textsuperscript{50,51,62}

**Virulence factors**

Both antibiotic resistance genes and virulence factors (genes) play crucial roles in the prevalence of antibiotic resistance. Investigating antibiotic resistance genes helps us to understand the molecular mechanisms behind resistance. This knowledge is essential for the development of new antibiotics that bypass or counteract these mechanisms. Pathogenic strains of *E. coli* and *K. pneumoniae* generally possess virulence factors that enhance their capacity to adapt to various environmental conditions, obtain nutrients and evade the host response. These virulence traits seem to be associated with clinical infection and can potentially impact the severity of the infection and clinical outcomes.\textsuperscript{65–67} Understanding these factors is important to determining the most effective interventions, antibiotic treatment and identifying outbreaks in clinical settings.\textsuperscript{17} The genes encoding virulence are often sorted by functionality into seven major categories.\textsuperscript{25,65} The most important virulence factors in *E. coli* are described in Figure 2, while a concise overview of the functionalities of the seven main types is described below, contextualized by our findings in paper II.

*Adhesin*

Adhesins such as fimbrial adhesins (e.g., pili) enable adherence to mucosal surfaces and are essential for the development of UTI.\textsuperscript{58,69} Pap-fimbriae are associated with febrile UTI. All *E. coli* strains sequenced in paper II, possessed Pap-fimbriae and Type 1 Fimbriae.

*Immune evasion (protectins)*

Bacterial infections trigger a robust host immune response. Typically, bacteria in the bloodstream are rapidly eliminated by the complement system or are marked for destruction by phagocytes. However, certain *E. coli* strains possess multiple mechanisms to evade these defenses, such as the K1 capsule that protects the bacteria and the genes *iss* that encodes a cell membrane-bound lipoprotein that increases serum survival. *Iss* has previously been reported to be
correlated with septic shock.\textsuperscript{65} In paper II, \textit{iss} and K1 capsule were present in all strains, regardless of whether they caused febrile or lower UTI.\textsuperscript{69,70}

\textit{Invasion}
Invasive virulence factors facilitate cellular invasion at various infection sites.\textsuperscript{71} In our cohort described in paper II, OmpA, OmpT, and FdeC were the most commonly found virulence factors. However, they were not associated with a particular outcome.

\textit{Iron-acquisition}
Iron-acquisition systems such as siderophores, are used by bacteria to acquire iron molecules that are essential nutrients for their survival. However, the urinary tract contains limited amounts of iron. Consequently, bacteria with an increased ability to absorb low amounts of iron gain an advantage in causing infection.\textsuperscript{68} The siderophore Aerobactin, Enterobactin and Chu occurred in almost all strains in paper II.

\textit{Proteases}
Proteases are associated with different virulence functions such as immunomodulation, toxicity and invasion. The proteases Pic, Sat and Tsh were analyzed in paper II, whereas Sat was the most frequently occurring protease.

\textit{Toxins}
Exotoxins such as hemolysin, are secreted by the bacteria and contribute to the destruction of host cells and facilitate bacterial invasion of tissues. The lysis of host cells releases nutrients, including iron, which the infecting bacteria can utilize.\textsuperscript{68} In our UTI cohort described in paper II, hemolysin was frequently detected and was associated with microbiological failure and relapse.

\textbf{High-risk clones}
Bacterial clones are closely related strains with the same ancestor. Successful high-risk clones are often MDR and have acquired multiple virulence factors that facilitate rapid transmission between hosts, as well as the ability to cause severe and/or recurrent infections. These clones are distributed worldwide.\textsuperscript{72,73} Genome sequencing of pathogenic \textit{E. coli} has revealed that specific clonal lineages are responsible for the majority of infections associated with antibiotic resistance, virulence, and clinical outbreaks globally.\textsuperscript{25} Multi-locus sequencing typing (MLST), is a widely applied method to type bacterial strains and identify sequence types (STs), enabling the characterization of genetic relationships among bacterial isolates. This method involves the analysis of DNA sequences in seven different housekeeping genes (i.e., genes that rarely mutate).\textsuperscript{74} Each species is defined by seven distinct housekeeping genes, and
information regarding registered STs is accessible in international databases. With the increasing availability of whole-genome sequencing (WGS), it has replaced the use of MLST. In Paper II, sequence types (STs) were determined using WGS data.

The *E. coli* ST131 high-risk clone remains key responsible for UTI and BSI caused by ESBL-producing *E. coli* and has spread globally like a pandemic. The *E. coli* ST131 is associated with multidrug resistance and possesses a significant number of virulence genes. The *E. coli* ST131 is divided into the clades A, B and C. The ST131 clade C is characterized by fluoroquinolone resistance and consists of two subclades: C1 and C2. C1 has acquired different CTX-M (*bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-27</sub> are the most common), whereas C2 (also called fimH30Rx) has acquired *bla*<sub>CTX-M-15</sub>. The subclade C2 is the dominant population among clade C and seems to be associated with recurrent UTI. The most prevalent *K. pneumoniae* clone associated with MDR and the production of KPC is ST258, closely followed by ST11, ST15 and ST101, which often harbor KPC genes and are associated with nosocomial spread.

Parenteral β-lactam antibiotics targeting *Enterobacteriales*

**Cephalosporins**

Cephalosporins are the first-line treatment for Gram-negative bacterial infections. Cephalosporins have continuously been improved and are divided into generations 1-5 based on their antibacterial spectrum and stability against resistance mechanisms. First-generation cephalosporins such as cefalexin, are administrated orally. Second-generation cephalosporins, including cefuroxime and exhibit an enhanced Gram-positive spectrum compared to later generations. Third-generation cephalosporins, e.g., extended-spectrum cephalosporins including cefotaxime, ceftriaxone and ceftazidime, are widely used. Fourth-generation cephalosporins e.g., cefepime, have a similar antibacterial spectrum as third-generation cephalosporins but demonstrate increased hydrolytic stability against AmpC enzymes. However, the use of cefepime remains limited in Sweden owing to low AmpC resistance. Fifth-generation cephalosporins, represented by ceftolozane in combination with tazobactam, offer improved efficacy in cases of porin loss and have an effect on ESBL<sub>LA</sub>. Cefiderocol, a new cephalosporine derivative introduced in 2019, consists of a cephalosporin compound attached to a siderophore. This siderophore specifically binds to iron and facilitates bacterial cell penetration through active iron transporters. Cefiderocol is currently a last-resort treatment option against Gram-negative bacteria, particularly against *Enterobacteriales* strains resistant to all other β-lactam antibiotics.
**Carbapenems**

Carbapenems, including meropenem, imipenem and ertapenem, have a very broad antibacterial spectrum and are stable against hydrolysis by ESBL-cephalosporinases (enzymes with resistance against extended-spectrum cephalosporins). However, they are susceptible to hydrolysis by carbapenemases.\textsuperscript{34,57} Meropenem and imipenem are considered gold standard therapy for severe infections caused by ESBL-producing Enterobacteriales, regardless of infection site, and have been associated with similar or superior clinical and microbiological outcomes compared to other broad-spectrum antibiotics.\textsuperscript{84–88} Ertapenem resistance is reported more frequently than resistance to other carbapenems, and the development of ertapenem resistance during treatment has been observed.\textsuperscript{89} Carbapenems are considered last-resort antibiotics that should be reserved for severe and difficult-to-treat infections. Additionally, given their very broad antibacterial spectrum, they are prone to cause major microbiome disturbances, further accelerating resistance development.\textsuperscript{12,90,91}

**Monobactams**

Aztreonam, the only monobactam in clinical use, has a Gram-negative aerobic spectrum but exhibits no activity against Gram-positive or anaerobic bacteria.\textsuperscript{34} It proves useful in cases of β-lactam allergy because cross-reactivity with other β-lactams is rare. A new combination therapy, aztreonam-avibactam, was approved by the EMA in March 2024\textsuperscript{56} and is anticipated for use in treating certain infections caused by carbapenemase-producing bacteria, due to aztreonam’s hydrolytic stability against MBLS.\textsuperscript{92}

**β-lactam-β-lactamase inhibitor combinations**

Combinations of β-lactam antibiotics with β-lactamase inhibitors (BLBLIs) have become increasingly important due to the higher prevalence of ESBL- and carbapenemase-producing bacteria. The β-lactamase-inhibitor inactivates the β-lactamase enzyme, thereby restoring the activity of the β-lactam antibiotic. The BLBLI combinations are sometimes categorized into two groups: the old ones (amoxicillin-clavulanic acid, piperacillin-tazobactam, ampicillin-sulbactam) and the newer ones (ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam).\textsuperscript{83} Tazobactam, clavulanic acid and sulbactam inhibit many class A β-lactamases. However, they have reduced effectiveness against strains that produce multiple β-lactamases and are ineffective against class A β-lactamases capable of inactivating carbapenem antibiotics (Table 2). Furthermore, these inhibitors generally exhibit insufficient activity against class B, C and D β-lactamases. The inhibitors, tazobactam, clavulanic acid and sulbactam, usually have no or low antibacterial activity when used alone against \textit{E. coli} and \textit{K. pneumoniae}.\textsuperscript{34} Avibactam, vaborbactam and relebactam are more potent ESBL inhibitors than the old inhibitors and have an effect both on ESBLs and some carbapenemases.
such as KPC but are not active against the MBL enzymes in class B (NDM, VIM, IMP). Furthermore, avibactam can inhibit class D enzymes that inactivate carbapenems (OXA-48).

In contrast to the older inhibitors, the more recent inhibitors inactivate most β-lactamase enzymes in a reversible manner. They may possess intrinsic antimicrobial activity. This is particularly true for avibactam. It is recommended, for antimicrobial stewardship considerations, to reserve the use of the newer BLBLIs for carbapenem-resistant Enterobacterales and other extensively resistant bacteria, rather than employing them for infections caused by ESBL-producing Enterobacterales.

Table 2. Spectrum of enzymatic inhibition by available β-lactam/β-lactamase inhibitor combinations.

<table>
<thead>
<tr>
<th>β-lactamase inhibitor</th>
<th>β-lactam partner</th>
<th>Ambler classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>Amoxicillin</td>
<td>x</td>
</tr>
<tr>
<td>Sulbactam</td>
<td>Ampicillin</td>
<td>x</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>Piperacillin</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avibactam</td>
<td>Ceftazidime</td>
<td>x</td>
</tr>
<tr>
<td>Vaborbactam</td>
<td>Meropenem</td>
<td>x</td>
</tr>
<tr>
<td>Relebactam</td>
<td>Imipenem</td>
<td>x</td>
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</table>

x Effective inhibitory activity.
* Variable inhibitory activity. Tazobacta generally has low but measurable activity against class C enzymes (AmpC) and can inhibit only certain OXA enzymes, (e.g., OXA-2 and OXA 32). Avibactam and relebactam are typically not potent inhibitors of most OXA-enzymes. Avibactam inhibits OXA-48.
Oral treatments against Enterobacteriales

Cefitubuten
Cefitubuten is not recommended for empirical treatment of ESBL infections due to a notably high resistance rate among these isolates, estimated at around 90%. Additionally, there is a lack of clinical evidence supporting its efficacy in treating ESBL infections. Nevertheless, cefitubuten could be considered as a treatment option for stable patients infected with a cefitubuten-susceptible strain, with the recommendation of using a higher dose of 400 mg q12h.\textsuperscript{94} Cefitubuten was used as a first-line antibiotic for febrile UTI in women and children in Sweden until its deregistration from the market in 2017. It is currently available under license and is used in pregnant women, children, and in cases where there are no other suitable oral antibiotics. Cefitubuten remains an attractive treatment option due to its relatively low resistance rate in non-ESBL-producing \textit{E. coli} (<10%) and limited microbiome impact compared to ciprofloxacin.\textsuperscript{95}

Ciprofloxacin
Ciprofloxacin is recommended as oral empirical treatment for clinically stable, low-risk patients with febrile UTI or as step-down treatment in severe infections according to ESCMID guidelines.\textsuperscript{57} However, it is not recommended as a first-line treatment for lower UTI due to concerns regarding microbiome disturbances and the emergence of resistance. Approximately 60-80\% of the ESBL-producing strains are resistant to ciprofloxacin in Sweden; hence ciprofloxacin as empirical treatment is not recommended for UTI.\textsuperscript{94}

Nitrofurantoin
Nitrofurantoin is a narrow-spectrum antibiotic active \textit{in vitro} against most ESBL-producing \textit{E. coli} and is indicated for treatment of lower UTI caused by \textit{E. coli} and \textit{E. faecalis}. Nitrofurantoin is recommended for empirical treatment of lower UTI in both men and women in Sweden due to its relatively limited microbiome impact and low resistance rates.\textsuperscript{94} During treatment, nitrofurantoin maintains low serum concentrations, with therapeutic levels primarily concentrated within the lower urinary tract.\textsuperscript{96,97}

Pivmecillinam
Pivmecillinam has been used in the Scandinavian countries since the 1980s.\textsuperscript{98,99} However, its usage remains predominantly confined to this region and pivmecillinam is currently under the Food and Drug Administration’s (FDA) review for approval, with a decision expected in April 2024.\textsuperscript{100} Long-term clinical experience in the Scandinavian countries supports its efficacy for treating lower UTI in women,\textsuperscript{101,102} with no clinically significant resistance reported despite widespread use.\textsuperscript{103,104} Mecillinam exposure often leads to the development of resistant mutants \textit{in vitro}.\textsuperscript{105} However, resistant mutants
disappear in vivo due to high fitness cost i.e. reduced ability to survive in the bladder of mecillinam-resistant strains.\textsuperscript{105} This explains the low prevalence of mecillinam resistance despite widespread use.

Mecillinam, administered as the oral prodrug pivmecillinam, is a narrow-spectrum antibiotic active in vitro against most ESBL-producing E. coli and also some carbapenemase-producing strains (NDM, OXA enzymes and KPC, while VIM hydrolyses mecillinam).\textsuperscript{106–108} It is a suitable therapeutic option for empirical treatment of lower UTI based on its relatively limited microbiome impact and low resistance rates.\textsuperscript{109–111} Pivmecillinam is a first-line antibiotic for lower UTI in both men and women in Sweden, and clinical antibiotic susceptibility breakpoints exist only for this indication.\textsuperscript{112} Higher (400mg q6-8h) than normal dosing or lowered (≤1 mg/L) clinical breakpoints may be required for systemic infections such as febrile UTI due to the relatively low drug concentrations achieved in the blood.\textsuperscript{113} However, while high drug concentrations in the blood are crucial in severely ill patients and at the initiation of therapy, adequate urine concentrations may be sufficient when used as an oral follow-up.

In some regions in Denmark and Norway, pivmecillinam is already recommended as first-line or second-line therapy for febrile UTI in clinically stable patients.\textsuperscript{114–116} It is also used as a treatment of lower UTI caused by ESBL-producing isolates and a higher rate of clinical cure has been observed when a higher dose of 400 mg q8h is applied compared to 200 mg q8h.\textsuperscript{107} In cases of febrile UTI caused by ESBL-producing strains, Region Hovedstaden’s guidelines in Denmark recommend a dosage of 800 mg q8h or 400 mg q6h. This regimen has demonstrated good tolerability and efficacy, leading to favorable clinical cure rates (personal communication with Niels Frimodt Møller and Katrine Harlung Hansen, Hvidovre Hospital, Region Hovedstaden, Denmark).\textsuperscript{117} A previous study investigating febrile UTI patients has similarly demonstrated good tolerability with intravenous administration of mecillinam at a dosage of 800 mg q8h.\textsuperscript{118}

**Trimethoprim-sulfamethoxazole (Co-trimoxazole)**

Trimethoprim-sulfamethoxazole can be considered for treatment of clinically stable patients with febrile UTI, if the bacterial strain is known to be susceptible.\textsuperscript{57,94} Trimethoprim-sulfamethoxazole is not recommended as a first-line treatment for lower UTI due to the risk of microbiome damage and resistance development. Moreover, its use is limited due to resistance rates exceeding 20% in most settings and approximately 60-70% of the ESBL-producing strains are resistant to trimethoprim-sulfamethoxazole in Sweden.\textsuperscript{113}
Knowledge gap in the treatment of UTI

UTI have become an increasingly significant medical challenge due to the increased prevalence of resistant bacteria.\textsuperscript{1,2} In recent years, a number of new parenteral antibiotics have been introduced for the treatment of more severe infections, but oral antibiotics are gradually losing their effectiveness and are not being replaced by new agents. More research is needed to increase our knowledge of how to use available antibiotics most effectively.\textsuperscript{10} We identified two major knowledge gaps: (i) treatment of lower UTI in men and, (ii) treatment of lower and febrile UTI caused by ESBL-producing \textit{E. coli} and \textit{Klebsiella} spp.

Pivmecillinam and nitrofurantoin were included in the Swedish national treatment guidelines for lower UTI in male patients in 2012.\textsuperscript{95} The change was made due to significantly lower resistance rates\textsuperscript{109} to these antibiotics compared to the previous first-line options, trimethoprim and ciprofloxacin, and presumed low microbiome impact. However, evidence regarding the efficacy of these treatments in male patients remains scarce,\textsuperscript{119} and there are concerns regarding their pharmacokinetic profiles. Notably, nitrofurantoin treatment is associated with low serum concentrations and limited tissue penetration in the prostate.\textsuperscript{120,121}

For the treatment of critically ill patients with BSI caused by either confirmed or suspected ESBL-producing bacteria, current evidence supports a carbapenem (meropenem or imipenem) as an empirical treatment.\textsuperscript{57} Conversely, piperacillin-tazobactam is recommended in less severe cases with urinary source of infection and with the higher dose of 4.5 g q6h applied.\textsuperscript{38} However, the clinical and microbiological outcome of piperacillin-tazobactam as treatment of febrile UTI caused by ESBL-producing Enterobacterales have not been investigated in a Swedish setting, and the applied treatment regimens for ESBL-producing UTI in Sweden are unknown. Oral treatment options for UTI caused by ESBL-producing bacteria are additionally limited due to frequent co-resistance to other classes of antibiotics such as trimethoprim-sulfamethoxazole and ciprofloxacin. This contributes to the overuse of carbapenems, such as ertapenem.\textsuperscript{122} The development of a novel oral carbapenem, tebipenem pivoxil hydrobromide, further increases the risk of carbapenem overuse.\textsuperscript{123} Although pivmecillinam is used for non-severe febrile UTI and as step-down treatment after initial intravenous treatment in Denmark and Norway, the available evidence supporting its efficacy is limited.\textsuperscript{107,115,116} Consequently, its inclusion as a first-line treatment for febrile UTI in European and Swedish guidelines is questionable. Concerns have also been raised regarding its effectiveness against ESBL-producing isolates, as well as uncertainties surrounding optimal dosing and treatment duration.
The intestinal microbiota

Function and importance

Recent evidence suggests that the human microbiome, the assemblage of microorganisms in and on our body, is a critical component of health. We host over 50% of the cells in our body, and the microbial inhabitants contribute to 100 times more genes than our human genome.\textsuperscript{124-126} The microbiota is distributed throughout our body, with a predominant concentration in the intestines, where a significant amount of host-microbe interaction occurs. Recent metagenomic analyses have confirmed that the intestinal microbiota consists of close to 3000 different bacterial species,\textsuperscript{127-129} with 99% of them being anaerobic. These bacterial species belong to different phyla, including Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia, which collectively represent over 90% of the total bacterial population (Figure 5).\textsuperscript{127}

![Figure 5. The gut microbiome composition in healthy children and adults. White – children, light blue - young adults, dark blue – adults, red – senior adults. By Justine W Debelius with data from Yatsunenko et al. (2012).\textsuperscript{130} Reproduced with permission from Justine W Debelius.](image)

Research findings indicate that the primary five phyla are relatively evenly distributed among healthy populations regardless of their origin, with higher levels of Firmicutes and Bacteroidetes in all groups (Figure 5).\textsuperscript{130} However, significant differences emerge when comparing children and adults in the same population, as highlighted in a comparative study involving healthy populations from the United States (U.S.), Malawi, and Venezuela (Figure 5). In general, the microbiota is established early in childhood by transmission of bacteria from other family members and remains relatively stable over time. However, various factors such as medication, age, lifestyle, diet, geographic location, and other environmental factors can influence the composition of the intestinal microbiota to varying degrees.\textsuperscript{16,130-136} Falony et al. have shown that
on a population level, some medications such as proton-pump inhibitors can have a significant impact on the intestinal microbiota.\textsuperscript{137,138}

The intestinal microbiota is very individual despite external influences. Each individual carries unique sets of bacteria and genes. The individual microbial identity has been identified in several studies of the intestinal microbiota,\textsuperscript{139,140} and no intervention beyond fecal transplant has been sufficient to alter the individual microbial identity. The microbiome has important biological functions for the human host. Perturbation of the intestinal microbiota has been associated with several long-term health complications such as allergies and inflammatory, immunological, and metabolic diseases.\textsuperscript{141–144} Additionally, microbiota has a vital function as a protective barrier against invasive pathogenic species.\textsuperscript{145} This phenomenon, known as colonization resistance, serves as a primary defense mechanism against both exogenously introduced infectious pathogens as well as the overgrowth of endogenously potential pathogens such as \textit{E. coli} and \textit{K. pneumoniae}.\textsuperscript{145,146} Colonization resistance is achieved through various mechanisms, including pathogen eradication (killing), enhancement of immune responses, and competition for limited nutrients.\textsuperscript{147}

Antibiotic-induced damage on the intestinal microbiota

Systemic antibiotic treatment inevitably has an impact on our intestinal microbiota. Such treatment can immediately disrupt the microbiome by eliminating susceptible bacteria and favouring the selection of antibiotic resistance genes (ARGs) and multidrug-resistant bacteria. These disturbances may result in short-term gastrointestinal side effects, dysbiosis, and an increased susceptibility to \textit{Clostridioides difficile} enteritis, along with reduced colonization resistance against invading pathogens.\textsuperscript{145,149} The microbiome disturbances can also result in long-term changes. Microbiome damage, characterized by reduced or altered microbial diversity and the loss of specific taxa has been found up to two years following brief exposures (1-7 days) to antibiotics such as oral ciprofloxacin, clindamycin, tetracyclines, and macrolides.\textsuperscript{12,141,150–155} Additionally, more persistent alterations in the microbiome have been associated with inflammatory, immunological, and metabolic disorders later in life such as cancer, heart disease and diabetes.\textsuperscript{156,157} Several studies indicate that exposure to antibiotics early in life might be correlated with the development of chronic diseases that emerge years after the initial antibiotic exposure.\textsuperscript{158,159} Furthermore, a recent study revealed a correlation between antibiotic treatment during pregnancy and the carriage of ARGs in newborns.\textsuperscript{131}

In treatment guidelines, it is often assumed that broad-spectrum antibiotics disrupt the composition and diversity of the intestinal microbiota to a greater extent than narrow-spectrum antibiotics. However, this is only a crude assumption since evidence regarding the impact of different antibiotics is limited.\textsuperscript{12} More knowledge is urgently needed to improve our understanding and
enable prediction of the microbiome impact of different antibiotics in guidelines and antimicrobial stewardship programs.

Most previous studies have used phenotypic culture-based methods to investigate the microbiome impact of antibiotics. Such culture-based methods allow the detection of clinically relevant resistant bacteria such as ESBL-producing Enterobacterales. However, these methods cannot fully capture the composition of the microbiota because unculturable commensals and the taxonomy of rare species are missed. Sequence-based metagenomic methods provide a better determination of selection pressure and characterization and quantification of the commensal bacteria, including the predominant anaerobic bacteria, as well as identifying the presence of ARGs (resistome). The intestinal microbiome serves as a well-established reservoir for numerous ARGs and functions as a central hub for their horizontal exchange. Antibiotic treatment can substantially impact both the intestinal microbiome and its resistome. The clinically most relevant antimicrobial selection pressure probably occurs in the intestinal microbiome and is an important factor in the emergence and dissemination of resistant pathogenic bacteria.

A recent meta-analysis investigated the current evidence on the impact of different antibiotics on the microbiota and found that it is difficult to draw definitive conclusions due to the heterogeneity in the study design. Many previous studies were limited by small sample sizes and short follow-up periods, thereby constraining definitive conclusions regarding the lasting impacts of antibiotics. Few of these studies accounted for potential confounding factors such as ongoing medication, prior antibiotic usage, age or hospitalization. Additionally, many studies were conducted several decades ago and used lower antibiotic dosages compared to current treatment guidelines.

The intestinal microbiota in patients undergoing hematopoietic stem cell transplantation

Patients undergoing hematopoietic stem cell transplantation (HSCT) for hematological diseases are at high risk of both acquisition of resistant bacteria such as ESBL-producing Enterobacterales, as well as microbiome perturbations. This vulnerability is due to factors such as underlying diseases, chemotherapy, and prolonged hospital stays, which compromise their immune systems and increase susceptibility to infections. Consequently, these patients often require antibiotic therapy. Most HSCT patients receive oral ciprofloxacin as prophylaxis, and patients with neutropenic fever are subjected to broad-spectrum intravenous treatment such as a carbapenem, piperacillin-tazobactam or ceftazidime. Some studies indicate that the composition of the microbiota can impact transplantation outcomes, including the occurrence of complications such as graft-versus-host disease (GVHD) and overall survival rates.
Early use of broad-spectrum antibiotics during HSCT has been associated with worse transplantation outcome. Additionally, antibiotic exposure prior to (within 3 weeks) chemotherapy is associated with poor transplantation outcome and reduced overall survival in patients diagnosed with various cancer types, including lymphoma and in patients undergoing CD19-CAR-T-cell cancer immunotherapy. However, antibiotic treatment prior to immunotherapy could be a surrogate for poor disease status. Knowledge of the relative effects of different antibiotics on the microbiome in this vulnerable patient group is, however limited. The enhanced understanding of the significant microbiome impact of antibiotic treatment underscores the need for increased attention to antimicrobial stewardship in this patient group. The characterization of the microbiome and resistome at baseline can contribute to personalized antimicrobial stewardship strategies. This approach could possibly identify patients who require closer monitoring and in-patient care (instead of out-patient care at home or in a patient hotel during the neutropenic phase, which is applied in many hematology wards), as well as the need for antibiotic treatment.

The provision of nutritional support for patients undergoing HSCT is debated. Most guidelines recommend enteral nutrition (EN). However, this recommendation is based on limited evidence and the majority of hematology wards provide total parenteral nutrition (TPN). The findings from a recent meta-analysis indicate that EN reduces the incidence of intestinal acute GVHD. These results underscore the importance of promoting EN as the primary nutritional support strategy for patients undergoing HSCT. However, additional investigations are warranted.
Aims

The overall aim of this thesis was to investigate the therapeutic effects of carbapenem-sparing and narrow-spectrum antibiotics, as well as oral antibiotics, as treatments for UTI, and to assess their impact on the intestinal microbiota.

Specific aims were:

I. To investigate the efficacy of narrow-spectrum antibiotics (nitrofurantoin and pivmecillinam) for lower UTI in men, with trimethoprim as a comparator.

II. To prospectively evaluate current treatment strategies and identify patient and pathogen factors associated with treatment outcome in UTI caused by ESBL-producing Enterobacterales.

III. To prospectively assess the impact on the microbiota of oral antibiotics that are commonly used for UTI (ceftibuten, ciprofloxacin, nitrofurantoin, pivmecillinam, and trimethoprim-sulfamethoxazole) in healthy adults.

IV. To prospectively assess the composition of the intestinal microbiota in patients undergoing HSCT due to hematological diseases, changes following oral and intravenous antibiotics prescribed as prophylaxis or treatment, and patient outcomes.
Methods

The methods used in the studies are fully described in the method section of each publication. Here, only a portion of those details is repeated to facilitate the understanding. A condensed version of the study design for each study is presented in the results section of this thesis.

Disk diffusion (paper II)
Antibiotic susceptibility testing (AST) was performed at the clinical microbiology laboratories in Sweden. The disk diffusion method, recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as the standard AST method, was employed. In this approach, Mueller-Hinton agar plates are evenly coated with a bacterial suspension using a swab, followed by placing a disk containing a predetermined amount of antibiotic on the surface of the inoculated plates. After 16-20 hours of incubation, the inhibition zones are measured using calipers. These zones are then interpreted as "Susceptible," "Susceptible with Increased Exposure," or "Resistant," based on breakpoints provided in EUCAST tables. Isolates with resistance to cefotaxime and/or ceftazidime were subjected to ESBL testing with the double disc synergy test (DDST), as described in EUCAST guidelines for the detection of resistance mechanisms.

Broth microdilution and agar dilution (paper II)
The reference method for determining AST is the determination of minimal inhibitory concentration (MIC) through broth microdilution (BMD), except for mecillinam and fosfomycin, where agar dilution is the reference method. In this method, a standardized inoculum is added into wells containing antibiotics at specific concentrations, with 2-fold dilution steps, including the concentration of 1 mg/L. The microtiter plate is incubated between 16-24 hours depending on the species. BMD is unavailable in all clinical laboratories due to the increased workload and large volumes of reagents. However, a commercially available product for BMD susceptibility testing is Sensititre™ DKMGN plates (Thermo Fisher Scientific, Waltham, MA, USA). These plates provide standard panels containing pre-prepared antibiotic dilutions in 96-well microtiter plates. While the Sensititre™ DKMGN plates were used
in paper II, an in-house microdilution method was used specifically for nitrofurantoin AST.

Agar dilution is the reference method for mecillinam and fosfomycin susceptibility testing. In agar dilution, antimicrobial agents at different concentrations are added to the agar before casting the plates, and approximately 5 x 10^5 CFU/mL are plated. An in-house agar dilution was used for mecillinam MIC in paper II. In both BMD and agar dilution, the antimicrobial susceptibility is defined by the MIC, which is the lowest concentration of antibiotic that effectively inhibits visible growth within the incubation period. The results were interpreted according to EUCAST clinical breakpoints version 13.0.177

Whole genome sequencing (paper II)
Genotypic characterization with whole genome sequencing (WGS) was performed on the collected bacterial isolates in paper II to detect resistance genes, virulence genes, and sequence types (STs). We selected previously described virulence genes associated with UTI.65 DNA was extracted by the MagNA Pure 96 System (F. Hoffmann-La Roche, Basel, Switzerland). WGS was performed using the HiSeq platform (Illumina Inc., San Diego, CA, USA). Read quality, screening for resistance genes and MLST were assessed using the in-house bioinformatic pipeline microSALT (https://github.com/Clinical-Genomics/microSALT), which use the ResFinder database v2.1.178 Raw reads were analyzed by the Virulence Finder database179 to identify virulence genes in E. coli. After assembly with SPAdes (version 3.9),180 contigs were submitted to the BIGSdb database (https://bigsdb.pasteur.fr/klebsiella) to retrieve virulence genes in Klebsiella spp.

Culture- versus sequencing-based methods to characterize bacteria (paper II)
Phenotypic methods, including disk diffusion, BMD, and agar dilution, can be used both to determine susceptibility and clinically relevant resistance such as ESBL-producing Enterobacterales. Valuable data on clones, resistance genes, and virulence genes can be acquired with WGS. In clinical settings, WGS is primarily used in outbreak management and pathogen surveillance.

However, as knowledge increases regarding the significance of specific sequence types (STs), resistance and virulence genes in infections, WGS holds the potential to evolve into a comprehensive molecular diagnostic tool for routine clinical practice.181,182 However, this requires robust bioinformatic capabilities and continuous development and validation against phenotypic methods. This is critical for detecting novel resistance mechanisms not yet cataloged in existing databases. Furthermore, with WGS, we can detect genes that are present without causing clinically relevant resistance.
Culture-based methods to assess the intestinal microbiota (paper III)

Quantitative culture analysis was performed on samples collected at baseline and 7, 14 and 90 days after initiation of antibiotic treatment. Selective agar plates with added antibiotics (ceftriaxone, ciprofloxacin, nitrofurantoin, pivmecillinam or trimethoprim-sulfamethoxazole) were used to assess the susceptibilities of isolates corresponding to the EUCAST clinical susceptibility breakpoints, version 13.0. Samples were weighed using a balance weight scale and 0.5 mg of faeces was suspended in 4.5 ml of tryptic soy broth. Then, the suspension was vortexed and serially diluted. From each dilution, 100 µl of the suspension was spread on chromogenic agar plates, with and without the studied antibiotics, and aerobically incubated overnight at 37 °C. Viable counts were read after 24 hours. The colony-forming units (CFU) counts were counted after overnight incubations and the counts were log-transformed for analysis. Additionally, selection on chromagar plates containing cefalosporin and ciprofloxacin (1 mg/L) were used to determine the fractions of isolates with ESBL production or resistance to ciprofloxacin, respectively, in the total population of Enterobacteriales.

Shotgun metagenomic sequencing (paper III)

Fecal samples were preserved in a DNA/RNA-shield and extracted using a combined physical and chemical lysis protocol. Libraries were prepared with the MGI FS DNA library prep kit and sequenced using a 2x100 paired-end approach on a DNBSeg G400 sequencer (Shenzhen MGI Tech Co., Ltd, China). The per-sample sequences were processed through a standard pipeline (StaG pipeline; https://github.com/ctmrbio/stag-mwc) which removes low-quality bases and any human DNA. Bacterial taxonomy was assigned using Kraken2 to map reads against the Genome Taxonomy Database, version 89,179,180. Antibiotic resistance genes were mapped using AMR++ in the MEGARes database.181 Diversity statistics were calculated using QIIME 2.182

16SrRNA sequencing and bioinformatics (paper IV)

Samples were extracted, amplified and processed as previously described by Hugerth et al.183 The 16S rRNA gene was amplified using primers targeting the V3-V4 hyper-viable regions using a one-step PCR protocol with 20 cycles and barcoded for another 10 cycles. For sequencing, we used the Illumina MiSeq platform, producing 2 x 300 bp paired-end reads according to the manufacturer’s instructions. Samples were demultiplexed using an in-house script. Demultiplexed samples were imported into QIIME 2 (v 2019.10) using the manifest format.184 Primers were trimmed using the q2-cutadapt plugin with default parameters (cutadapt v 2.6).185 Paired-end sequences were merged and
the reads were quality filtered using the vsearch and quality filtering plugins, respectively with default parameters (vsearch v. 2.7.0).\textsuperscript{186,187} Sequences were denoised using the deblur algorithm trimmed to 400 nt.\textsuperscript{188} A phylogenetic tree was built using fragment insertion into the Silva reference tree backbone with the SEPP algorithm.\textsuperscript{189,190} Taxonomy was assigned using a region-specific naïve Bayesian classifier built using the Silva QIIME 2 compatible reference database; sequences that could not be assigned at kingdom level were excluded.\textsuperscript{191} The data was rarefied to 1250 sequences/sample for diversity calculations and samples with fewer reads were excluded.

Untargeted metagenomic sequencing versus 16SrRNA-sequencing (paper III, IV)

Untargeted metagenomic sequencing and 16S rRNA sequencing are two different approaches used in microbiome analysis. Untargeted metagenomic sequencing sequences all genes present in the sample, including human DNA, although fecal samples contain 2\% human DNA. The human DNA is then removed \textit{in silico} (computationally). This method offers deeper sequencing compared to amplicon analysis (16SrRNA), providing taxonomy down to the species level and enabling resistome analysis (antibiotic resistance genes).\textsuperscript{192} Untargeted metagenomic sequencing provides information on the entire microbial community, including less abundant and non-bacterial microorganisms and allows for functional analysis (functional genes) and metabolic pathways.\textsuperscript{193} However, untargeted metagenomic is more computationally intensive and expensive compared to amplicon sequencing. The increased resolution and functional capacity may not provide additional utility over what is available with 16rRNA sequencing, especially if the goal is ecological characterization. 16S rRNA gene sequencing specifically targets and amplifies a segment (most often the V3-4 region) of the 16S ribosomal RNA gene, which is present in all bacteria, but varies sufficiently to distinguish between different taxa. The amplified gene fragments are then sequenced to identify bacterial taxa. Due to the amplification step, amplicon sequencing is more suitable for samples with low microbial DNA content, as observed in the fecal samples analyzed in paper IV.

Ethical considerations

All studies were approved by the Uppsala regional ethical review boards. In studies II-IV, which were based on prospective data, all participants approved written informed consent prior to enrollment. Participation in the studies had no impact on the care provided to individual patients. Study I, based on retrospective data from a UTI cohort, investigated clinical outcomes of UTI treatment. Obtaining consent without potential bias was deemed impossible.
Consequently, the ethical review approved to the study with a waiver of consent from participants. The primary ethical concern was the lack of autonomy and potential privacy breaches, as data had to be gathered from patients' medical records. To mitigate any adverse effects on individual participants, several precautionary measures were implemented. Firstly, only essential data was obtained, and all information was pseudonymized and securely stored in a password-protected file. Secondly, access to the data was restricted to authorized researchers only. Lastly, the results were presented at an aggregated level to mitigate any potential for identifying individual participants. Study III raised ethical considerations due to the administration of oral antibiotics to participants who did not have infections or a requirement for antibiotic treatment. However, we did conclude that the potential benefits of this study would outweigh any potential harm to participants, as exposure to antibiotics in healthy, young individuals was not anticipated to result in long-term effects on the intestinal microbiota or acquired resistance. Moreover, the insights gained from this study could significantly influence future treatment guidelines and decrease emergence of resistance. In summary, it was judged that the beneficial impact of these studies would outweigh the potential harm subjected to any participant.
Results and discussion

Paper I

Retrospective evaluation of nitrofurantoin and pivmecillinam for the treatment of lower urinary tract infections in men

When the study was initiated, nitrofurantoin and pivmecillinam had recently been introduced into the national UTI treatment guidelines for male patients with lower UTI.\textsuperscript{95} This change was made due to notably lower resistance rates to these antibiotics compared to previous first-line options, trimethoprim and ciprofloxacin.\textsuperscript{109} However, the evidence regarding their efficacy in male patients remains limited, and concerns persist regarding their pharmacokinetic profiles and the attainment of sufficient tissue concentrations in the prostate.\textsuperscript{94,120} Additionally, underlying predisposing factors for UTI such as urogenital disease and urinary tract catheter are more common among men with UTI compared to women, leading to a higher frequency of recurrent infections.\textsuperscript{194} Thus, our objective was to investigate the efficacy of nitrofurantoin and pivmecillinam as treatments for lower UTI in men, with trimethoprim as a comparator.

All adult male patients prescribed nitrofurantoin, pivmecillinam or trimethoprim and diagnosed with lower UTI in Uppsala County during 2012 were retrospectively identified. Data on patient characteristics, antibiotic treatments, clinical outcome and microbiological results were obtained from the medical records. We assessed associations between antibiotic treatment, patient factors and pathogens on treatment failure, rate of new antibiotic prescriptions, and relapse within three months. Treatment failure was defined as prescription of a new antibiotic during the intended treatment period due to clinical deterioration or persistent UTI symptoms. Relapse was defined as identification of the same species with similar antibiotic susceptibilities compared to the initial episode.

We identified male patients (n=629) with lower UTI. After exclusion due to no culture, no significant growth, fever, absence of UTI symptoms or growth of >1 bacterial species, 171 patients were included (nitrofurantoin n=69, pivmecillinam n=57, trimethoprim n=45). One case of treatment failure was observed with nitrofurantoin and four with pivmecillinam, while no treatment failure was noted with trimethoprim. A new prescription within three months was more frequently observed with nitrofurantoin (34%, 23/69) and
pivmecillinam (30%, 17/57), compared to trimethoprim (22%, 10/45). Additionally, relapse within three months was more commonly observed in patients treated with nitrofurantoin (15%, 10/69), or pivmecillinam (17%, 10/57), compared to trimethoprim (7%, 3/45). However, the observed differences were not statistically significant. The clinical outcomes with nitrofurantoin and pivmecillinam were acceptable in comparison with trimethoprim and in line with previous studies with other established treatment options such as ciprofloxacin.\textsuperscript{195,196} Notably, over 95% of \textit{E. coli} isolates were susceptible to nitrofurantoin and pivmecillinam, in contrast to the 69% susceptibility observed to ciprofloxacin and trimethoprim. Urinary catheterization was associated with a higher risk for new antibiotic prescriptions (p=0.02), and prostate cancer was associated with a higher incidence of relapse (p=0.04).

No statistically significant association was observed between treatment outcome and dose or duration. For nitrofurantoin the dose 50 mg q8h was used. For pivmecillinam, 200 mg q8h was used in most cases, and a third of the patients were prescribed a lower dosage of 200 mg q12h. However, it's worth noting that higher dosages, e.g., 100 mg q8h of nitrofurantoin and 400 mg q8h of pivmecillinam, are used in other countries.\textsuperscript{102,119,194} Notably, a comparative analysis of 5- versus 7-day regimens of pivmecillinam for men with lower UTI in Denmark demonstrated no significant differences in treatment outcomes.\textsuperscript{119} Therefore, the use of the higher dose of 400 mg q8h for 5-10 days may be a preferable treatment for lower UTI in men. An increased dose had potentially improved the clinical cure rate for pivmecillinam in this study.

In conclusion, nitrofurantoin and pivmecillinam are suitable for empirical treatment of lower UTI in men, given their high activity against \textit{E. coli}, minimal resistance development, as well as limited impact on the intestinal microbiota.
Paper II

Treatment, outcomes and characterization of pathogens in urinary tract infections caused by ESBL-producing Enterobacterales: a prospective multicenter study

Treatment options for UTI caused by ESBL-producing Enterobacterales are scarce due to their frequent co-resistance to multiple antibiotic classes, and evidence to support therapeutic decisions is limited. We aimed to evaluate clinical and microbiological treatment outcomes and relapse in patients with UTI caused by ESBL-producing Enterobacterales.

This prospective observational multi-center study was conducted in collaboration with local investigators at 15 infectious diseases hospital departments in Sweden. Patients with UTI caused by ESBL-producing Enterobacterales were recruited by the local investigators and were included following written informed consent (Figure 6). Data on patient characteristics and treatments were collected from the medical records. The first follow-up, 10-14 days after completion of treatment, included clinical assessment of recovery and sampling for urine culture. A second follow-up was conducted after 90 days to capture relapse with the same species and with similar antibiotic susceptibilities compared to the initial episode. This involved conducting phone consultations, reviewing medical records, and review of microbiological results in cases of relapse. Bacterial isolates were subjected to MIC determination with broth microdilution (agar dilution for mecillinam) and whole genome sequencing to detect resistance genes, virulence genes and sequence types (STs).

![Study design in paper II](https://www.biorender.com)

Figure 6. Study design in paper II. Created with BioRender.com.
Recurrent UTI was defined as >1 UTI within the last 6 months or ≥3 within the past 12 months. Clinical cure was defined as resolution of UTI symptoms, while microbiological cure was defined as no bacteria growth or growths <10^3 CFU/mL in urine sampled 10-14 days after the end of treatment. Relapse was defined as the recurrence of UTI symptoms within 3 months and the presence of significant bacterial growth in the urine, consistent with the bacterial species identified in the initial episode and with similar susceptibility.

The final analysis included 235 patients. Carbapenem and piperacillin-tazobactam as empirical intravenous treatment were associated with similar clinical outcomes, 83%, 34/41 versus 82%, 23/28, respectively. Notably, the majority of cases received a suboptimal dose of piperacillin-tazobactam (4.5g q8h), which is lower compared to the current dosing recommendations for the treatment of ESBL-infections (4.5g q6h).

Resistance rates of 11% among *E. coli* strains and 18% among *K. pneumoniae* strains were observed against piperacillin-tazobactam. Notably, resistance rates were higher among blood isolates, with 26% resistance observed in *E. coli* and 40% in *K. pneumoniae* strains. These findings highlight the challenge of using piperacillin-tazobactam as empirical therapy for severe infections, leading to the recommendation of carbapenems as the preferred empirical treatment for the critically ill. Oral nitrofurantoin and pivmecillinam showed high clinical cure rates in patients with lower UTI, 79%, 44/56 and 81%, 42/52, respectively. Regarding pivmecillinam, only 38% (63/164) of the collected strains had a MIC ≤1 mg/L. Pivmecillinam was used as treatment for febrile UTI in 10 cases, resulting in a clinical cure rate of 100% (10/10) and a microbiological cure rate of 80% (8/10).

Complicating patient factors e.g., diabetes mellitus, urogenital disease, urinary tract catheter and recurrent infection were associated with disease presentation (febrile UTI). Recurrent infection was associated with clinical failure (p=0.003) and relapse (p=0.004), whereas diabetes mellitus and urological disease were associated with relapse (p=0.03, p=0.002, respectively). Moreover, bacterial genetic features (ST131 in *E. coli* and haemolysin) were associated with microbiological failure or relapse, in accordance with previous publications.197

CTX-M-15, CTX-M-27, and TEM were identified as the predominant β-lactamases in *E. coli* and CTX-M-15 and SHV in *K. pneumoniae*. OXA-1 was found in 32% (52/164) of the *E. coli* strains, all of which were CTX-M-15 producers. The vast majority of the *E. coli* strains belonged to ST131, and a smaller proportion to ST69. *E. coli* ST131 clade C, subgroup C2 was notably the most prevalent, particularly in cases of febrile UTI (30%, 19/64 versus 16%, 16/100).

In conclusion, non-carbapenem treatments performed well and should be advocated in patients who are not critically ill. Complicating patient factors and recurrent infection were associated with febrile UTI. ST131 in *E. coli* and
haemolysin were associated with microbiological failure or relapse. With the increased availability of whole genome sequencing, integrating genetic characterisation into routine practice has become feasible. In the near future, antibiotic resistance genes, virulence genes and STs will likely be considered when making clinical decisions and may prove useful in guiding individualized treatment and follow-up.
Paper III

Impact of nitrofurantoin, pivmecillinam, trimethoprim-sulfamethoxazole, ceftibuten and ciprofloxacin on the intestinal microbiota and resistome: a randomized controlled trial with healthy adults

Any systemic antibiotic treatment is likely to cause disturbances in the intestinal microbiota. In some cases, this will result in gastrointestinal side effects or enteritis caused by Clostridioides difficile. However, most often perturbations in the intestinal microbiota, including selection of resistance genes and multidrug-resistant bacteria, will be asymptomatic. The presumed microbiome impact is the basis of treatment decisions and guidelines in many common infections when there are several options with similar efficacy with regard to clinical and microbiological outcomes. Additionally, it is often assumed that broad-spectrum antibiotics disrupt the intestinal microbiota to a greater extent than narrow-spectrum antibiotics. However, this is only a crude assumption. Yet, evidence of the relative impact of different antibiotics has not been systematically evaluated using the recent sequence-based metagenomic methods for characterization of the intestinal microbiota. We aimed to investigate the microbiome impact of five commonly used antibiotics for the treatment of UTI.

![Figure 7. Study design in paper III. Created with BioRender.com. Abbreviation: Trimethoprim-sulfamethoxazole; co-trimoxazole.](image)

We conducted a randomized, controlled trial with healthy adults. Participants were block-randomized in a 1:1:1:1:1 ratio stratified by sex to antibiotics that are commonly used for UTI (ceftibuten, ciprofloxacin, nitrofurantoin, pivmecillinam, trimethoprim-sulfamethoxazole) for 5 days at standard dosing, or no treatment (Figure 7). Fecal samples collected before and at 7, 14, 30, 90,
180, and 365 days after start of therapy were analyzed by untargeted metagenomic sequencing for characterizing alfa- and beta diversity of the intestinal microbiota, taxonomy and antibiotic resistance genes (resistome). Additionally, samples collected before and at 7, 14 and 90 days after initiation of treatment were analyzed using phenotypic culturing methods to detect selection of resistant Enterobacterales. Prior to enrollment and after 12 months of follow-up, all participants completed a questionnaire to capture factors that may have influenced the composition of the intestinal microbiome and the acquisition of resistant bacteria. A q-value <0.1 (used in the taxonomy methods) and a p-value <0.05 were considered significant.

The final analysis included 86 participants. The baseline characteristics were evenly distributed between the groups. All antibiotics, except nitrofurantoin, induced significant changes in the intestinal microbiome. Ciprofloxacin caused the most dramatic and immediate disturbances in alfa- (p<0.01) and beta diversity (p<0.01), as well as taxonomy (q<0.1). Some of these changes persisted, with a 10.96% reduction in number of observed species (alpha diversity) after 12 months compared to the pre-treatment baseline sample. A sustained perturbation in beta diversity was observed over 90 days post-treatment (p<0.01). The other broad-spectrum antibiotics, ceftibuten and trimethoprim-sulfamethoxazole, also showed significant effects, ceftibuten in alpha- and beta diversity (p<0.05), and trimethoprim-sulfamethoxazole in taxonomy (q<0.1). However, these changes were less pronounced and short-term (<90 days). Specifically, ciprofloxacin influenced the abundance of species within the Firmicutes phylum, resulting in an increase in Lachnospiraceae and a decrease in Ruminococcaceae. Collectively, broad-spectrum antibiotics showed a significantly greater impact on the microbiome compared to the narrow-spectrum antibiotics nitrofurantoin and pivmecillinam. Additionally, phenotypic culture analyses detected resistant Enterobacterales most frequently in the trimethoprim-sulfamethoxazole group. The low rate of selection of resistant Enterobacterales, as well as low frequency of ARGs in all groups, could be attributed to a limited colonization of ARGs and resistant strains within this young and healthy study population. A higher frequency of resistance would have been expected in an older study population with a higher prevalence of comorbidities.

In conclusion, ciprofloxacin showed significant, immediate and long-term disruption of the intestinal microbiota in terms of diversity and taxonomy, and stands out in comparison with the other studied antibiotics regardless of their antimicrobial spectrum. Consequently, ciprofloxacin is considered a less favorable treatment option for UTI.
Paper IV

Antibiotic-induced microbiome disturbances in patients with hematological disease undergoing stem cell transplantation: a prospective observational study

Increasing evidence suggests that the composition of the microbiota can impact transplantation outcomes, including the occurrence of complications such as graft-versus-host disease (GVHD) and mortality.\textsuperscript{163–165} However, our knowledge on the relative effects of different antibiotics on the microbiome in this vulnerable patient group is limited.\textsuperscript{12,169} We aimed to explore the microbiome impact of oral versus intravenous antibiotics in patients with hematological diseases undergoing HSCT. Finally, we wanted to investigate associations between the intestinal microbiome at baseline and patient outcomes.

In this prospective observational study, we included adult patients admitted to the hematology ward for hematopoietic allogeneic-HSCT (allo-HSCT) or autologous-HSCT (auto-HSCT). Fecal samples were collected prior to chemotherapy, weekly for 4 weeks (or until hospital discharge), and at the 6-month follow-up. The samples were analyzed using 16S rRNA sequencing to characterize the gut microbiome diversity. Additionally, phenotypic screening was performed at the same timepoints to detect ESBL- or carbapenemase-producing Enterobacterales. Data on patient demographics, medical condition, treatment and outcome (three years follow-up) was extracted from the medical records (Figure 8).

Figure 8. Study design in paper IV. Created with BioRender.com. Abbreviation: MRG: multidrug-resistant Gram-negative bacteria (only ESBL).

The final analysis included 88 patients (allo-HSCT, n=35 and auto-HSCT, n=53). Most patients (80/88) received ciprofloxacin prophylaxis during the neutropenic phase, and 73\% (64/88) received broad-spectrum intravenous antibiotics (carbapenem, ceftazidime, piperacillin-tazobactam, and vancomycin).
Baseline microbiome diversity was significantly lower in allo-HSCT patients compared to auto-HSCT patients (p<0.001), likely due to a higher frequency of recent antibiotic treatment in this group or poorer disease status. Moreover, a low microbiome diversity was associated with future occurrences of neutropenic fever, intravenous antibiotic therapy (p<0.05), and TPN during hospitalization (p<0.001). We observed immediate changes in the intestinal microbiome diversity with oral ciprofloxacin prophylaxis (p<0.05), while no significant changes were noted with intravenous broad-spectrum antibiotics. Our findings in this study align with the results in Paper III, indicating that oral ciprofloxacin exerts a pronounced impact on the intestinal microbiota. This observation contributes to the ongoing discussion of whether ciprofloxacin prophylaxis during the neutropenic phase following HSCT is beneficial or not. The greater impact of oral ciprofloxacin on the microbiome, compared to intravenous antibiotics, may be attributed to the high concentrations of ciprofloxacin found in the feces. This finding also challenges the antimicrobial stewardship concept of an early switch from intravenous to oral administration to reduce selection of resistance. Further research, including randomized trials, is warranted to validate our results.

It is optimal to conduct ESBL-screening before chemotherapy, as most screenings in our study yielded negative results after chemotherapy. This observation is consistent with the sequencing results, which showed reduced DNA content after chemotherapy, antibiotic treatment and TPN. The decrease in DNA content after TPN may be attributed to decreased nutrition in the intestine during TPN administration.

In conclusion, significant perturbations in the intestinal microbiota were found already at baseline especially in the allo-HSCT cohort. A low diversity at baseline was associated with neutropenic fever, intravenous antibiotic treatment and TPN during hospitalization. Prophylactic oral ciprofloxacin demonstrated a significant impact on the microbiome, whereas we did not detect any significant impact of intravenous antibiotics. These findings add to the discussion of whether ciprofloxacin prophylaxis should be used in these patients.
Conclusion

Overall, this thesis provides new insights into therapeutic efficacy of narrow-spectrum and carbapenem-sparing antibiotics for the treatment of UTI caused by ESBL-producing Enterobacterales or non-ESBL-producing bacteria. Furthermore, it increases our knowledge of how different antibiotics, commonly used to treat UTI, affect the intestinal microbiota.

More specifically:

I. Nitrofurantoin and pivmecillinam are suitable for empirical treatment of lower UTI in men, considering their high activity against *E. coli* and limited impact on the intestinal microbiota and resistance development.

II. Carbapenem-sparing antibiotics are effective treatments for UTI caused by ESBL-producing Enterobacterales and should be recommended for non-critically ill patients. Certain bacterial genetic features (ST131 in *E. coli* and haemolysin), were associated with microbiological failure or relapse.

III. Ciprofloxacin demonstrated significant, immediate and long-term disruption of the intestinal microbiota in terms of diversity and taxonomy and stands out in comparison with other antibiotics regardless of their antimicrobial spectrum. Consequently, ciprofloxacin is a less favorable treatment option for UTI.

IV. A low microbiome diversity at baseline is associated with neutropenic fever, antibiotic treatment and the use of TPN during HSCT. Prophylactic oral ciprofloxacin demonstrated a more pronounced impact on the microbiome compared to intravenous antibiotics.
Future perspectives

The results from these studies have contributed to a better understanding of the treatment of UTI caused by ESBL and non-ESBL-producing bacteria. Additionally, they have provided new insights into how different antibiotics commonly used to treat UTI affect the intestinal microbiota. To the best of our knowledge, paper III represents the first RCT to provide a parallel assessment of the impact on the microbiome induced by five frequently prescribed antibiotics for UTI, using untargeted metagenomic sequencing. Both papers III and IV showed a significant impact of ciprofloxacin on the intestinal microbiome. Consequently, ciprofloxacin appears to be a less favorable treatment option for UTI. These results could potentially influence both national and international treatment guidelines.

The results from paper II suggest the potential use of oral antibiotics for febrile UTI caused by ESBL-producing bacteria. Specifically, pivmecillinam emerges as a promising narrow-spectrum option, offering ecological advantages over ciprofloxacin and trimethoprim-sulphamethoxazole (paper III). Consequently, there is a need for a randomized, controlled, open-label, non-inferiority clinical trial to assess the efficacy of high-dose pivmecillinam as a step-down oral therapy for patients with febrile UTI caused by ESBL- or non-ESBL-producing *E. coli*.

Moreover, it is important to reduce the use of ciprofloxacin as a treatment for non-severe febrile UTI in primary healthcare settings. To achieve this goal, it is essential to evaluate the efficacy of pivmecillinam and other narrow-spectrum antibiotics as empirical treatment for non-severe febrile UTI. Once alternative treatment options have been systematically evaluated, antibiotic stewardship initiatives for UTI in primary healthcare could involve modifying the reporting of antimicrobial susceptibility testing (AST) results such as excluding AST for ciprofloxacin. Additionally, the inclusion of older and narrow-spectrum antibiotics is essential in the advancement of new diagnostic tools. Numerous companies and researchers are currently dedicated to developing rapid AST methods to determine bacterial susceptibility within a few hours. However, several of these methods do not include older antibiotics such as pivmecillinam, nitrofurantoin, and aztreonam; instead, they prioritize newer BLBLI combinations. Additionally, the Sensititre DKMGN plates used for BMD in paper II did not include nitrofurantoin, necessitating the use of an in-house microdilution method specifically for nitrofurantoin susceptibility.
testing. This may potentially result in decreased use of older antibiotics in favor of newer antibiotics that should be reserved for more difficult-to-treat infections.

The findings in paper IV raise questions about the benefit of ciprofloxacin as antibiotic prophylaxis during the neutropenic phase of HSCT. Currently, there is an ongoing discussion regarding the use of prophylaxis, with different clinical practices observed across hospitals, both within Sweden and internationally. More research is needed on this topic, such as an RCT comparing ciprofloxacin prophylaxis to no prophylaxis but early recognition and treatment of infections in hospitals with similar resistance rates. Additionally, investigating the relative impact of oral versus intravenous antibiotics on the microbiome, warrants further investigation, ideally in randomized studies.

Another RCT was conducted as part of this thesis. We assessed the impact of phenoxybenzylpenicillin, amoxicillin, and amoxicillin-clavulanic acid on the intestinal microbiota and resistome in healthy adult volunteers. A total of 106 patients were randomly assigned to receive one of these treatments or placebo (no treatment). Patient inclusion, one-year follow-up, and untargeted metagenomic analysis have been completed, while bioinformatic and phenotypic culture analyses are currently ongoing.

There is a need for academic-driven RCTs of old, already approved antibiotics to evaluate their efficacy for indications beyond their initial approval.\textsuperscript{10} However, these RCTs are expensive, receive inadequate funding, are time-consuming, and are challenging to execute in terms of patient recruitment. Collaborations among different hospitals and countries are required to facilitate the implementation of randomized clinical treatment trials and enhance the power of the studies. This could be achieved through the establishment of a united MDR Gram-negative database in real-time, where patient registration could start at the microbiology laboratory upon the detection of MDR Gram-negative bacteria.\textsuperscript{109}

och resistensgener, de här metoderna används i studierna som ingår i avhandlingen.


**Studie 1** syftade till att undersöka effekten av antibiotika med smalt bakteriellt spektrum (effekt mot få sjukdomsframkallande bakterier och liten påverkan på tarmfloran) som behandling av UVI hos män.

**Studie 2** syftade till att kartlägga hur UVI orsakat av ESBL-producerande bakterier behandlas i Sverige. Samband mellan olika antibiotikabehandlingar och behandlingsresultatet (symptomfre샘 samt ny UVI inom 3 månader) undersöks samt behandlingsresultat kopplat till bakteriens gener och komplicerande patientfaktorer som exempelvis diabetes och urinkateter.

**I studie 3** jämfördes effekten på tarmfloran av flera vanliga antibiotika som används vid behandling av UVI. Friska förövare fick antibiotika under 5 dagar och tarmfloran undersökes både före och upp till ett år efter antibiotikakuren.

**I studie 4** studerades förändringar i tarmfloran hos patienter som behandlas med stemcelltransplantation. Hur tarmfloran påverkades av olika antibiotika och om det fanns samband mellan tarmflorans sammansättning och behandlingsutfall eller biverkningar undersökes.

Sammanfattningsvis visar våra resultat att:

I. Antibiotikasorterna nitrofurantoin och pivmecillinam fungerar bra som behandling vid nedre UVI hos män, pga. god behandlingseffekt vid infektion orsakad av *Eschericha coli* bakterier och liten påverkan på tarmfloran.

II. Det fungerar bra att använda andra antibiotikasorter än karbapenem som behandling av UVI orsakad av ESBL-producerande bakterier till patienter som inte är kritiskt sjuka. Genom att analysera (sekvensera) den sjukdomsframkallande bakteriens gener kan man vägleda valet av rätt antibiotika och uppföljning av patienter med UVI.

III. Ciprofloxacin har en påtaglig och långvarig (upp till 1 år) påverkan på tarmfloran jämfört med de andra antibiotikasorterna som ingick i studien. Ciprofloxacin bör undvikas vid behandling av UVI när det finns andra alternativa antibiotika som har lika god behandlingseffekt.

IV. Tarmfloran innan behandling med cellgifter vid stamcellstransplantation påverkas av grundsjukdom och ökar benägenheten för infektion och antibiotikabehandling under vårdtiden. Ciprofloxacin i tabletform som förebyggande behandling mot infektion hade en tydlig negativ effekt på tarmfloran, även i jämförelse med andra breda antibiotikasorter som ges intravenöst.
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