Aus der Medizinischen Universitätsklinik und Poliklinik Tübingen

Abteilung Innere Medizin I

(Schwerpunkt: Gastroenterologie, Gastrointestinale Onkologie, Hepatologie, Infektiologie und Geriatrie)

Outcome of sepsis due to Pseudomonas aeruginosa: Impact of antibiotic resistance and therapy

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Maurer, Susanna Hedwig

2020

Dekan:	Professor Dr. I. B. Autenrieth
1. Berichterstatter:	Professor Dr. E. Tacconelli
2. Berichterstatter:	Professor Dr. C. Wolz

Tag der Disputation: 24.02.2020

Meiner Familie

Table of contents

List of tables

List of figures

List of abbreviations

Abbr.	Abbreviation
AMK	Amikacin
ARDS	Acute respiratory distress syndrome
AZ	Aztreonam
BSI	Bloodstream infection
CAP	Community-acquired pneumonia
CDC	Centre for Disease Control and Prevention (USA)
CEF	Ceftazidim
CF	Cystic fibrosis
CI	Confidence interval
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CNS	Central nervous system
COL	Colistin
CVL	Central venous line
def	definitive
DIC	Disseminated intravascular coagulation
E. coli	*Escherichia coli*
ECDC	European Centre for Disease Prevention and Control
ECCMID	The European Congress of Clinical Microbiology and Infectious Diseases
Eg	Echthymia gangraenosum
emp	empirical
ERCP	Endoscopic retrograde cholangiopancreaticography
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FOS	Fosfomycin
GM	Gentamicin
HAP	Hospital-acquired pneumonia
HIV	Human immunodeficiency virus
HR	Hazard ratio

ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IMP	Active on imipenem
IMP	Imipenem
L, μmol, ml, dL	Liter, deciliter, milliliter, microliter
MALDI-TOF	Matrix Assisted Laser Desorption Ionization – Time of Flight
MDR	Multidrug resistant
MER	Meropenem
mg	milligram
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin susceptible *Staphylococcus aureus*
NNIS	National Nosocomial Infections Surveillance System
P. aeruginosa	*Pseudomonas aeruginosa*
PIP	Piperacillin
PIP/TAZ	Piperacillin/Tazobactam
SCOPE	Surveillance and Control of Pathogens of Epidemiologic Importance
SD	Standard deviation
spp.	Species
US	United States of America
UTI	Urinary tract infection
VAP	Ventilator-associated pneumonia
VIM	Verona integron-encoded metallo-β-lactamases
XDR	Extensively drug-resistant

1. Introduction

1.1 *Pseudomonas aeruginosa*

1.1.1. Microbiology

Pseudomonas aeruginosa (*P. aeruginosa)* was first extracted from green pus and described by French pharmacist Gessard in 1882 (1). This bacterium belongs to the family of *Pseudomonadaceae*. It is a gram-negative bacterium that appears in a rod-like shape, carrying a monotrichous flagella and several pili. *P. aeruginosa* does not derive its energy from fermentation of glucose. It thrives aerobically but can also grow under anaerobic conditions in the presence of nitrate. Thus, it is a facultative anaerobic nonfermenter. It is not very fastidious concerning its nutrition, making its identification in the laboratory uncomplicated. *P. aeruginosa* strains produce a variety of pigments such as pyrubin, pyomelanin, pyoverdin and pyocyanin. If pyoverdin and pyocyanin are produced the colonies shows a characteristical blue-green discolouration. The latin word *aeruginosa* is translated into „copper-rust“ or verdigris and describes its typical blue-green color of laboratory cultures of the species (2). Wound infections due to *P. aeruginosa* can often be identified by its „grapelike“ odor, which is produced by most strains (3). The organism can survive temperatures as high as 50°C and it can grow in distilled water. Water with a pH of 4.5 or lower does not allow the survival of *P. aeruginosa* (3).

The pathogen causes a wide variety of infections in humans. Just as varied as the diseases *P. aeruginosa* causes, it produces and possesses a wide range of virulence factors, including endotoxins, exotoxins, type III secreted toxins, pili, flagella, proteases, phospolipases, iron-binding proteins, exopolysaccharides, the ability to produce biofilms and elaboration of pyocyanin (3).

Most strains are able to produce an extracellular polysaccharide called alginate. It serves to protect the bacterium in different environmental conditions but also from the patient’s immune system. The production of these so-called biofilms plays a major role in the pathogenesis of cystic fibrosis (CF) patients. In CF-patients *P. aeruginosa* invades the thick mucus layers in the lungs. Despite limited oxygen levels in the mucus, the bacterium has the ability to produce an alginate (4), consequently shielding itself

from the body's immune system (5) and it protects the pathogen from antibiotic treatment.

P. aeruginosa is not the only species of the Pseudomonas genus known to cause infection in humans, albeit it is the most prominent bacterium. Pseudomonas spp. other than aeruginosa include *Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas cepacia, Pseudomonas stutzeri* and *Pseudomonas putrefaciens*, just to name a few (6). They are occasionally isolated from human clinical specimens and can result in opportunistic infections (7, 8). However, non-aeruginosa Pseudomonas spp. are associated with a lower degree of virulence and infections and generally milder in course (7, 9, 10).

1.1.2 Epidemiology

P. aeruginosa is an opportunistic pathogen that primarily causes infection in immunocompromised hosts. It is a nosocomial pathogen that mostly infects hospitalized patients. It is also responsible for community-acquired infections in patients with severe underlying diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD) or a debilitated immune system (11). Even though it rarely causes disease in healthy humans, it can lead to numerous diseases in such a setting, especially when the patient is exposed to moist environment.

Bathing in contaminated swimming pools, hot tubs and whirlpools may lead to skin infections, referred to as folliculitis (12, 13). One of the leading causes for acute otitis externa, commonly known as „swimmer's ear" is *P. aeruginosa* (14). Patients who use particularly extended-wear contact lenses are at risk for sight-threatening infections due to *P. aeruginosa*. When infected they may suffer from ulcerative keratitis and endophaltmitis (15, 16). The bacterium can be found in contact lens solutions and it adheres to the contact lens surface more easily than other pathogens (17, 18). However, *P. aeruginosa* also plays a substantial role in the infection of puncture wounds and endocarditis linked to intravenous drug users (19).

Data from the National Nosocomial Infections Surveillance (NNIS) System recorded in 2003 has shown the distribution of gram-negative and gram-positive bacilli in hospitals

in the United States (US): *P. aeruginosa* is the second most common microorganism isolated in nosocomial pneumonia (18.1% of cases), accounts for 16.3% of cases of urinary tract infections, is the fifth most common cause of surgical site infection (9.5%) and has been isolated in blood stream infections in almost 4% of the cases (20). In hospitals, *P.aeruginosa* can be found in numerous reservoirs: hospital equipment such as cleaning solutions, mops, respiratory ventilators and surgical equipment. Furthermore, it has been isolated from sinks, drains, toilets and showers and is also reintroduced into the hospital's environment through water used for flowers in patient's rooms and spreads by contact of unsanitized hands (3). Nosocomial outbreaks due to unclean medical equipment have been described (21, 22). A severe outbreak of *P. aeruginosa* occurred during the winter of 2001-2002 in Norway involving 231 patients in 24 hospitals. Due to contaminated moist mouth-swabs and receipt of mechanical ventilation, 71 patients (31%) with severe underlying diseases died during hospitalization (22).

Nosocomial infections are often associated with hospitalization in the intensive care unit (ICU), medical devices (e.g. mechanial ventilation, central venous catheter), previous antibiotic treatment and surgery (23).

Outside of the hospital, *P. aeruginosa* lives ubiquitously in our environment: it can be found in soil, plants and water and also colonizes healthy animals and is part of the normal human flora or so-called microbiota. Up to 7% of healthy humans carry this bacterium in the throat, nasal mucosa, or on the skin. Transmission may occur through various modes, such as from patient to patient, from reservoir to patient and by colonization with subsequent autoinfection with the acquired strain (24).

1.1.3 High-risk patients

P. aeruginosa ist characterized as an opportunistic pathogen – a term that is used to describe organisms that may exist as part of the normal human flora and that are "capable of causing disease only when the host's resistance is lowered" (25). *P. aeruginosa* is known to infect mostly immunocompromised patients. The state of impaired immune response may be either a consequence of an underlying disease and of the use of certain therapies that reduce or alter the patients' immune system. Classically,

patients with neutropenic conditions were seen to be at high risk for *P. aeruginosa* bacteremia (26). Neutropenia is known as a condition with abnormally low neutrophile granulocytes (neutrophile count ≤500/µl). Neutrophile granulocytes play an important role in the host defense against pathogens. *P. aeruginosa* employs a wide array of virulence factors to invade the host and to evade the host's immunological defense. Not only does it posses factors that inhibit phagocytosis by neutrophils (27), it also secretes leukocidin that kills neutrophils (28). Although *P. aeruginosa* infections were not frequently reported prior to 1960, cancer and immunosuppressive treatments were associated with an increased frequency of infection (29). In the late 1960s and the 1970s when effective antipseudomonal antbiotics were unavailable, *P. aeruginosa* was a common cause for infection in neutropenic patients with incidence rates reaching 55% (30). The incidence of *P. aeruginosa* changed after the introduction of carbenicillin and the pathogen spectrum in immunocompromised patients shifted from gram-negative to predominantly gram-positive microorganisms (31). Currently, the epidemiological situation seems to shift again, with several reports indicating a significant rise in gram-negative infections in patients with underlying hematological malignancies (31-33), with high mortality rates reaching up to almost 40% for *P. aeruginosa* bacteremia (31). Trecarichi et al. (25) prospectively analyzed 575 bloodstream infections in patients with hematological malignancies and observed an increase in gram-negative infections with *P. aeruginosa, Escherichia coli (E. Coli)* and *Klebsiella pneumoniae* being the most prominent isolates.

A number of studies have identified several patient groups at high risk for *P. aeruginosa* bacteremia due to an impaired immune system. Buhl et al. reviewed risk factors for acquisition of extensively drug-resistant (XDR) *P. aeruginosa* strains. Patients with a debilitated immune function, prior solid-organ transplantation and hematological malignancies were prone to infection or colonization (34).

Although effective infection control measures and improved therapy procedures have led to a decrease in incidence of bacteremia due to *P. aeruginosa* in burn wound patients (35), *P. aeruginosa* is still the most frequent cause for burn wound infections in many centers (36). Pathophysiological knowledge may explain why this subgroup of patients is at risk for *P. aeruginosa* bacteremia. Pathogenesis of burn wound infection is based on an impaired immune system and the loss of the skin's barrier function. Studies

have shown that patients with extensive burn wounds share compromised neutrophile functions and T-lymphocyte dysfunctions (37, 38). These mechanisms may predispose the patient for serious infection.

1.1.4 Resistance

P. aeruginosa is naturally (intrinsically) resistant to several antibiotics and it can also develop resistance towards multiple classes of antibacterial agents.

P. aeruginosa is naturally resistant against the following antibiotics: penicillin G, A, M, 1st and 2nd generation cephalosporins, some 3rd generation cephalosporins, ertapenem, kanamycin, tetracyclines, macrolides, cotrimoxazole and glcopeptides (39). Responsible for the pathogens intrinsic resistance are mechanisms such as expression of efflux pumps, low permeability of its outer membrane and naturally occurring AmpC β-lactamases, only to name a few. Acquired resistance can result from mutation or from exogenous resistance determinants and can be achieved by a number of mechanisms, such as degrading enzymes (e.g. carbapenemases such as IMP and VIM (40)), reduced permeability and active efflux (41).

While the prevalence of infections caused by *P. aeruginosa* has remained relatively stable, the prevalence of resistant isolates has increased in 2003 compared with 1998 (42). Likewise, a national surveillance study of ICU patients identified an increase of multidrug-resistant (MDR) *P. aeruginosa* strains from 4% in 1993 to 14% in 2002 (43). In a survey of microbiological data from over 200 hospitals in the United States, the incidence of MDR among *P. aeruginosa* pneumonia isolates was found to be 22% and 15% among bloodstream isolates (44). The emergence of MDR strains makes treatment of *P. aeruginosa* even more difficult.

In order to create a uniformed and standardized characterization of resistance pattern, a joint-initiative by the ECDC and the CDC proposed a definition both for XDR and MDR *P. aeruginosa* strains (45). Beforehand, it is important to remember that the 17 available antipseudomonal agents can be catalogued into the following 8 antimicrobial categories: antipseudomonal penicillins + β-lactamase inhibitors, monobactams, antipseudomonal cephalosporins, antipseudomonal carbapenems, antipseudomonal fluorochinolones, aminoglycosides, phosphoric acids and polymyxins. MDR *P.*

aeruginosa strains are resistant to at least one agent in at least three out of the eight categories. On the other hand, XDR *P. aeruginosa* strains show resistance to at least one agent in the minimum of six out of the eight categories.

MDR *P. aeruginosa* strains show higher mortality rates when compared to multidrug-susceptible strains (46, 47) which associated with increased patient morbidity (48). Risk factors associated with multidrug resistant *P. aeruginosa* strains include the following: Bedridden status, ICU stay, presence of invasive devices, prior use of certain antibiotics (including broad-spectrum cephalosporins, aminoglycosides, carbapenems, fluoroquinolones), Diabetes mellitus, malignant disease, undergoing surgery and HIV-infection (48, 49).

Buhl et al. reviewed the impact of therapeutic factors, patient-related factors, environmental factors and medical devices on risk for aquisition and colonization of XDR *P. aeruginosa* strains. Prior exposure to certain antibiotics such as fluorochinolones, carbapenem and amikacin were identified as a risk factor for acquisition for XDR *P. aeruginosa*, leading the authors to suggest that antibiotic therapy may lead to the selection of resistant strains. Furthermore, the use of medical devices (e.g. urinary catheter, mechanical ventilation, central venous catheter) were found to be independent risk factors for acquisition and colonization of XDR *P. aeruginosa*. Wet hospital reservoirs such as sinks were often suspected to be the source of exposition for patients (34).

Available clinical data suggests that the emergence of MDR *P. aeruginosa* results in greater risk of death, longer duration of hospitalization (50) and an increase in surgery required for treatment (51), which consequently has a burdensome impact on healthcare costs (52).

1.2 Bacteremia due to *P. aeruginosa*

1.2.1 Epidemiology and clinical characteristics

Incidence of bacteremia of *P. aeruginosa* has changed considerably over the past decades. Kerby et al. noted 91 cases of bacteremia due to *P. aeruginosa* in the world literature (53). Before 1950 only 1% of bacteremic cases were caused by this pathogen.

After 1950 the incidence increased to 7%-18%, causing mortality rates from 37%-77% (26). Numbers published in the 1970s have shown mortality rates of pseudomonas bloodstream infections surpassing 50% (54). A US nationwide prospective surveillance study (Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE)) analyzed 24.179 cases of BSI in 49 hospitals during a 7-year period (1995-2002) and concluded that 4% of the bloodstream infections were associated to *P. aeruginosa*, making it the 3rd leading cause of gram-negative infections (55). Several risk factors for BSI due to *P. aeruginosa* have been identified as neutropenia or other immunodeficiency (e.g. HIV and bone marrow transplants), severe burns, pancreatobiliary tract disease, urinary catheters or central venous lines, advanced age and recent hospitalization (56, 57). Typically, neutropenic and burn wound patients have been considered to be affected the most by Pseudomonas infection. However, most recent data has shown that the most frequent sources of bacteremia are the urinary and respiratory tract. This may be due to the use of indwelling urinary catheters and respiratory ventilators. Also, nosocomial outbrakes due to contaminated medical equipment used for ERCP (endoscopic retrograde cholangiopancreaticography) have been described (58, 59).

BSI due to *P. aeruginosa* may present itself as benign transient bacteremia (8). Transient bacteremia may lead to fever; however it is typically asymptomatic and first and foremost describes bacteria circulating in the blood system without any pathogenic value. Tachypnea, Tachykardia, mental disorientation and high fever may suggest development of sepsis. Common complications are respiratory failure due to pneumonia or acute respiratory distress syndrom (ARDS), development of DIC (disseminated vascular coagulation), renal failure and acute encephalopathia (60). Sepsis due to *P. aeruginosa* does not differ from sepsis due to other gram-negative bacteria in its clinical manifestation (28). Additional symptoms vary from site of infection. Certain patients with *P. aeruginosa* bacteremia develop a skin lesion known as Ecthyma gangraenosum (Eg). Eg is not pathognomic for *P. aeruginosa*, however, it is most frequently described in the setting of *P. aeruginosa* bacteremia in neutropenic patients. These patients present skin lesions as portal of entry. In its early stages the edema can be found around the lesion, progressing to a painful erythematous macula. Eventually, the macula turns into a necrotic ulcer. The lesion is a consequence of diffuse invasion of the

microorganism, particularly in the media and adventitia of the blood vessels. Eg is rarely occurs in mucosal areas and is usually found in the axilla, the gluteal and perianal region (28).

1.2.2 Therapy of bloodstream infection

Antibiotic therapy is the mainstay in the treatment of *P. aeruginosa* infections. The following antibiotics serve as therapy for *P. aeruginosa* infections **(Tab.1)**:

Table 1 - Antipseudomonal agents

Class	Agent/(Abbr.)
Penicillins	Piperacillin **(PIP)**
	Piperacillin/Tazobactam **(PIP/TAZ)**
	Ticarcillin-Clavulanate (not available in Germany)
Cephalosporins	Ceftazidime **(CEF)**
	Cefepime
Monobactam	Aztreonam **(AZ)**
Fluorochinolones	Ciprofloxacin **(CIP)**
	Levofloxacin
Carbapenems	Meropenem **(MER)**
	Doripenem
	Imipenem **(IMP)**
Aminoglycosides	Gentamicin **(GM)**
	Tobramycin
	Amikacin **(AMK)**
Polymyxins	Colistin **(COL)**
	Polymyxin B

When *P. aeruginosa* bloodstream infection (BSI) is suspected, antimicrobial susceptibility testing should be initiated after drawing of blood cultures. Initial therapy is typically given as empiric therapy. Adequate empiric therapy should include agents

that cover *P. aeruginosa*, show the lowest local resistance rates within an institution, are in accordance to patient allergy history and hospital guidelines and have to be chosen depending on site of infection. It is also important to apply the antibiotic in a timely manner. A study has shown that a delay of more than 52 hours of administering the appropriate drug from the time the blood culture is drawn has at least doubled the 30-day mortality (61). Thus, for successful therapy the interval between first positive blood culture and administration of the antibiotic needs to be kept as short as possible. Typically, a combination of an anitpseudomonal β-lactam (penicillin, cephalosporin or carbapenem) with either an aminoglycoside or a fluorochinolone is chosen as first-line empirical treatment (62, 63). Once laboratory results are available, antibiotic treatment shall be adjusted according to susceptibility results. The clinician also needs to take optimal dosing intervals into consideration. Due to their time-dependent activity, β-lactam antibiotics should be applied frequently or by continuous infusion (64). Agents with concentration-dependent activity (e.g. aminoglycosides) shall be given as a single total daily dose (65). In addition to systemic antibiotic therapy, the primary site of infection needs to be addressed and infected catheters should be removed and obstructions and abscesses drained.

Due to poor penetration into the central nervous system, lungs and abscesses, aminoglycosides are commonly avoided when infections involve these sites. Aminoglycosides are often combined with β-lactam antibiotics in order to enhance their antibacterial activity. β-lactam antibiotics work by inhibiting cell wall synthesis, consequently leading to open pores in the bacterial wall, allowing the aminoglycoside to penetrate more effectively (66).

Fluorochinolones prevent the bacterial cell from duplicating by inhibiting certain enzymes (e.g. gyrase) and are the only antipseudomonal class of antibiotics that can be given orally (67).

In the past, colistin and polymyxin B were typically agents of last resort due to fear of nephro- und neurotoxicity (68-70). However, it is suggested that colistin is effective against multidrug resistant bacteria (71, 72) and therefore may be administered when choices are limited. Moreover, nephrotoxicity is often reversible and neurotoxicity occurs rarely (73).

1.2.3 Combination versus monotherapy

There has been controversial debate on whether combination therapy is superior to monotherapy. Around 1972, the outcome for neutropenic patients presenting with *P. aeruginosa* bacteremia was dismal due to scarceness of antipseudomonal agents. At that time, the common antipseudomonal agents were gentamicin and polymyxins (74). During that time two studies revealed that therapy with carbenicillin improved the outcome in neutropenic patients with *P. aeruginosa* bacteremia (74, 75). Several retrospective analyses suggested that a combination of synergistic antibacterial agents for gram-negative pathogens would lead to better outcomes in neutropenic patients (76, 77). Thus, combination therapy was set as the standard approach for the treatment of *P. aeruginosa* infections. At a time when ceftazidime was arguably the best antipseudomonal agent, a study conducted in 1986 showed successful treatment with ceftazidime monotherapy (78). In the following years several studies addressed this issue and compared monotherapy to combination therapy in *P. aeruginosa* patients (79-81). However, no clear conclusion could be drawn from the wide range of studies conducted in the past 20-30 years due to the paucity of well-controlled studies using clinically important end points. Experts today favor the use of combination therapy for *P. aeruginosa*. The IDSA (Infectious Diseases Society of America) concluded in 2002 that empirical monotherapy in high-risk patients suffering from *P. aeruginosa* bacteremia present the same efficiency as empirical combination therapy (82). It is also important to acknowledge that the drawbacks of combination therapy are linked to higher costs and an increase of toxicity. For example, a systematic review and meta-analysis of randomized trials found nephrotoxicity to be more common in combination therapy (83).

1.2.4 Prognosis of bloodstream infections due to *P. aeruginosa*

Prognosis for *P. aeruginosa* BSI infections remains poor despite advances in therapy over the past decades. Wisplinghoff et al. analyzed 24,179 cases of nosocomial BSI in US hospitals. The authors found crude mortality rates of 27.6% for non-ICU patients and 47% for ICU patients with BSI due to *P. aeruginosa* (55). A prospective study conducted in 2001-2002 compared hospital mortality of bloodstream infections due to

Staphyloccocus aureus and *P. aeruginosa*. They found mortality with *P. aeruginosa* to be significantly higher in contrast to mortality due to Methicillin-sensitive *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (30.6%, 16.2%, 13.5% respectively) (84).

Investigation has shown that several risk factors are associated with higher mortality in patients with *P. aeruginosa* bacteremia. In a study where 133 patients with *P. aeruginosa* bacteremia were examined, four variables influencing outcome were defined as development of septic shock, granulocyte count under 500/mm3, inappropriate antibiotic therapy and development of septic metastasis (85). Furthermore, drug resistance also negatively influences survival (86, 87). A study of 100 episodes of *P. aeruginosa* demonstrated that underlying host disease is directly related to the patient's survival (88).

1.3 Other types of *P. aeruginosa* infections

1.3.1 Respiratory tract infections

P. aeruginosa has been reported to cause infections in various sites of the body. However, its role in lung diseases is of particular importance.

P. aeruginosa is a common pathogen causing hospital-acquired pneumonia (HAP), where the incidence has almost doubled in the years from 1975 to 2003, from 9.6% to 18.1% respectively (20). Mode of transmission is through aspiration of endogenous oral flora, via aspiration of the pathogens through contaminated ventilator tubing and other medical devices or through hematogenous dissemination (89, 90).

It is also one of the most common bacteria causing ventilator-associated pneumonia (VAP) (91), with attributal mortality rates as high as 13% (92). Per definition VAP is a pneumonia that develops 48 to 72 hours after intubation, where the patient's oropharyngeal tract is in direct contact with respiratory devices.

Eventhough community-acquired pneumonia (CAP) due to *P. aeruginosa* in otherwise healthy patients is rare, *P. aeruginosa* infection in this setting is not neglectable due to high crude mortality rates reaching up to 61.1% (93) . Nonetheless, *P. aeruginosa* CAP does occur more frequently in patients with underlying risk factors such as chronic

obstructive pulmonary lung diseases (COPD), HIV infection and structural lung diseases such as cystic fibrosis (94, 95).

P. aeruginosa is also the key bacterial agent of cystic fibrosis (96). Most patients get chronically infected with *P. aeruginosa* strains during childhood. The Cystic Fibrosis Foundation's 2015 Patient Registry Annual Data Report found prevalence of patients cultured positive for *P. aeruginosa* to have declined over the past ten years. In 1995 50.7% of CF-patients showed positive blood cultures for *P. aeruginosa* compared to a 30.4% in the year of 2015 (97).

Pneumonia due to *P. aeruginosa* is clinically not clearly distinguishable from other pathogens and infected patients typically present with fever and purulent cough (23).

Generally, diagnosis of pneumonia is based on radiological findings, identification of clinical symptoms and results from microbiological pathogen testing. *P. aeruginosa* pneumonia does not display a specific feature in chest radiology (98). In order to obtain cultures from the lung it is suggested to perform invasive procedures such as bronchoalveolar lavage (23). *P. aeruginosa* is rarely isolated in blood cultures from pneumonia patients (98).

The American Thoracic Society and the Infectious Diseases Society of America advocates the use of combination therapy with either a β-lactam or carbapenem in combination with either a fluorochinolone oder an aminoglycoside (99). Parenteral monotherapy with an aminoglycoside is not recommended because these agents perform poorly in the acidic environment of the lung. (23). Inhaled antibiotics may be useful to treat resistant strains. Despite the fear of toxic side effects, pneumonia due to MDR *P. aeruginosa* has been successfully treated with aerosolized colistin (99).

1.3.2 Burn wound infections

In the 1960s and 70s *P. aeruginosa* posed one of the most significant threats in burn wound infections. At that time and in contrast to today, as many as 10% of burn wound patients suffered from *P. aeruginosa* bacteremia (100). Today, the occurrence of *P. aeruginosa* in burn wound patients has decreased. In a large study 1400 burn wound patients were examined and showed that roughly 1% of the patients suffered from *P. aerugnosa* sepsis (101). However, *P. aeruginosa* remains the most frequent

migroorganism isolated from burn wounds, following *Acinobacter* and *Escherichia coli* (102).

Typically, 48 hours after thermal injury, gram-positive pathogens like staphylococci that have survived colonize the burn wound. Subsequently other gram-negative pathogens and yeast infect the avascularized necrotic burn eschar (103, 104). After such invasion, microorganisms can proliferate in necrotic tissue and invade the blood system, leading to secondary bacteremia.

Diagnosis of burn wound infection is achieved through examination of clinical signs and of burn wound swabs in the laboratory. In order to distinguish between human bacterial flora and infection, a colony count of $\geq 10^5$ organisms per gram tissue is indicative of burn wound infection. Urinary samples, respiratory and blood cultures may also be used for diagnosis (105).

Treatment of *P. aeruginosa* burn wound infections includes topical and systemic application of antimicrobial agents and aggressive surgical debridement of the necrotic tissue. Topical agents such as silver nitrate have strong bacteriostatic activity against gram-negative bacteria such as *P. aeruginosa* (106).

Although incidence rates for *P. aeruginosa* in burn wound patients have considerably decreased, mortality remains alarmingly high, reaching rates up to 77% in some burn centers (100, 102).

1.3.3 Ear infections

P. aeruginosa is known to cause acute otitis externa ("swimmer's ear") and the most frequent pathogen in malignant otitis externa (26).

Swimmer's ear typically occurs in children and under moist or humid conditions. Pain, itchiness, mucoid discharge and hearing loss are typical clinical signs (107). Therapy consists of local application of solutions containing an aminoglycoside (23).

Malignant otitis externa is a more dreaded infection, initially affecting the ear canal and cartilage of the ear (23). Subsequently, the infection proceeds to the soft tissue of the retromandibular area and cranial nerves. Nerve palsy, osteomyelitis, brain abscesses and dural vein thrombosis are typical complications (28).

Diagnosis consists of isolation of the pathogen from ear exsudate and a nuclear imaging technique with technetium 99 of the bone. Treatment consists mainly of antibiotic treatment; nonetheless débridement or abscess drainage may be required. Antipseudomonal agents such as penicillins, aminoglycosides or cephalosporins are applied intravenously for a duration of 6 to 8 weeks. Alternatively, ciprofloxacin can be applied orally (108).

1.3.4 Urinary tract infections

Urinary tract infections (UTI) caused by *P. aeruginosa* are generally hospital-acquired. *P. aeruginosa* accounts for approximately 7% of UTI in this setting and ranks third in causes for hospital-acquired UTI, following *Escherichia Coli* and Enterococci (109).
These infections typically occur in male patients, after longer stays in other hospitals and are associated with prior penicillin use and indwelling urinary catheters (110).
Typical clinical features for UTI such as dysuria, hematuria, fever, suprapubic and flank pain are not any different when caused by *P. aeruginosa*.
During the course of treatment, foreign bodies (e.g. indwelling catheters, stents and stones) should be removed and an antibiotic such as ciprofloxacin (23) for systemic treatment should be applied.
Due to *P. aeruginosa*'s propensity to form a biofilm on the catheters' surface, UTI due to *P. aeruginosa* is often associated with persistent and recurrent episodes (111).

1.3.5 Infections of the central nervous system

Primary infections of the central nervous system (CNS) due to *P. aeruginosa* are uncommon. This pathogen is mostly involved in secondary infections – infections that occur in connection to head trauma and in the course of surgical procedures (112). Typical clinical syndromes are meningitis, brain abscesses, subdural and epidural infections (23).
The profile of the cerebrospinal fluid of *P. aeruginosa* is similar to that of other bacterial meningitis. Symptoms of this entity include neck stiffness, fever and altered mental status and are not any different to those of other gram-negative pathogens (113).

Once the pathogen has been identified, an intravenous antimicrobial therapy with either cefepim or ceftazidim is suggested. Alternatively, aztreonam, ciprofloxacin or meropenem can be applied (114). It is occasionally necessary to install an antimicrobial agent by the intraventricular route (114) in patients with difficult or persistent infections. In addition, it is required to drain abscesses and empyemas and to remove foreign bodies such as ventriculostomy tubes.

1.4 Objectives of the thesis

P. aeruginosa is one of the most common causes for nosocomial infections, and despite advances in treatment options, mortality for BSI due to *P. aeruginosa* remains high (115). *P. aeruginosa* seldom causes infections in otherwise healthy patients, however, immunocompromised patients, patients carrying medical devices and ICU patients are at high risk for BSI due to *P. aeruginosa* (116-118).

P. aeruginosa employs a wide array of virulence factors, which may in part explain the deleterious impact on survival. In addition to its high intrinsic resistance towards many antibiotics, *P. aeruginosa* has the capacity to rapidly develop resistance during antimicrobial therapy (23). Rates of antipseudomonal resistance continue to rise worldwide (20, 119), limiting the choices of effective therapeutic options.

In order to adequately treat patients with suspected BSI due to *P. aeruginosa*, the physician needs to determine who is at risk for infection with this pathogen. To date, experts have not reached consensus on how to adequately treat BSI due to *P. aeruginosa* (120-122). In addition, the clinician must find a balance between choosing the best treatment options for improved outcome but at the same time limiting the spread and increase in resistance and drug toxicity.

In this regard, our study aimed to examine:

1. All-cause hospital mortality among patients with BSI due to *P. aeruginosa* and risk factors associated with increased mortality,
2. Impact of appropriate and inappropriate antipseudomonal therapy on mortality,
3. Whether antipseudomonal combination therapy is superior to monotherapy,
4. Impact of carbapenem resistance on mortality in *P. aeruginosa* BSI.

2. Material & Methods

2.1 Study design

We conducted a study as part of an international, multicenter, retrospective, cohort-study in 19 hospitals in 10 countries **(Tab. 2)** (project leaders: Dr. Dafna Yahav and Prof. Leonard Leobovici in Tel-Aviv/Israel). Aim of the international study was to compare treatment options for *P. aeruginosa* bacteremia. A preliminary summary of the study has been presented at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in April 2018 in Seville, Spain. We obtained written permission to use the data for our own analysis.

The institutional review board in Germany approved the study. Informed consent of the patients was not required due to the retrospective nature of the study. Patient data was extracted in a pseudonymised form.

We reviewed medical records and microbiological laboratory results of patients hospitalized during the 1st of January 2009 until the 31st of October 2015. We included patients aged ≥ 18 years old and who had a positive blood culture for *P. aeruginosa*. Polymicrobial infections and recurrent episodes in the same patient were excluded from the study.

Table 2 - List of participants of the international, multicenter study

Country	Centre
Australia	The University of Queensland, Centre for Clincial Research, Brisbane
England	North Bristol NHS Trust, Southmead Hospital
France	Centre Hospitalier Regional Universitaire de Nancy, Nancy
Germany	University Hospital Tübingen, Tübingen
Greece	University Hospital Heraklion, Iraklio
Israel	HaEmek Medical Center, Afula Rambam Health Care Campus, Haifa Sourasky Medical Center, Tel Aviv Soroka Medical Center, Beersheba
Italy	Bolzano Central Hospital, Bolzano

Slovenia	University Medical Centre, Ljubljana
Spain	Hospitales Universitario Virgen Macarena, Sevilla Hospital De Sant Pau, Barcelona Hospitales Universitario Virgen del Rocio, Sevilla Hospital Universitario Son Espases, Palma de Mallorca Hospital Universitario Reina Sofia, Cordoba Hospital Universitario Marqués de Valdecilla, Santander Hospital Universitario y Politecnico de La Fe, Valencia
Sweden	Karolinska University Hospital, Solna

2.2 Setting

University Hospital Tübingen is a 1,500-bed hospital that provides specialized and general treatment in all medical specialties including medical, surgical and intensive care. It serves both as teaching hospital of Tübingen University and District Hospital for the Town and the Administrative District of Tübingen.

2.3 Data collection

Demographic, clinical and microbiological data were collected from electronic medical and archived records for all patients. Patients were identified by positive blood culture for *P. aeruginosa* in microbiological laboratory records. Microbiological data was extracted from SAP-sytem, Lauris-system and in part from swisslab-system. Clinical and demographical data were collected from SAP-system and Lauris-System. The data was then entered into an Excel-table. When specific data could not be obtained from the electronic records, the cell in the Excel-table was left blank. Variables were encoded as binary variables (e.g. no=0, yes=1), except the variables ‘age’, ‘date of hospitalization’ and ‘date of death’.

Following variables were collected for each patient:

- Age
- Gender
- Date of hospital admission
- Department of hospitalization

    - Medical
    - Surgical
    - ICU
- Place of acquisition (of infection)
    - Nosocomial
    - Community-acquired
- Previous hospitalization in the previous 90 days
- Medical devices upon admission:
    - Endotracheal tube
    - Central venous line
    - Nasogastric tube
    - Prosthesis
- Predisposing conditions at admission:
    - Neutropenia
    - Organ transplant
    - Chemotherapy
    - Corticosteroid use
    - Surgery
- Microbiological susceptibility data:
    - Gentamicin (GM)
    - Piperacillin (PIP)
    - Piperacillin/tazobactam (PIP/TAZ)
    - Ceftazidime (CEF)
    - Ciprofloxacin (CIP)
    - Meropenem (MER),
    - Fosfomycin (FOS)
    - Aztreonam (AZ)
    - Colistin (COL)
    - Amikacin (AMK)
    - Imipienem (IMP)
- Antipseudomonal agents for empirical therapy

2.4 Definitions

Table 3 - Variable definitions

Variable	Definition
Pseudomonas aeruginosa bacteremia	Isolation of the pathogen in a blood culture.
Department of hospitalization	Clinical department where blood for the initial blood cultures was taken. ‚ICU' included the ICU of surgical and medical department.
Nosocomial infection	Onset of bacteremia ≥48 hours after hospitalization.
Community-acquired infection	Cases that were not marked as ‚nosocomial infection' were considered community-acquired.
Source of bacteremia	Sources were determined by the physician responsible for data extraction based on the information from the medical records. When a localized infection could not be determined, the source of bacteremia was categorized as ‚unknown'. In many cases the treating physician did not document the source. Bacteremia in the presence of any central line and in the absence of another source where categorized as ‚line-associated'.
Neutropenia	Absolute neutrophile count below 500 cells/mm^3
Organ transplant	Included solid organ transplant and autologous/allogeinic hematopoetic stem cell transplant in the previous 30 days.
Chemotherapy	Any type of cytotoxic therapy in the

	previous 30 days.
Corticosteroid use	Administration of ≥ 10 mg/d prednisone in the previous 30 days.
Surgery	Any type of surgery - except percutaneous procedures and angiography - in the previous 30 days.
Previous hospitalization	Hospitalization in the previous 90 days as an in-patient before the current hospitalization.
Multidrug resistance	Resistance to two or more antipseudomonal drugs.
Carbapenem resistance	*P. aeruginosa* was considered as carbapenem resistant if the strain was resistant to either imipenem or meropenem
Empirical therapy	Administration of antipseudomonal treatment within 24h of first positive blood culture.
Antipseudomonal monotherapy	Administration of one of the following agents: gentamicin, amikacin, ceftazidim, ciprofloxacin, levofloxacin, ofloxacin, piperacillin, piperacillin/tazobactam, imipenem, meropenem, doripenem, aztreonam, cefepim, tobramycin and fosfomycin.
Appropriate monotherapy	At least one prescribed antibiotic had to match the in vitro susceptibility of the respective *P. aeruginosa* isolate.
Antipseudomonal combination therapy	Administration of one of the following combination options: (I) piperacillin, piperacillin/tazobactam, meropenem, ceftazidim, doripenem, colistin, cefepim was combined with either

	a fluorchinolone (levofloxacin, ciprofloxacin) or an aminoglycoside (amikacin, gentamicin, tobramycin), (II) meropenem and piperacillin/tazobactam, (III) cefepim and meropenem, (IV) colistin and piperacillin/tazobactam, (V) colistin and cefepim, (VI) meropenem and ceftazidim, (VII) colistin and doripenem.
Appropriate combination therapy	Both antipseudomonal agents had to match in vitro susceptibility of the respective *P. aeruginosa* isolate.
Inappropriate therapy	Administration of an antipseudomonal agent to which the pathogen was tested resistant in vitro.

2.5 Microbiological testing

Blood was obtained from every patient and inoculated into a two-bottle set. Although it is recommended to follow standard protocol and collect blood from at least two locations (123, 124), in practice the microbiological laboratory may also receive one blood culture set.

In University Hospital of Tübingen blood cultures are tested using *BD BACTEC™ Instrumented Blood Culture Systems (Becton, Dickinson and Company,* New Jersey, USA). Gram staining and subculture was performed. Subsequently, microorganisms were examined according to routine bacteriological procedures. Species were identified by the means of a linear MALDI-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Antimicrobial resistance was routinely tested by microbroth method on a *Vitek 2 system* (*bioMérieux*, Marcy l'Étoile, France). The following antimicrobials were tested in our center: gentamicin, amikacin, piperacillin, piperacillin/tazobactam, ceftazidim, ciprofloxacin, meropenem, fosfomycin, colistin, imipenem and aminoglycoside. We

considered intermediate suscpeptibility tested isolates to be resistant. Susceptibility was interpreted following EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines with the exception of colistin to which susceptibility was evaluated according to CLSI (Clinical and Laboratory Standards Institute) recommendations (125, 126).

In the microbiological institute in Tübingen blood culture bottles are examined during the week 7.30 a.m. to 5.30 p.m. and on weekends 7.30 a.m. to 3 p.m. Laboratory results are transferred into the hospital computer system (SAP-system), which is accessible to the physician. However, the laboratory also provides on-call service for the physician.

2.6 Statistical analysis

Data were analyzed using STATA 14.2 for Windows (StataCorp, College Station, TX, USA) Software. Descriptive analysis of the study population is presented as proportion for categorical variables and as mean with standard deviation for continuous variables. Factors associated with risk of mortality among patients admitted to the hospital with *P. aeruginosa* infection were assessed using survival Cox proportional hazard model, wherein the total person-time at risk was calculated as the total days under surveillance (time from the day patient tested positive for *P. aeruginosa* in blood culture until discharge/death). Factors identified in the univariate analysis at the significance level of 0.30 and clinically relevant variables were considered for inclusion in the full multivariate models. Multivariate analysis was performed using backward stepwise method. The risk is presented as hazard ratio (HR) with 95% confidence interval (CI). Kaplan-Meier estimates of median survival time were calculated and compared for patients who received appropriate and inappropriate empirical and definitive therapy.

Significance was assumed at a 5% level.

Survival analysis is commonly used for statistical analysis of clinical trials. In survival analysis, time-to-event is recorded, whereas the event (outcome) may be death, time to hospitalization, relapse, recovery etc. This method of analysis includes patients who reach the chosen outcome and those who fail to complete the trial (censored data). This allows us to take into consideration as much information as possible, in order to optimize the power or validity of the study. Data from the survival analysis may then be

depicted in Kaplan-Meier curves. There are several models that can be used for time-to-event analysis, such as log-rank test and Cox proportional hazard model (127). Cox proportional hazard model is a survival regression model that identifies differences in survival with respect to treatment and prognostic factors. This model gives an estimate of the hazard ratio (128). Hazard ratio is seen as a type of relative risk that compares survival times between two groups of patients (129).

3. Results

3.1 Study population

A total of 104 patients presenting *P. aeruginosa* bacteremia during a hospitalization from the 1st of January 2009 until the 31st of October 2015 were included in the study. The mean age was 63.7 ± 14 (standard deviation). In our cohort 68 patients were male and 36 were female. Most frequent department of hospitalization was the medical department (67 patients, 64.4%), followed by the ICU (29 patients, 27.9%) and the surgical department (8 patients, 7.7%). A total of 74 (71.2%) infections were nosocomially acquired, whereas the remainder cases were considered as community-acquired (28.8%). The presumed sources of bacteremia were pulmonary (21 patients, 20.6%), urinary (11 patients, 10.8%), line-associated (10 patients, 9.8%) and in 45 (58.5%) cases the source of infection was unknown. 'Unknown' source of infection was determined during data extraction when the treating physician did not document the source or could not determine a source of infection. In our cohort 20 patients (19.2%) were neutropenic, 13 patients (12.5%) had undergone an organ transplant and 19 patients (18.3%) had surgery in the previous 30 days. Of 101 patients, 19 patients (18.8%) had received corticosteroid therapy in the previous 30 days and of 102 patients, 19 patients (18.6%) had received chemotherapy in the previous 30 days. Of 103 patients 67 patients (64.4%) had been hospitalized in the previous 90 days. In the last three cases and in further cases below, data could not be obtained due to missing medical records, thus reducing the sample size. Furthermore, we recorded medical devices that patients were carrying upon admission. 10 (9.6%) patients had an endotracheal tube, 36 (34.6) patients had a central venous line (CVL), 6 (5.8%) had a nasogastric tube, 15 (14.4%) had an indwelling urinary catheter and 32 (31.1%) had a prosthesis. Clinical and epidemiological characteristics are shown in **Tab. 4**.

Empiral therapy was analysed in 92 patients; in 12 patients data was incomplete. Of 71 patients who received appropriate empirical therapy, 47 patients (51.1%) received appropriate empirical monotherapy and 24 patients (26.1%) received appropriate empirical combination therapy. Of the 21 patients who received inappropriate empirical

therapy, 13 patients (14.1%) received monotherapy and 8 patients (8.7%) received combination therapy.

Table 4 - Clinical and epidemiological characteristics of the study population

Characteristics (n=104)	Patients
Age, years (mean±SD)	63.7±14
Sex	
Male	68 (65.4)
Female	36 (34.6)
Department of hospitalization	
Surgical	8 (7.7)
Medical	67(64.4)
ICU	29 (27.9)
Place of acquisition	
Community-acquired	30 (28.8)
Nosocomial	74 (71.2)
Source of bacteremia (n=101)*	
Unknown	50 (49.5)
Line-associated	10 (9.9)
Pulmonary	27 (26.7)
Urinary	14 (13.9)
Predisposing condition	
Neutropenia	20 (19.2)
Organ transplant	13 (12.5)
Chemotherapy within previous 30 days (n=102)*	19 (18.6)
Corticosteroid use within previous 30 days (n=101)*	19 (18.8)
Surgery within previous 30 days	19 (18.3)
Hospitalization within previous 90 days (n=103)*	67 (64.4)
Invasiv devices upon admission	
Endotracheal device	10 (9.6)
Central venous line	36 (34.6)

Nasogastric tube	6 (5.8)
Urinary catheter	15 (14.4)
Any prosthesis (n=103)*	32 (31.1)
Treatment	
Empirical therapy (n=92)*	
Appropriate empirical therapy	71 (77.2)
Appropriate empirical monotherapy	47 (51.1)
Appropriate empirical combination therapy	24 (26.1)
Inappropriate empirical therapy	21 (22.8)
Inappropriate empirical monotherapy	13 (14.1)
Inappropriate empirical combination therapy	8 (8.7)

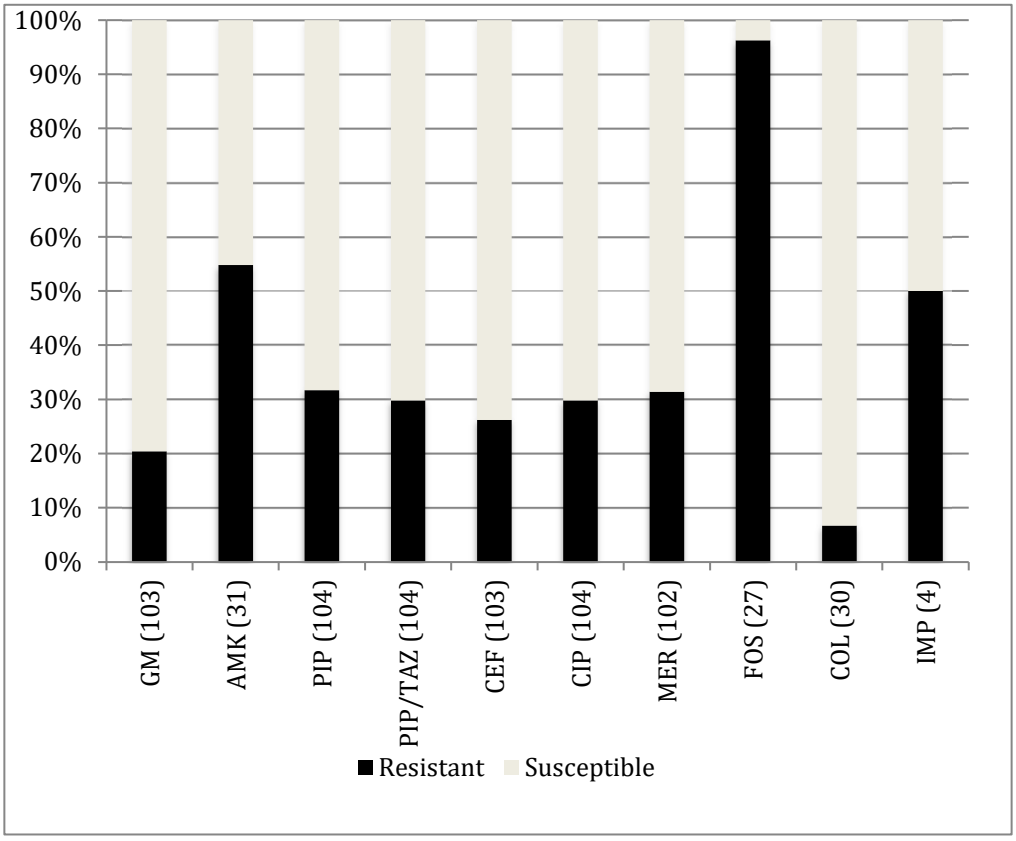
SD=standard deviation. ICU=intensive care unit. CVL=central venous line.
The coloumn ‚patients' is expressed in total numbers (with percentages in brackets), while ‚age' as the only continuous variable is presented as mean age (with standard deviation in brackets).
*With regard to certain charateristics, some data of the sample size n=104 was either missing in the medical records or was not of relevance for our study and thus the sample size was smaller (smaller than 104) in these cases.

3.2 Microbiological characteristics

Susceptibility patterns of the *P. aeruginosa* blood isolates are shown in **Fig. 1**.

Of 103 isolates that were tested for the susceptibility of gentamcin, 21 (20.4%) were resistant to gentamicin. Of the 31 isolates tested for amikacin, 17 (54.8%) were resistant. Of 104 isolates tested for piperacillin, 33 (31.7%). Of 104 isolates tested for piperacillin/tazobactam, 31 (29.8%) were resistant. Of 103 isolates tested for ceftazidim, 27 (26.2%) were resistant. Of 104 isolates tested for ciprofloxacin, 31 (29.8%) were resistant. Of 102 isolates tested for meropenem, 32 (31.4%) were resistant. Of 27 isolates tested for fosfomycin, 26 (96.3%) were resistant. Of 30 isolates tested for colistin, 2 (6.7%) were resistant. Of 4 isolates tested for imipenem, 2 (50%) were resistant.

Figure 1 - Percentage distribution of the susceptibility profile of *P. aeruginosa* BSI



Resistance rates for gentamicin, amikacin, piperacillin, piperacillin/tazobactam, ceftazidim, ciprofloxacin, meropenem, fosfomycin, colistin and imipenem were 20.4%,

54.8%, 31.7%, 29.8%, 26.2%, 29.8%, 31.4%, 96.3%, 6.7% and 50%, respectively. The x-axis shows the antibiotics and their respective sample sizes.
The y-axis shows the percentage of the tested isolates shown as susceptible (black) and resistant (grey).
Numbers in brackets describe the amount of *P. aeruginosa* strains tested.

3.3 Univariate analysis of risk factors associated with all-cause hospital mortality

Among all patients, the overall mortality was 37.5% (39/104). Among neutropenic patients all-cause mortality was 60% (12/20) and among non-neutropenic patients 32% (27/84). All-cause hospital mortality among patients with pulmonary-associated infections was 47% (10/21) and if the source of infection was unknown, the all-cause hospital mortality was 51% (23/45). In contrast, patients with line-associated infections had a comparatively lower all-cause hospital mortality of 10% (1/10). Risk factors for all-cause hospital mortality are presented in **Tab. 5**. According to univariate analysis, mortality was associated with chemotherapy (adjusted HR 2.12 [95% CI 1-4.5]; p=0.04), neutropenia (adjusted HR 2.54 [95% CI 1.25-5.18], p=0.01), an unknown source of infection (adjusted HR 2.45 [95% CI 1.28-4.69]; p=0.006), multi-drug resistant strains (adjusted HR 3.05 [95% CI 1.53-6.08]; p=0.001), inappropriate empirical therapy (adjusted HR 2.24 [95% CI 1.12-4.49]; p=0.02) and inappropriate empirical monotherapy (adjusted HR 3.13 [95% CI 1.16-8.44]; p=0.02).

Table 5 - Risk factors for all-cause hospital mortality in *P. aeruginosa* BSI based on univariate analysis

Variable	Survivors, (%)	Non-survivors, (%)	HR (95% CI)	p-value[a]
Age (n=104)				
Age ≤55 years	15 (55.6)	12 (44.4)	1.0 (reference)	
Age ≥75 years	18 (90)	2 (10)	0.24 (0.05-1.07)	0.06

Variabel	Survivors, (%)[b]	Non-survivors, (%)[b]	HR (95% CI)	p-value[a]
Sex (n=104)				
Male	44 (64.7)	24 (35.3)	1.0 (reference)	
Female	21 (58.3)	15 (41.7)	1.25 (0.66-2.40)	0.48
Devices upon admission (n=104)				
Endotracheal	7 (70)	3 (30)	1.33 (0.40-4.40)	0.64
CVL	23 (63.9)	13 (36.1)	1.02 (0.52-1.99)	0.94
Nasogastric tube	4 (66.6)	2 (33.3)	1.55 (0.36-6.55)	0.55
Urinary catheter	12 (80)	3 (20)	0.58 (0.18-1.91)	0.38
Any prosthesis	25 (78.1)	7 (21.9)	0.61 (0.26-1.40)	0.25
Predisposing condition				
Chemotherapy in the last 30 days (n=102)	9 (47.4)	10 (52.6)	2.12 81-4.5)	**0.04**
Corticosteroid use in the last 30 days (n=101)	10 (52.6)	9 (47.4)	1.83 (0.84-3.97)	0.12
Surgery in the last 30 days (n=104)	14 (73.7)	5 (26.3)	0.59 (0.23-1.53)	0.28
Non-neutropenic	57 (68)	27 (32)	1.0 (reference)	
Neutropenia (n=104)	8 (40)	12 (60)	2.54 (1.25-5.18)	**0.01**
Organ transplant (n=104)	7 (53.8)	6 (46.2)	1.05 (0.44-2.53)	0.9
Source of bacteremia (n=101)				
Urinary	11 (78.6)	3 (21.4)	1.0 (reference)	
Unknown	23 (46.0)	27 (54.0)	2.45 (1.28-4.69)	**0.006**
Line-associated	9 (90)	1 (10)	0.20 (0.03-1.48)	0.11
Pulmonary	12(44.5)	15 (55.5)	1.05 (0.51-2.18)	
Place of acquisition (n=104)				
Community-acquired infection	19 (29.2)	11 (28.2)	1.0 (reference)	
Nosocomial infection	46 (70.8)	28 (71.8)	0.68 (0.33-1.40)	0.3

Variabel	Survivors, (%)[b]	Non-survivors, (%)[b]	HR (95% CI)	p-value[a]
Antibiotic susceptibility				
Gentamicin				
Susceptible	58 (70)	24 (30)	1.0 (reference)	
Resistant	6 (28.6)	15 (71.4)	2.34 (1.22-4.46)	**0.01**
Amikacin				
Susceptible	6 (42.9)	8 (57.1)	1.0 (reference)	
Resistant	4 (23.5)	13 (76.5)	1.48 (0.60-3.64)	0.38
Piperacillin				
Susceptible	52 (73)	19 (26)	1.0 (reference)	
Resistant	13 (39.4)	20 (60.6)	2.40 (1.28-4.51)	**0.006**
Piperacillin /Tazobactam				
Susceptible	55 (77.5)	18 (22.5)	1.0 (reference)	
Resistant	10 (32.3)	21 (67.7)	2.78 (1.48-5.23)	**0.001**
Ceftazidim				
Susceptible	53 (69.7)	23 (30.3)	1.0 (reference)	
Resistant	11 (40.7)	16 (59.3)	1.88 (1.0-3.56)	**0.05**
Ciprofloxacin				
Susceptible	53 (72.6)	20 (27.4)	1.0 (reference)	
Resistant	12 (38.7)	19 (61.3)	1.98 (1.05-3.72)	**0.03**
Meropenem				
Susceptible	51 (72.8)	19 (27.2)	1.0 (reference)	
Resistant	12 (37.5)	20 (62.5)	2.35 (1.25-4.42)	**0.008**
Fosfomycin				
Susceptible	0 (-)	1 (100)	-	-
Resistant	7 (27)	19 (73)	-	-

Variabel	Survivors, (%)[b]	Non-survivors, (%)[b]	HR (95% CI)	p-value[a]
Colistin				
Susceptible	10 (35.7)	18 (64.3)	-	-
Resistant	0 (-)	2 (100)	-	-
Imipenem				
Susceptible	2 (100)	(-)	-	-
Resistant	1 (50)	1 (50)	-	-
Resistance (n=104)				
No drug resistance	40 (77)	12 (23)	1.0 (reference)	
Any drug resistance	25 (48)	27 (52)	2.21 (1.12-4.37)	**0.02**
Single drug resistance	13 (86.7)	2 (13.7)	0.05 (0.11-2.23)	0.36
Multi-drug resistance	12 (32.4)	25 (67.6)	3.05 (1.53-6.08)	**0.001**
Treatment				
Empirical therapy (n=92)				
Appropriate empirical therapy	50 (70.4)	21 (29.6)	1.0 (reference)	
Inappropriate empirical therapy	8 (38)	13 (62)	2.24 (1.12-4.49)	**0.02**
Appropriate empirical monotherapy	37 (79)	10 (21)	1.0 (reference)	
Inappropriate empirical monotherapy	6 (46)	7 (54)	3.13 (1.16-8.44)	**0.02**
Appropriate empirical combination therapy	13 (55)	11 (45)	1.0 (reference)	
Inappropriate empirical combination therapy	2 (25)	6 (75)	1.67 (0.61-4.53)	0.31

HR=hazard ratio. CI=confidence interval. CVL=central venous line. [a]Statistically significant values have been marked in boldface.[b]Percentages in parentheses are based on the total number of each variabel.
Our overall study population included 104 patients. Due to incomplete medical records *n* decreased in some cases.

3.4 Multivariate analysis

Multivariate analysis using Cox hazard model, demonstrated that multi-drug resistance (adjusted HR 3.40 [95% CI 1.28-9.03], p=0.01) is an independent risk factors for all-cause mortality **(Tab.6)**.

Table 6 - Independent risk factors for all-cause mortality for 104 patients with *P. aeruginosa* BSI according to multivariate analysis using Cox hazard model

Risk factor	HR (95%CI)	p-value
Drug resistance		
No drug resistance	1.0 (reference)	
Multi-drug resistance	3.40 (1.28-9.03)	**0.01**
Unknown source of infection	2.24 (0.94-5.30)	0.06

HR= hazard ratio. CI= confidence interval. .

3.5 Impact of carbapenem resistance on mortality in *P. aeruginosa* bacteremia

For a more comprehensible presentation, patients with strains resistant to any drug except carbapenem were marked as group A, patients with strains resistant to only carbapenem as group B and patients with strains resistant to at least two antipseudomonal drugs and one carbapenem as group C (**Tab**. **7**).

Of the 104 patients, there were 17 patients (16.4%) with strains resistant to any antipseudomonal drug except carbapenem (group A). 33 patients (31.7%) with *P. aeruginosa* strains that were resistant only to carbapenems (group B) and in 29 cases (27.9%) strains were resistant to one carbapenem and at least two other antipseudomonal drugs (group C). All-cause hospital mortality rate for patients in group A was 35.3% (6/17), whereas mortality rates for group B and C were higher: 60.6% (20/33) and 69% (20/29), respectively. In univariate analysis for all-cause mortality, group A tended to be associated with mortality, however not statistically significant (adjusted HR 1.47 [95% CI 0.55-3.93], p=0.44). Compared to patients without carbapenem resistance, patients in group B were significantly associated with mortality

(adjusted HR 2.31 [95% CI 1.13-3.98], p=0.02). The same association was observed in group C (adjusted HR 2.79 [95% CI 1.36-5.73], p=0.005). When analyzing resistance by various drug combinations, our results demonstrate that carbapenem resistance was the driving force for mortality

Table 7 - Influence of carbapenem resistance on mortality using univariate analysis

Resistance by drug combination	No. of survivors (%)	No. of non-survivors (%)	HR (95% CI)	p-value[a]
No drug resistance	40 (38.5)	12 (11.5)	1.0 (reference)	
Group A: Resistance to any antipseudomonal drug except carbapenem	11 (10.6)	6 (5.8)	1.47 (0.55-3.93)	0.44
No carbapenem resistance	51 (49.0)	19 (18.3)	1.0 (reference)	
Group B: Resistance to carbapenems	13 (12.5)	20 (19.2)	2.13 (1.13-3.98)	**0.02**
No drug resistance	40 (38.5)	12 (11.4)	1.0 (reference)	
Group C: Resistance to 1 carbapenem and at least 2 other antipseudomonal drugs	9 (8.7)	20 (19.2)	2.79 (1.36-5.73)	**0.005**

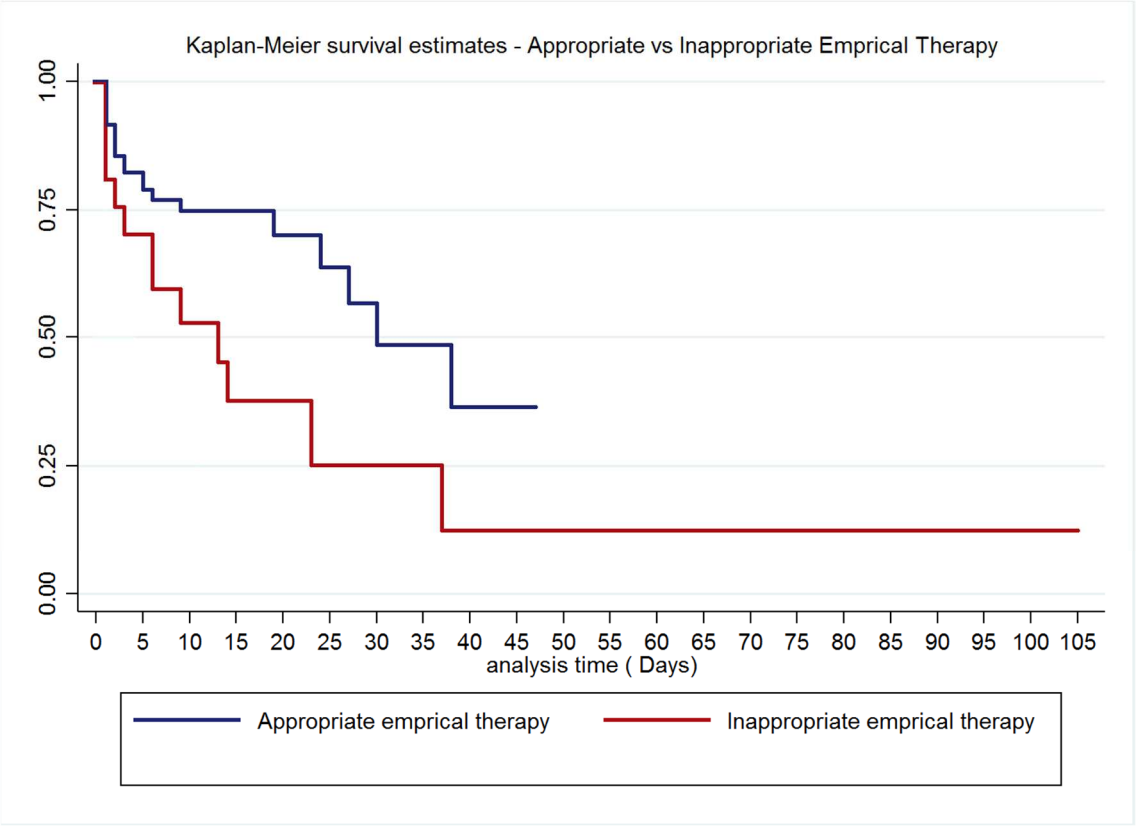
For a more comprehensible presentation drug resistances were categorized into three groups (A-C). [a] Statistically significant values have been marked in boldface.
Percentages in brackets are based on our overall study population of 104 patients.

3.6 Survival analysis

The association between inappropriate empirical therapy and resistance was further explored by a survival curve.

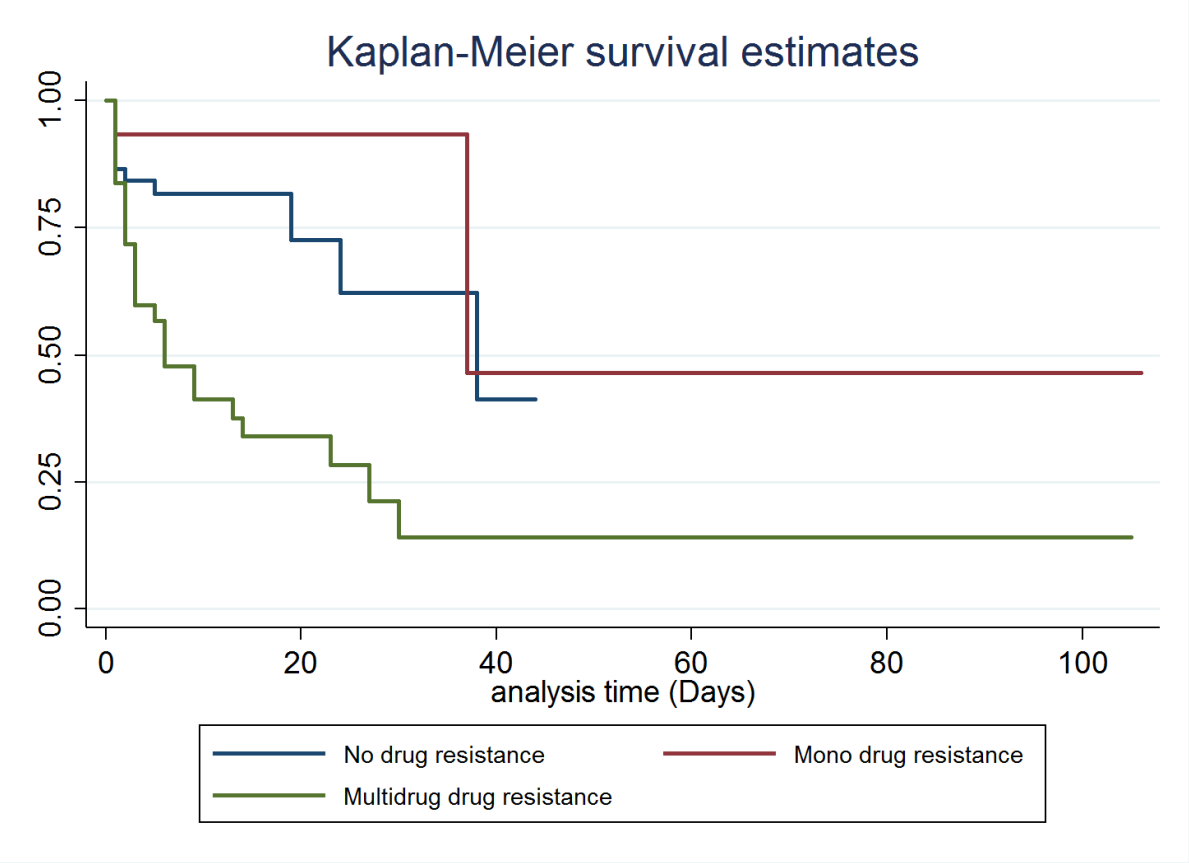
Kaplan-Meier survival curve (log-rank p=0.0008) showed that patients receiving inappropriate empiric therapy had a lower chance of survival when compared to patients receiving appropriate empirical therapy **Fig. 1**.

Kaplan-Meier survival curve (long-rank p=0.5) for drug resistance showed that patients without any drug resistance had a higher chance of survival (median survival time=38 days), compared to patients with a single drug resistance (median survival time=37 days), followed by patients with multiple drug resistance (median survival time=6 days) **Fig. 2.**



The y-axis shows the survival percentage and the x-axis shows survival time in days. n= 101, median survival time for 'appropriate empirical therapy'=30 days, median survival time for 'inappropriate empirical therapy'=13 days, median survival time for 'no empirical therapy'=3 days; p=0.0014.
Blue= Appropriate empirical therapy. Red= Inappropriate empirical therapy.

Figure 2 - Kaplan-Meier survival analysis for all-cause hospital mortality according to the receipt of empirical antimicrobial therapy (n=101)



The y-axis shows the survival percentage and the x-axis shows survival time in days. n= 104, median survival time for 'no drug resistance'=38 days, median survival time for 'single drug resistance'=37 days, median survival time for 'multidrug resistance'=6 days; p=0.5
Blue= No drug resistance. Red= Single drug resistance. Green= Multi drug resistance.

Figure 3 - Kaplan-Meier analysis for all-cause hospital mortality according to antimicrobial resistance (n=104)

4. Discussion

This retrospective study, analyzing patients with BSI due to *P. aeruginosa* in the time period of 1st of January 2009 until the 31st of October 2015, sought to determine risk factors for BSI due to *P. aeruginosa* and to investigate the impact of resistance and antibiotic therapy on mortality.

Univariate analysis demonstrated that chemotherapy, neutropenia, unknown source of bacteremia, multidrug resistance, carbapenem resistance and inappropriate empirical therapy was associated with increased all-cause hospital mortality. Resistance to ≥2 agents (multidrug resistance) was an independent risk factors for worse outcome.

4.1 Risk factors for BSI due to *P. aeruginosa*

All-cause hospital mortality was found to be 37.5%. This could be corroborated with mortality rates from recently published studies with mortality ranging between 34% and almost 60% (118, 130). It is also important to note, that mortality rates may show regional variation.

Identification of risk factors provides several advantages for clinical practice. First of all, it helps the physician to identify potentially infected patients more quickly and initiate aggressive therapy. It also helps to understand epidemiology and pathogenesis of the pathogen.

Our study did not conclude that older age is a risk factor for mortality. In fact, according to our results patients aged 75 or above had a higher chance of survival in comparison to younger patients. This finding does not only contradict other studies (118, 131) but also clinical understanding that older patients typically bear preexisting comorbidities, resulting in higher mortality. In our study, patients in the age group ≥75, out of 20 patients only 2 (5.1%) died. In comparison to the younger age groups of ≤55, 56-64 and 65-74, 30.7%, 33.3% and 30.7% died, respectively. This suggests that this subgroup of older patients in the study was comparatively healthy. Chamot et al. also unexpectedly found older patients to be associated with better survival. The authors concluded that lack of predisposing conditions (e.g. no neutropenia, no steroid treatment) of this subgroup resulted in a better outcome when compared to younger patients. (132).

Indeed, this observation was also applicable to the study-subgroup of older patients, in that the surviving older patients were neither neutropenic nor had they received chemotherapy in the last 30 days.

Patients with *P. aeruginosa* BSI admitted to ICU had a non-statistically significant 1.56 fold greater likelihood of dying, when compared to patients in medical and surgical wards. Patients in the ICU are typically severely ill, have a debilitated immune system, often receive corticosteroids and cytotoxic agents, thus making them more susceptible to infections with opportunist pathogens such as *P. aeruginosa*.

Results from our study also suggest that patients who had been hospitalized in the last 90 days had a greater risk of dying. This finding almost achieved statistical significance. (p=0.06). Previously hospitalized patients may be more severely ill and may have received antipseudomonal antibiotics during previous hospital stays. Previous studies have identified prior antibiotic therapy as a risk factor for higher mortality in *P. aeruginosa* (133-135). Kollef et al. suggest that prior administration of an antibiotic predisposes a patient to colonization with a strain resistant against the previously administered antibiotic, therefore leading to increased mortality (136).

We sought to investigate the impact of medical devices on mortality and concluded that endotracheal devices, CVL and nasogastric tube tended to be associated with lower survival, however not yielding statistically significant results. Urinary catheters and prosthesis tended to be associated with improved outcome, albeit without statistical significance.

Medical devices (e.g. mechanical ventilator, arterial and venous lines, urinary catheter etc.) have been established as risk factors for *P. aeruginosa* BSI (57, 130). They predispose to this type of infection, as they disrupt the normal barrier of the human skin and are adept to biofilm-formation on the surface of the devices (137).

We could identify primary source of BSI in 42 cases, whereas in 45 cases the primary source of BSI remained unknown. Of the known primary sources of infection, the respiratory tract was the most frequent source of infection. We could not determine significant association with higher mortality in the cases of pulmonary source, urinary source and line-associated infections. This may be a result of the small numbers of cases that could be investigated. Moreover, if the source of infection was unknown patients had a 2.45 fold greater risk of dying (p=0.006). This result is in accordance with other

studies (118, 122). Patients with unknown source of infection and pulmonary source of infection had higher mortality rates than patients with line-associated infections (51% vs. 47% vs. 10% respectively). This may indicate that treatment options such as removal of potentially infected venous lines are of utmost importance in BSI due to *P. aeruginosa.* Although unknown source of infection was found to be an independent risk factor associated with mortality, this needs to be interpreted with caution for our center. In many cases physicians did not document the source of infection. These cases were thus marked as 'unknown source of infection', subsequently leading to reporting-bias.

Chemotherapy and presence of neutropenia were both significantly associated with increased mortality. Neutropenic patients had higher mortality in comparison to non-neutropenic patients (60% vs. 32%, respectively). Neutropenic patients had a 2.54-fold lower chance for survival (p=0.01). Thus, according to our data, neutropenia is a risk factor for *P. aeruginosa* BSI. A previously published study yielded similar results. Cattaneo et al. examined 441 episodes of BSI in patients with underlying hematological diseases and found *P. aeruginosa* to be the only pathogen to be independently associated with mortality (138). Kim et al. studied febrile neutropenic adolescents and children and noted high mortality of approximately 39% for *P. aeruginosa* BSI (31). In the past years *P. aeruginosa* and other gram-negative pathogens have been reaching high rates of incidence. Interestingly, MDR strains have also been rising alarmingly in hematological wards. This may have resulted in increased mortality rates in hematological patients (139). Cattaneo and colleagues also suggest the rise of MDR in *P. aeruginosa* may be a result of the widespread use of fluorochinolones as prophylaxis in hematological patients (138).

During extraction of data, the investigator could only evaluate nosocomial or hospital-acquired infections, as community-acquired infections and healthcare-acquired infections are not marked in the patients' files. Traditionally, community-acquired infections are defined as infections that occur within 48 hours of hospital admission. In addition, the patient may not have been in contact with healthcare service. Healthcare-acquired infections also occur within 48 hours of admission. In this case, previous contact to healthcare service (also including nursing homes and long-term care facilities) is included in the definition (140).

4.2 Impact of appropriate versus inappropriate therapy on mortality

Our study demonstrated a statistically significant association between administration of inappropriate initial antipseudomonal therapy and all-cause hospital mortality and clearly suggest a benefit in survival for patients receiving appropriate empirical therapy.
When a bloodstream infection is suspected, an antibiotic treatment must be administrated within few hours and before the susceptibility profile is known. The clinician has to balance preventing death from infection and an unnecessary overuse of antibiotics, which would increase resistance rates in healthcare and community settings. Superfluous use of antibiotics also increases the risk for adverse events. In addition it may have an unfavorable economic impact. Despite high mortality rates for patients receiving inappropriate therapy (80, 136, 141), there have been conflicting results on the impact of empirical therapy on the outcome mainly due to low quality studies.
In 1999, Kollef et al. published a study that investigated the effect of inappropriate antimicrobial therapy on the outcome of critically ill patients. The authors found inappropriate antimicrobial treatment to be the most important independent risk factor for higher hospital mortality (adjusted OR 4.26 [95% CI 3.35-5.44], p=≤0.001). However, this study did not evaluate the impact specifically for *P. aeruginosa* BSI (136). Several other studies also suggest that appropriate therapy is associated with improved survival (142-147). Leibovici et al. examined 3440 patients with bloodstream infections in a single-center study and inappropriate empirical treatment was found to be an independent risk factor for mortality in multivariate analysis (OR=1.6 [95% CI 1.0-2.5]). Benefit for survival could be seen in patient groups with good (young, no underlying disease) and bad (advanced age, with low functional capacity) prognosis. When treated with inappropriate empirical therapy, risk for fatality was highest for *Klebsellia pneumoniae* (OR=3.0 [95% CI 1.7-5.1]). Risk for fatality for *P. aeruginosa* was lower in comparison (OR=1.2 [95% 0.7-1.9]). In this study, empirical therapy was deemed appropriate when the pathogen was found to be susceptible in vitro to the agent. The authors highlighted the importance of conducting future studies with more specific definitions, as more factors (e.g. route of administration, dosage) influence appropriateness (145). Several other studies found an association between inappropriate empirical therapy and mortality (142-144), however they did not focus exclusively on *P. aeruginosa* BSI.

Micek et al. conducted a single-center study in Israel and retrospectively examined the impact of appropriate empirical therapy on 305 patients with BSI due to *P. aeruginosa.* The authors of this large-scale study found inappropriate empirical treatment to be an independent prognostic factor for hospital mortality (146). Appropriate initial therapy was defined as the antipseudomonal agent to which the strain was susceptible in vitro. Their analysis also showed that administering a combination therapy as empirical treatment increased the chance of receiving appropriate therapy.

Two recently published studies suggested that appropriate empirical therapy only significantly improved survival in specific subgroups of patients. In a multicenter study conducted in Israel, Schechner et al. examined the impact of therapy on in-hospital mortality in patients with *P. aeruginosa* BSI upon hospital admission. Multivariate analysis showed that inappropriate initial therapy was associated with increased risk of death only in patients with severe sepsis or septic shock (OR= 1.8, p=0.051). In univariate analysis, inappropriate initial treatment tended to be associated with mortality in patients without severe sepsis of septic shock, however without statistical significance (122). Kang et al. examined a total of 286 patients with BSI caused by gram-negative resistant strains, including 74 patients with *P. aeruginosa* BSI. Their data showed an association between inappropriate empirical therapy and mortality in patients with lung-associated bacteremia, peritoneum-associated bacteremia and when source of bacteremia was unknown (high-risk source) and no association with pancreaticobiliary-associated, urinary tract associated and catheter-related bacteremia (low-risk source) (133). Two studies yielded similar results (80, 147). Results from another study conducted by Kang et al. suggested that a delay in appropriate empirical therapy for patients with *P. aeruginosa* infections of the pancreaticobiliary tract did not deteriorate survival significantly (147). Vidal et al. established that inappropriate empirical therapy influenced outcome in patients with *P. aeruginosa* BSI only when intravenous line-associated infections were excluded from analysis (80). These reports not only highlight the importance of treatment options such as surgical decompression of obstruction or removal of infected venous lines, but also that empirical antipseudomonal therapy is beneficial for patients presenting severe illness (e.g. septic shock) and when high-risk sources of bacteremia are involved.

Other studies failed to find an association between inappropriate empirical therapy and lower survival (121, 148). In a single-center study Zaragoza and colleagues (148) prospectively examined the impact of inappropriate empirical therapy on ICU-patients with BSI due to various pathogens. Of the 166 patients, only two patients with *P. aeruginosa* infections had received inappropriate emipirical therapy. They could not establish an association between inadequacy and mortality. Nonetheless, the authors emphasized the low prevalence of patients with pulmonary-associated infections and abdominal-associated infections (high-risk source of infection) that had been treated inadequately. In addition, the study included various pathogens. Osih et al. (121) also examined the impact of adequate empirical therapy on 167 patients with BSI due to *P. aeruginosa*. They studied adequate empirical therapy at three different time points, in order to clearly distinguish between empirical and definitive therapy. The authors argued that it is difficult to clearly distinguish between empirical and definitive therapy retrospectively, for in many cases the clinician changes antimicrobial agents during therapy according to preliminary blood culture results. However, this therapy regimen cannot be labeled as definitive therapy. This is only true for agents that have been applied according to final susceptibility results. Therefore, the authors examined therapies that were applied in the time frame of (i) 24h and (ii) 24h-48h after blood cultures were drawn and (iii) after susceptibility results were known. They also assessed severity of illness at a time point 24h before the first blood culture was collected, thus assessing severity of illness at a time before presentation of bacteremia. This allowed them to properly control for severity of illness as a predictor for mortality. Their results showed a non-statistical significant trend towards decreasing mortality and length of stay for appropriate empirical therapy but no clear benefit for survival could be found. However, it is essential to point out that the authors did not exclude polymicrobial infections and could not assure that these infections were appropriately treated as well, subsequently influencing mortality.

Our study clearly suggests an association between inappropriate empirical therapy and increased mortality and these results are consistent with those of a meta-analysis published in 2010. Paul et al. (149) reviewed prospective studies that examined the effect of appropriate empirical therapy on all-cause mortality in patients with sepsis. Although the authors could determine an association between appropriate empirical

therapy and improved outcome, the OR ranged from 0-15, pointing to a highly variable effect on mortality. The studies were heterogeneous in nature, in patient populations, in types of infections and microorganisms. Most importantly, definitions for adequacy of empirical were not identical. The authors emphasized the need for new studies employing uniform methodologies. McGregor and colleagues examined study methods used to assess the benefit of appropriate empirical therapy (150). The authors made several suggestions to improve designs of future studies: i) Definition for appropriateness should not only include in vitro susceptibility but also dosage, route and pattern of administration. ii) Empirical and definitive treatment should be examined separately. iii) To avoid confounding effects on mortality, severity of illness should be assessed before onset of bacteremia. iv) Mortality should be defined more specifically (e.g. 30-day mortality (149)).

4.3 Monotherapy versus combination therapy

In our study, we also attempted to examine the impact of antipseudomonal monotherapy versus combination therapy on mortality. There are several arguments supporting the administration of combination therapy **(Tab. 8)**. It is suggested that a higher killing rate may be achieved by synergism between antibiotics (76, 151, 152). It has been shown that bactericidal activity of antipseudomonal β-lactam agents may be enhanced by an addition of an aminoglycoside (151). Additionally, combination therapy may lower the risk of receiving inappropriate therapy (146). It has also been suggested that the use of combination therapy may reduce the risk of emergence of resistance (153). On the other hand, monotherapy may be associated with lower risk for adverse events, especially when aminoglycosides are avoided (154). Also emergence of fungal superinfections and increased costs may be a disadvantage of combination therapy (155).

Table 8 - Potential advantages and disadvantages for combination therapy in *P. aeruginosa* BSI

Advantages	Disadvantages
Lower risk for administration of inappropriate empirical	Increased costs
Lower risk for emergence of resistance	Increased risk for superinfection
Possible synergism	Increased risk for drug toxicity

Patients receiving appropriate empirical combination therapy showed higher mortality rates than the patients who received appropriate empirical monotherapy (45% vs. 21%, n=24 vs. n=47, respectively). When considering mortality rates, monotherapy is favorable to combination therapy as empirical treatment. However, a comparison of monotherapy to combination therapy may lead to skewed results, for clinicians may treat severely ill patients with worse outcome more frequently with combination therapy. This may explain higher mortality rates for empirical combination therapy in our study. Peña and colleagues have highlighted this aspect in displaying that combination therapy was more frequently administered in high-risk sources of bacteremia and when clinical presentation was severe (156).

Observational studies examining the impact of combination therapy have yielded conflicting results. To the best of my knowledge, Peña et al. conducted the largest cohort-study of 593 patients with *P. aeruginosa* bacteremia in regard to antipseudomonal therapy. Antipseudomonal therapy was classified as appropriate when the strain matched in vitro susceptibility and pattern of administration and dosage were in line with medical standards. The authors could not identify a survival benefit for combination therapy during empirical or definitive treatment. Howbeit, the authors included different combination regimens (e.g. β-lactam and aminoglycoside, colistin and aminoglycoside). It is therefore not possible to examine the impact of synergism, for synergistic interactions might be class dependent (156). Bowers et al. conducted an international, multicenter study where 384 patients were included in order to study the impact of appropriate empirical combination therapy on outcome (157). According to their data, no survival difference for empirical combination or monotherapy could be found. Chamot et al. included a cohort of 115 patients with bacteremia due to *P.*

aeruginosa, showing lower survival for patients receiving appropriate empirical monotherapy (adjusted HR, 3.7 [95% CI, 1.0-14.1]) (132).

It is difficult to compare results from observational studies. First and foremost, authors take different definitions for adequacy of treatment into account. As mentioned before, it is problematic to make predictions on synergism when different types of combination therapies are included in one study. Some studies accepted aminoglycosides as an appropriate therapy regimen (141, 158). However aminoglcoside monotherapy for *P. aeruginosa* is not recommended except in the case of UTI. In addition, study populations are variable in comorbidities and severity of underlying diseases and small cohort sizes can lead to a diminished statistical validity. In order to compensate for small sample sizes, several authors have conducted meta-analyses to study the impact of combination and monotherapy on survival. Paul et al. (159) reviewed 7863 sepsis patients from various studies to examine whether β-lactam-aminoglycoside combination therapy was superior to β-lactam monotherapy. The authors did not find an advantage for combination therapy. Moreover, they found an increase in renal damage when combination therapy was administered. However, the soubgroup of *P. aeruginosa* patients was underpowered to show an effect. A second meta-analysis, conducted by Vardakas and colleagues, investigated the effect of β-lactam-aminoglycoside or fluorochinolone combination therapy and that of β-lactam monotherapy in *P. aeruginosa* infections on outcome. In total, 174 patients were included. The authors could not determine a benefit for combination therapy, neither for β-lactam and fluorochinolone nor for β-lactam and aminoglycoside (120).

To date presumed advantages for combination therapy remain questionable. Several investigators suggest in vitro synergism between drugs for improved outcome (151). However, these results could not be confirmed in in vivo studies (79). The assertion that application of combination therapy increases the chance for appropriate therapy has been examined by Micek and colleagues (146) and needs to be confirmed in future studies. It is plausible to conclude that combination therapy may reduce the risk for resistance, for this is true for other infectious diseases such as tuberculosis (160). However, there is no clear evidence for *P. aeruginosa* infections (161, 162). In contrast, a recently published study suggested that combination therapy for *P. aeruginosa* infections might select for broad-spectrum resistance (163). As mentioned above, Paul

and colleagues identified an increased risk for renal damage for combination therapy with an aminoglycoside in sepsis patients (161). Another meta-analysis examining combination therapy in cancer patients with neutropenia yielded similar results: drug toxicity was associated with combination therapy, specifically leading to an increase in renal damage (164). In addition, antipseudomonal empirical therapy is also applied to patients that may not be infected with *P. aeruginosa*, thus exposing this subgroup of patients to an unnecessary risk for drug toxicity.

In regard to the factors mentioned above and taking the results of my study into consideration, there is no clear benefit for combination therapy in *P. aeruginosa* bacteremia. More observational studies with larger study groups, precise and concordant definitions are needed to examine whether combination therapy is superior to monotherapy. Additionally, apart from mortality, outcomes should also include emergence of resistance, adverse events and development of bacterial and fungal superinfections.

4.4 Carbapenem resistance

In our cohort of 104 patients with *P. aeruginosa* BSI, carbapenem resistance was significantly associated with increased all-cause mortality. Patients with a carbapenem-resistant strain had a 2.13 fold higher chance of dying (p=0.02) than patients with carbapenem-susceptible strains. To further explore this association, we added further resistances to the analysis of carbapenem resistance (Group C) and found that the rates for mortality were similar to those for carbapenem resistance and that the impact on outcome did not differ substantially (**Tab. 7**). This observation emphasizes the impact of carbapenem resistance on mortality for patients with *P. aeruginosa* BSI. In contrast to non-resistant strains, having any drug resistance except carbapenem did not have a significant impact on mortality. Our data therefore clearly suggests a significant influence of carbapenem resistance on mortality.

Carbapenems are potent drugs of choice against serious *P. aeruginosa* infections but alarmingly high prevalence of carbapenem resistance worldwide (165, 166) is threatening their role as drug of choice for MDR *P. aeruginosa* infections (167). The detrimental impact of antimicrobial resistance on patient outcomes such as length of

hospital stay and rising economic costs have been established (168). Nonetheless, the impact of carbapenem resistance on mortality has been questioned (87, 169).

Suarez et al. retrospectively compared 88 episodes of carbapenem-resistant strains to 33 episodes of carbapenem-susceptible strains in patients with *P. aeruginosa* bacteremia (169). The authors discovered similar attributable mortality (33% vs. 30%, p=0.69) and interestingly, initial slower deaths for patients with carbapenem resistance. The authors explained this association with a potential lower virulence of resistant strains, for there has been some in vitro evidence of a more damaging immune response when infection with susceptible *P. aeurginosa* strains occurred (170). As the former study is retrospective in nature and the small cohort size may have influenced lower statistical power for attributal mortality, the results should be treated with caution. In a prospective multicenter study 638 episodes of *P. aeruginosa* BSI were analyzed and although the authors could find a significant association between carbapenem resistance and mortality, this effect was not as detrimental in the first days of infection (87). To date, the complex interactions between resistance and bacterial fitness remain unclear and need to be further explored (171).

A recently published meta-analysis that included 6660 patients with *P. aeruginosa* infections found a significant association between carbapenem-resistant *P. aeruginosa* and higher mortality, in both univariate and multivariate analysis.

In regard to our results there is evidence for an association between carbapenem resistance and increased mortality in *P. aeruginosa* infections.

Several studies have investigated risk factors for carbapenem resistance in *P. aeruginosa* BSI. Prior carbapenem exposure and medical devices were found to be strong risk factors for carbapenem resistance (172, 173). As incidence of MDR *P. aeruginosa* infections is on the rise (43), MDR infections have been associated with an increase in mortality, morbidity and economic costs (174). Tumbarello et al. found MDR to be an independent risk factor for mortality in patients with *P. aeruginosa* BSI and prior use of antibiotics as a risk factor for MDR (175). In a systematic review Falagas and colleagues established prior carbapenem use as one of the main risk factors for MDR in *P. aeruginosa (176).*

It is also important to point out that mechanisms of resistance of the clinical isolates of *P. aeruginosa* were not available to us and our results may not be applicable to other

centers, as there can be differences in resistance mechanisms leading to different resitance phenotypes.
In light of these results it seems important that hospital stewardship programs target usage of carbapenems to reduce the spread of resistant strains and reduce overall mortality associated with severe infections due to MDR gram negatives.

4.5 Conclusion

This retrospective study shows high mortality rates for patients with *P. aeruginosa* BSI. Infection with *P. aeruginosa* resistant to two or more antipseudomonal agents (multidrug resistance) was a risk factor for mortality in BSI. We could not find a survival benefit for combination therapy.
On the basis of the results of this study, empirical therapy of BSI in patients with risk factors for gram-negatives etiology needs to include at least one antibiotic active against *P. aeruginosa* based on local resistance rates and settings.

5. Abstract

Background:

Pseudomonas aeruginosa is one of the most prevalent causes for bloodstream infections due to gram-negative bacteria, resulting in high mortality rates, especially in patients with severe underlying disease. In addition to its intrinsic resistance, this pathogen acquires resistance rapidly. Currently the resistance rates in *P. aeruginosa* are rapidly increasing in hospitalized patients worldwide. Of even more concern, the increase in the incidence of multidrug resistant strains leaves very few treatment options.

Methods:

In our study we sought to define overall mortality rate and risk factors associated with mortality in bloodstream infections due to *Pseudomonas aeruginosa.* Secondary outcomes were the mortality rates according to appropriateness of antipseudomonal therapy, combination versus monotherapy, and presence of multidrug resistance defined as resistance to at least two antipseudomonal agents.

We conducted a retrospective cohort study in the University Hospital of Tübingen of inpatients with bloodstream infections due to *Pseudomonas aeruginosa* between January 2009 and October 2015. We collected epidemiological, medical and microbiological data from medical records and analyzed the data using survival analysis. Univariate and multivariate analysis was performed using Cox proportional hazard model.

Results:

The final cohort comprised 104 patients with *Pseudomonas aeruginosa* bloodstream infections. Overall all-cause hospital mortality was 37.5 %. Univariate risk factor analysis showed factors, which significantly increased mortality: chemotherapy (adjusted HR 2.12 [95% CI 1.0-4.5], p=0.04), neutropenia (adjusted HR 2.54 [95% CI 1.25-5.18], p=0.01), unknown source of infection (adjusted HR 2.45 [95% CI 1.28-4.69], p=0.006) and multidrug resistance (adjusted HR 3.05 [95% CI 1.53-6.08], p=0.001). Empirical inappropriate treatment was significantly associated with poor outcome (adjusted HR 2.24 [95% CI 1.12-4.49], p=0.02). All-cause mortality for patients receiving appropriate empirical monotherapy, inappropriate empirical

monotherapy, appropriate empirical combination therapy and inappropriate empirical combination therapy were 21% (10/47), 53% (7/13), 45% (11/24) and 75% (6/8) respectively. Carbapenem resistance was significantly associated with mortality in univariate analysis (adjusted HR 2.13 [95% CI 1.13-3.98], p=0.02).

Multivariate analysis demonstrated that multidrug resistance (adjusted HR 3.40 [95% CI 1.28-9.03], P=0.01) were independent risk factors for mortality.

Conclusion

The data show that BSI due to *P. aeruginosa* is still associated with mortality. Major risk factor for mortality is infection with a strain resistant to two or more antipseudomonal agents. The study does not show a benefit for combination therapy on survival. These data contribute to the existing evidence on impact of empirical therapy on mortality of patients with BSI due to *P. aeruginosa* and provide important information for hospital stewardship.

6. Zusammenfassung

Hintergrund:

Pseudomonas aeruginosa zählt zu den häufigsten Ursachen einer gram-negativ bedingten bakteriellen Sepsis. Hohe Mortalitätszahlen sind besonders bei Patienten mit schweren Grunderkrankungen zu verzeichnen. Charakteristisch für das Bakterium ist nicht nur die hohe intrinsiche Antibiotikaresistenz, sondern auch die Fähigkeit zusätzlich Resistenzmechanismen zu erlangen. Die weltweit steigenden Resistenzraten, insbesondere die der multiresistenten Stämme, erschweren eine adäquate Therapie hospitalisierter Patienten.

Methodik:

Im Rahmen dieser Dissertation wurden die Gesamtmortalität und Risikofaktoren von Patienten mit einer durch das *Pseudomonas aeruginosa* Bakterium verursachte Sepsis untersucht. Als sekündäre Zielvariabeln wurde die Mortalität in Bezug auf adäquate antimikrobielle Therapie, antimikrobielle Kombinations- bzw. Monotherapie und Multiresistenz (definiert als Resistenz gegen mindestens zwei Pseudomonas-wirksame Antibiotika) untersucht.

In die retrospektive Kohortenstudie wurden Patienten eingeschlossen, die sich im Zeitraum Januar 2009 bis Oktober 2015 in stationärer Behandlung am Universitätsklinikum in Tübingen befanden. Es wurden epidemiologische, medizinische und mikrobiologische Daten aus den Patientenakten erhoben und im Rahmen einer Ereigniszeitanalyse wurde das Cox-Regressionsmodell zur uni- und multivariaten Analyse der Daten herangezogen.

Ergebnisse:

Insgesamt konnten Daten von 104 Patienten mit einer *Pseudomonas aeruginosa* Blutstrominfektion analysiert werden. Die Gesamtmortalität der Kohorte betrug 37.5 %. Die univariate Analyse zeigte einen signifikanten Zusammenhang zwischen der Mortalität und folgenden Risikofaktoren: Chemotherapie (adjusted HR 2.12 [95% CI 1.0-4.5], p=0.04), Neutropenie (adjusted HR 2.54 [95% CI 1.25-5.18], p=0.01), unbekannte Infektionsquelle (adjusted HR 2.45 [95% CI 1.28-4.69], p=0.006), Multiresistenz (adjusted HR 3.05 [95% CI 1.53-6.08], p=0.001) und inadäquate

empirische Antibiotika-Therapie (adjusted HR 2.24 [95% CI 1.12-4.49], p=0.02). Die Gesamtmortalität für Patienten mit einer adäquaten empirischen Monotherapie, einer inadäquaten empirischen Monotherapie, einer adäquaten empirischen Kombinationstherapie und einer inadäquaten empirischen Kombinationstherapie waren wie folgt: 21% (10/47), 53% (7/13), 45% (11/24) and 75% (6/8).

Die multivariate Datenanalyse konnte Multiresistenz (adjusted HR 3.40 [95% CI 1.28-9.03], P=0.01) als einen unabhängigen Risikofaktor für erhöhte Mortalität feststellen.

Zusammenfassung:

Die Ergebnisse der Analyse heben die hohe Mortalität für Sepsis durch *Pseudomonas aeruginosa* hervor. Stämme mit mindestens zwei Resistenzen stellen den Hauptrisikofaktor für erhöhte Mortalität dar. Die Studie kann keine therapeutische Überlegenheit einer antimikrobiellen Kombinationstherapie gegenüber einer Monotherapie feststellen.

Die Ergebnisse unterstützen die bisherige Beweislage für die Auswirkung von empirischer Antibiotikatherapie auf die Mortalität für Patienten mit einer *Pseudomonas aeruginosa* Sepsis und liefern wichtige Daten für das Verständnis einer rationalen Antibiotikaanwendung („Antibiotic Stewardship“).

7. Literature

1. Konrad Botzenhart GD. Ecology and Epidemiology of Pseudomoans aeruginosa. 1993. In: *Pseudomonas aeruginosa* as an Opportunistic Pathogen [Internet]. Springer USInfectious Agents and Pathogenesis; [pp 1-18].

2. Lewis CT, Charles Short. Latin dictionary (founded on Andrew's edition of Freund's Latin dictionary): Oxford; 1879.

3. Gerald B. Pier RR. *Pseudomonas aeruginosa*. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 2. Sixth ed2005. p. 1727-3662.

4. Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J Clin Invest. 2002;109(3):317-25.

5. Hoiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future Microbiol. 2010;5(11):1663-74.

6. Iglewski BH. Pseudomonas. In: S. B, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston1996.

7. Naiel Bisharat TG, Yoram Keness. 10-years hospital experience in Pseudomonas stutzeri and literature review. The Open Infectious Diseases Journal. 2012;6:21-4.

8. Hilmar Wisplinghoff HS. Pseudomonas spp., Acinobacter spp. and miscellaneous Gram-negative bacilli. In: Jonathan Cohen SMO, William G. Powderly, editor. Infectious Diseases. 32010.

9. Kim SE, Park SH, Park HB, Park KH, Kim SH, Jung SI, et al. Nosocomial Pseudomonas putida Bacteremia: High Rates of Carbapenem Resistance and Mortality. Chonnam Med J. 2012;48(2):91-5.

10. Von Graevenitz A. WJ. Pathogenic significance of Pseudomonas fluorescens and Pseudomonas putida. The Yale Hournal of Biology and Medicine. 1971;44(3):265-73.

11. Lee CC, Lee CH, Hong MY. Risk factors and outcome of *Pseudomonas aeruginosa* bacteremia among adults visiting the ED. Am J Emerg Med. 2012;30(6):852-60.

12. Silverman AR, Nieland ML. Hot tub dermatitis: a familial outbreak of Pseudomonas folliculitis. J Am Acad Dermatol. 1983;8(2):153-6.

13. Ratnam S, Hogan K, March SB, Butler RW. Whirlpool-associated folliculitis caused by *Pseudomonas aeruginosa*: report of an outbreak and review. J Clin Microbiol. 1986;23(3):655-9.

14. Schaefer P, Baugh RF. Acute otitis externa: an update. Am Fam Physician. 2012;86(11):1055-61.

15. Fleiszig SM, Evans DJ. The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa*. Clin Exp Optom. 2002;85(5):271-8.

16. Koch JM, Refojo MF, Hanninen LA, Leong FL, Kenyon KR. Experimental *Pseudomonas aeruginosa* keratitis from extended wear of soft contact lenses. Arch Ophthalmol. 1990;108(10):1453-9.

17. Duran JA, Refojo MF, Gipson IK, Kenyon KR. Pseudomonas attachment to new hydrogel contact lenses. Arch Ophthalmol. 1987;105(1):106-9.

18. Willcox MD. *Pseudomonas aeruginosa* infection and inflammation during contact lens wear: a review. Optom Vis Sci. 2007;84(4):273-8.

19. Rajashekaraiah KR, Dhawan VK, Rice TW, McCulley D, Kallick CA. Increasing incidence of Pseudomonas endocarditis among parenteral drug abusers. Drug Alcohol Depend. 1980;6(4):227-30.

20. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(6):848-54.

21. Srinivasan A, Wolfenden LL, Song X, Mackie K, Hartsell TL, Jones HD, et al. An outbreak of *Pseudomonas aeruginosa* infections associated with flexible bronchoscopes. N Engl J Med. 2003;348(3):221-7.

22. Iversen BG, Jacobsen T, Eriksen HM, Bukholm G, Melby KK, Nygard K, et al. An outbreak of *Pseudomonas aeruginosa* infection caused by contaminated mouth swabs. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007;44(6):794-801.

23. Gerald B. Pier RR. *Pseudomonas aeruginosa*. In: Mandell GL, Douglas, R. G., & Bennett, J. E., editor. Mandell Douglas and Bennett's principles and practice of infectious diseases. 2. 6 ed2005. p. 2587-615.

24. Morrison AJ, Jr., Wenzel RP. Epidemiology of infections due to *Pseudomonas aeruginosa*. Rev Infect Dis. 1984;6 Suppl 3:S627-42.

25. Farlex Partner Medical Dictionary. 2012. opportunistic pathogen.

26. Bodey GP, Bolivar R, Fainstein V, Jadeja L. Infections caused by *Pseudomonas aeruginosa*. Rev Infect Dis. 1983;5(2):279-313.

27. Gould IM, Wise R. *Pseudomonas aeruginosa*: clinical manifestations and management. Lancet. 1985;2(8466):1224-7.

28. Artenstein DAW, Cross AS. Serious Infections Caused by *Pseudomonas aeruginosa*. Journal of Intensive Care Medicine. 1994;9(1):34-51.

29. Forkner CE, Jr., Frei E, 3rd, Edgcomb JH, Utz JP. Pseudomonas septicemia; observations on twenty-three cases. Am J Med. 1958;25(6):877-89.

30. Gaya H, Tattersall MH, Hutchinson RM, Spiers AS. Changing patterns of infection in cancer patients. Eur J Cancer. 1973;9(6):401-6.

31. Kim HS, Park BK, Kim SK, Han SB, Lee JW, Lee DG, et al. Clinical characteristics and outcomes of *Pseudomonas aeruginosa* bacteremia in febrile neutropenic children and adolescents with the impact of antibiotic resistance: a retrospective study. BMC Infect Dis. 2017;17(1):500.

32. Lee JH, Kim SK, Kim SK, Han SB, Lee JW, Lee DG, et al. Increase in Antibiotic-Resistant Gram-Negative Bacterial Infections in Febrile Neutropenic Children. Infect Chemother. 2016;48(3):181-9.

33. Trecarichi EM, Pagano L, Candoni A, Pastore D, Cattaneo C, Fanci R, et al. Current epidemiology and antimicrobial resistance data for bacterial bloodstream infections in patients with hematologic malignancies: an Italian multicentre prospective survey. Clin Microbiol Infect. 2015;21(4):337-43.

34. Buhl M, Peter S, Willmann M. Prevalence and risk factors associated with colonization and infection of extensively drug-resistant *Pseudomonas aeruginosa*: a systematic review. Expert Rev Anti Infect Ther. 2015;13(9):1159-70.

35. Mayhall CG. The epidemiology of burn wound infections: then and now. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003;37(4):543-50.

36. Altoparlak U, Erol S, Akcay MN, Celebi F, Kadanali A. The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. Burns. 2004;30(7):660-4.

37. Bjerknes R, Vindenes H, Laerum OD. Altered neutrophil functions in patients with large burns. Blood Cells. 1990;16(1):127-41; discussion 42-3.

38. Schwacha MG, Ayala A, Chaudry IH. Insights into the role of gammadelta T lymphocytes in the immunopathogenic response to thermal injury. J Leukoc Biol. 2000;67(5):644-50.

39. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002;34(5):634-40.

40. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. Hippokratia. 2012;16(4):303-7.

41. Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. Clin Microbiol Infect. 2005;11 Suppl 4:17-32.

42. National Nosocomial Infections Surveillance S. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004;32(8):470-85.

43. Obritsch MD, Fish DN, MacLaren R, Jung R. National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrobial agents and chemotherapy. 2004;48(12):4606-10.

44. Zilberberg MD, Shorr AF. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* and carbapenem-resistant Enterobacteriaceae among specimens from hospitalized patients with pneumonia and bloodstream infections in the United States from 2000 to 2009. J Hosp Med. 2013;8(10):559-63.

45. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

46. Cao B, Wang H, Sun H, Zhu Y, Chen M. Risk factors and clinical outcomes of nosocomial multi-drug resistant *Pseudomonas aeruginosa* infections. J Hosp Infect. 2004;57(2):112-8.

47. Tam VH, Rogers CA, Chang KT, Weston JS, Caeiro JP, Garey KW. Impact of multidrug-resistant *Pseudomonas aeruginosa* bacteremia on patient outcomes. Antimicrobial agents and chemotherapy. 2010;54(9):3717-22.

48. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. Antimicrob Agents Chemother. 2006;50(1):43-8.

49. Tacconelli E, Tumbarello M, Bertagnolio S, Citton R, Spanu T, Fadda G, et al. Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: analysis of trends in prevalence and epidemiology. Emerg Infect Dis. 2002;8(2):220-1.

50. Carmeli Y, Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. Arch Intern Med. 1999;159(10):1127-32.

51. Harris A, Torres-Viera C, Venkataraman L, DeGirolami P, Samore M, Carmeli Y. Epidemiology and clinical outcomes of patients with multiresistant *Pseudomonas*

aeruginosa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1999;28(5):1128-33.

52. Hirsch EB, Tam VH. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.

53. Kerby GP. *Pseudomonas aeruginosa* bacteremia; summary of literature, with report of a case. Am J Dis Child. 1947;74(5):610-5.

54. Whitecar JP, Jr., Luna M, Bodey GP. Pseudomonas bacteremia in patients with malignant diseases. The American journal of the medical sciences. 1970;60(4):216-23.

55. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(3):309-17.

56. Willmann M, Klimek AM, Vogel W, Liese J, Marschal M, Autenrieth IB, et al. Clinical and treatment-related risk factors for nosocomial colonisation with extensively drug-resistant *Pseudomonas aeruginosa* in a haematological patient population: a matched case control study. BMC Infect Dis. 2014;14:650.

57. Schechner V, Nobre V, Kaye KS, Leshno M, Giladi M, Rohner P, et al. Gram-negative bacteremia upon hospital admission: when should *Pseudomonas aeruginosa* be suspected? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(5):580-6.

58. Fraser TG, Reiner S, Malczynski M, Yarnold PR, Warren J, Noskin GA. Multidrug-resistant *Pseudomonas aeruginosa* cholangitis after endoscopic retrograde cholangiopancreatography: failure of routine endoscope cultures to prevent an outbreak. Infect Control Hosp Epidemiol. 2004;25(10):856-9.

59. Kovaleva J, Peters FT, van der Mei HC, Degener JE. Transmission of infection by flexible gastrointestinal endoscopy and bronchoscopy. Clin Microbiol Rev. 2013;26(2):231-54.

60. Herold G. Innere Medizin 2017 : eine vorlesungsorientierte Darstellung ; unter Berücksichtigung des Gegenstandskataloges für die Ärztliche Prüfung ; mit ICD 10-Schlüssel im Text und Stichwortverzeichnis: Selbstverl.; 2017.

61. Lodise TP, Jr., Patel N, Kwa A, Graves J, Furuno JP, Graffunder E, et al. Predictors of 30-day mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrobial agents and chemotherapy. 2007;51(10):3510-5.

62. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, et al. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect. 2007;13(6):560-78.

63. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med. 2008;36(1):296-327.

64. Bassetti M, Righi E, Viscoli C. *Pseudomonas aeruginosa* serious infections: mono or combination antimicrobial therapy? Curr Med Chem. 2008;15(5):517-22.

65. Dudley MN, Zinner SH. Single daily dosing of amikacin in an in-vitro model. J Antimicrob Chemother. 1991;27 Suppl C:15-9.

66. Höck M, Fille M. Aminoglykoside. In: Suerbaum S, Burchard G-D, Kaufmann SHE, Schulz TF, editors. Medizinische Mikrobiologie und Infektiologie. Berlin, Heidelberg: Springer Berlin Heidelberg; 2016. p. 745-7.

67. Giamarellou H, Galanakis N, Dendrinos C, Stefanou J, Daphnis E, Daikos GK. Evaluation of ciprofloxacin in the treatment of *Pseudomonas aeruginosa* infections. Eur J Clin Microbiol. 1986;5(2):232-5.

68. M., Fille M. Weitere antibakterielle Substanzen. In: Suerbaum S, Burchard G-D, Kaufmann SHE, Schulz TF, editors. Medizinische Mikrobiologie und Infektiologie. Berlin, Heidelberg: Springer Berlin Heidelberg; 2016. p. 767-74.

69. Pogue JM, Lee J, Marchaim D, Yee V, Zhao JJ, Chopra T, et al. Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;53(9):879-84.

70. Brown JM, Dorman DC, Roy LP. Acute renal failure due to overdosage of colistin. The Medical journal of Australia. 1970;2(20):923-4.

71. Furtado GH, d'Azevedo PA, Santos AF, Gales AC, Pignatari AC, Medeiros EA. Intravenous polymyxin B for the treatment of nosocomial pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa*. Int J Antimicrob Agents. 2007;30(4):315-9.

72. Kallel H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, et al. Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: a matched case-control study. Intensive Care Med. 2007;33(7):1162-7.

73. Martis N, Leroy S, Blanc V. Colistin in multi-drug resistant *Pseudomonas aeruginosa* blood-stream infections: a narrative review for the clinician. J Infect. 2014;69(1):1-12.

74. Bodey GP, Whitecar JP, Jr., Middleman E, Rodriguez V. Carbenicillin therapy for pseudomonas infections. JAMA. 1971;218(1):62-6.

75. Schimpff S, Satterlee W, Young VM, Serpick A. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. N Engl J Med. 1971;284(19):1061-5.

76. Klastersky J, Zinner SH. Synergistic combinations of antibiotics in gram-negative bacillary infections. Rev Infect Dis. 1982;4(2):294-301.

77. De Jongh CA, Joshi JH, Newman KA, Moody MR, Wharton R, Standiford HC, et al. Antibiotic synergism and response in gram-negative bacteremia in granulocytopenic cancer patients. Am J Med. 1986;80(5C):96-100.

78. Verhagen C, de Pauw BE, Donnelly JP, Williams KJ, de Witte T, Janssen TH. Ceftazidime alone for treating *Pseudomonas aeruginosa* septicaemia in neutropenic patients. J Infect. 1986;13(2):125-31.

79. Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: outcome correlations in a prospective study of 200 patients. Am J Med. 1989;87(5):540-6.

80. Vidal F, Mensa J, Almela M, Martinez JA, Marco F, Casals C, et al. Epidemiology and outcome of *Pseudomonas aeruginosa* bacteremia, with special emphasis on the influence of antibiotic treatment. Analysis of 189 episodes. Arch Intern Med. 1996;156(18):2121-6.

81. Siegman-Igra Y, Ravona R, Primerman H, Giladi M. *Pseudomonas aeruginosa* bacteremia: an analysis of 123 episodes, with particular emphasis on the effect of antibiotic therapy. Int J Infect Dis. 1998;2(4):211-5.

82. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002;34(6):730-51.

83. Paul M, Benuri-Silbiger I, Soares-Weiser K, Leibovici L. Beta lactam monotherapy versus beta lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomised trials. BMJ. 2004;328(7441):668.

84. Osmon S, Ward S, Fraser VJ, Kollef MH. Hospital mortality for patients with bacteremia due to Staphylococcus aureus or *Pseudomonas aeruginosa*. Chest. 2004;125(2):607-16.

85. Bisbe J, Gatell JM, Puig J, Mallolas J, Martinez JA, Jimenez de Anta MT, et al. *Pseudomonas aeruginosa* bacteremia: univariate and multivariate analyses of factors influencing the prognosis in 133 episodes. Rev Infect Dis. 1988;10(3):629-35.

86. Marra AR, Pereira CA, Gales AC, Menezes LC, Cal RG, de Souza JM, et al. Bloodstream infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: epidemiology, microbiology, and clinical outcomes. Antimicrobial agents and chemotherapy. 2006;50(1):388-90.

87. Pena C, Suarez C, Gozalo M, Murillas J, Almirante B, Pomar V, et al. Prospective multicenter study of the impact of carbapenem resistance on mortality in

Pseudomonas aeruginosa bloodstream infections. Antimicrobial agents and chemotherapy. 2012;56(3):1265-72.

88. Baltch AL, Hammer M, Smith RP, Sutphen N. *Pseudomonas aeruginosa* bacteremia: susceptibility of 100 blood culture isolates to seven antimicrobial agents and its clinical significance. J Lab Clin Med. 1979;94(2):201-14.

89. Kumar PD, Ravakhah K, West BC. Disseminated *Pseudomonas aeruginosa* and necrotizing pneumonia with complete recovery. South Med J. 2001;94(2):229-32.

90. Valles J, Mariscal D, Cortes P, Coll P, Villagra A, Diaz E, et al. Patterns of colonization by *Pseudomonas aeruginosa* in intubated patients: a 3-year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia. Intensive Care Med. 2004;30(9):1768-75.

91. Kollef MH, Morrow LE, Niederman MS, Leeper KV, Anzueto A, Benz-Scott L, et al. Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia. Chest. 2006;129(5):1210-8.

92. Rello J, Jubert P, Valles J, Artigas A, Rue M, Niederman MS. Evaluation of outcome for intubated patients with pneumonia due to *Pseudomonas aeruginosa*. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1996;23(5):973-8.

93. Fine MJ, Smith MA, Carson CA, Mutha SS, Sankey SS, Weissfeld LA, et al. Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. JAMA. 1996;275(2):134-41.

94. Arancibia F, Bauer TT, Ewig S, Mensa J, Gonzalez J, Niederman MS, et al. Community-acquired pneumonia due to gram-negative bacteria and *Pseudomonas aeruginosa*: incidence, risk, and prognosis. Arch Intern Med. 2002;162(16):1849-58.

95. Madeddu G, Porqueddu EM, Cambosu F, Saba F, Fois AG, Pirina P, et al. Bacterial community acquired pneumonia in HIV-infected inpatients in the highly active antiretroviral therapy era. Infection. 2008;36(3):231-6.

96. Sanders DB, Fink AK. Background and Epidemiology. Pediatric Clinics of North America. 2016;63(4):567-84.

97. Cystic Fibrosis Foundation Patient Registry 2015 Annual Data Report Bethesda, Maryland [

98. Fujitani S, Sun HY, Yu VL, Weingarten JA. Pneumonia due to *Pseudomonas aeruginosa*: part I: epidemiology, clinical diagnosis, and source. Chest. 2011;139(4):909-19.

99. American Thoracic S, Infectious Diseases Society of A. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005;171(4):388-416.

100. McManus AT, Mason AD, Jr., McManus WF, Pruitt BA, Jr. Twenty-five year review of *Pseudomonas aeruginosa* bacteremia in a burn center. Eur J Clin Microbiol. 1985;4(2):219-23.

101. Gang RK, Bang RL, Sanyal SC, Mokaddas E, Lari AR. *Pseudomonas aeruginosa* septicaemia in burns. Burns. 1999;25(7):611-6.

102. Estahbanati HK, Kashani PP, Ghanaatpisheh F. Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. Burns. 2002;28(4):340-8.

103. Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. Burns. 2004;30(4):357-61.

104. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev. 2006;19(2):403-34.

105. Tredget EE, Shankowsky HA, Rennie R, Burrell RE, Logsetty S. Pseudomonas infections in the thermally injured patient. Burns. 2004;30(1):3-26.

106. Aziz Japoni; Farshad SA, Alborzi. *Pseudomonas aeruginosa*: Burn Infection, Treatment and Antibacterial Resistance. Iranian Red Crescent Medical Journal. 2009/07/01;11.

107. Hawke M, Wong J, Krajden S. Clinical and microbiological features of otitis externa. J Otolaryngol. 1984;13(5):289-95.

108. Carfrae MJ, Kesser BW. Malignant otitis externa. Otolaryngol Clin North Am. 2008;41(3):537-49, viii-ix.

109. Bouza E, San Juan R, Munoz P, Voss A, Kluytmans J, Co-operative Group of the European Study Group on Nosocomial I. A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI-004 study). European Study Group on Nosocomial Infection. Clin Microbiol Infect. 2001;7(10):532-42.

110. Djordjevic Z, Folic MM, Zivic Z, Markovic V, Jankovic SM. Nosocomial urinary tract infections caused by *Pseudomonas aeruginosa* and Acinetobacter species: sensitivity to antibiotics and risk factors. Am J Infect Control. 2013;41(12):1182-7.

111. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. Urinary tract infections caused by *Pseudomonas aeruginosa*: a minireview. J Infect Public Health. 2009;2(3):101-11.

112. Wise BL, Mathis JL, Jawetz E. Infections of the central nervous system due to *Pseudomonas aeruginosa*. J Neurosurg. 1969;31(4):432-4.

113. Campa MB, Mauro; Friedman, Herman. *Pseudomonas aeruginosa* as an opportunistic pathogen / edited by Mario Campa, Mauro Bendinelli and Herman Friedman1993.

114. Tunkel ARK, Barry J.; Kaufman, Bruce A.; Roos, Karen L.; Whitley, W. Michael. Practice Guidelines for the Management of Bacterial Meningitis. Clinical Infectious Diseases. 2004;39(9).

115. Iversen BG, Brantsaeter AB, Aavitsland P. Nationwide study of invasive *Pseudomonas aeruginosa* infection in Norway: importance of underlying disease. J Infect. 2008;57(2):139-46.

116. Kim YJ, Jun YH, Kim YR, Park KG, Park YJ, Kang JY, et al. Risk factors for mortality in patients with *Pseudomonas aeruginosa* bacteremia; retrospective study of impact of combination antimicrobial therapy. BMC Infect Dis. 2014;14:161.

117. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Clinical features and outcome of patients with community-acquired *Pseudomonas aeruginosa* bacteraemia. Clin Microbiol Infect. 2005;11(5):415-8.

118. Aliaga L, Mediavilla JD, Cobo F. A clinical index predicting mortality with *Pseudomonas aeruginosa* bacteraemia. J Med Microbiol. 2002;51(7):615-9.

119. Weist K, Hogberg LD. ECDC publishes 2015 surveillance data on antimicrobial resistance and antimicrobial consumption in Europe. Euro Surveill. 2016;21(46).

120. Vardakas KZ, Tansarli GS, Bliziotis IA, Falagas ME. beta-Lactam plus aminoglycoside or fluoroquinolone combination versus beta-lactam monotherapy for *Pseudomonas aeruginosa* infections: a meta-analysis. Int J Antimicrob Agents. 2013;41(4):301-10.

121. Osih RB, McGregor JC, Rich SE, Moore AC, Furuno JP, Perencevich EN, et al. Impact of empiric antibiotic therapy on outcomes in patients with *Pseudomonas aeruginosa* bacteremia. Antimicrobial agents and chemotherapy. 2007;51(3):839-44.

122. Schechner V, Gottesman T, Schwartz O, Korem M, Maor Y, Rahav G, et al. *Pseudomonas aeruginosa* bacteremia upon hospital admission: risk factors for mortality and influence of inadequate empirical antimicrobial therapy. Diagn Microbiol Infect Dis. 2011;71(1):38-45.

123. Kirn TJ, Weinstein MP. Update on blood cultures: how to obtain, process, report, and interpret. Clin Microbiol Infect. 2013;19(6):513-20.

124. Towns ML, Jarvis WR, Hsueh PR. Guidelines on blood cultures. J Microbiol Immunol Infect. 2010;43(4):347-9.

125. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters.Version 6.0, 2016. Available from: http://www.eucast.org.

126. CLSI. Performance Standards for Antimicrobial Suscpetibility Testing. In: M100 Cs, editor. 27th ed ed. Wayne, PA: Clinical and Laboratory Standards Institute2017.

127. Spruance SL, Reid JE, Grace M, Samore M. Hazard ratio in clinical trials. Antimicrobial agents and chemotherapy. 2004;48(8):2787-92.

128. Singh R, Mukhopadhyay K. Survival analysis in clinical trials: Basics and must know areas. Perspect Clin Res. 2011;2(4):145-8.

129. Zwiener IB, Maria; Hommel, Gerhard. Überlebenszeitanalysen. Dtsch Arztebl International. 2011;108:163-9.

130. Vitkauskiene A, Skrodeniene E, Dambrauskiene A, Macas A, Sakalauskas R. *Pseudomonas aeruginosa* bacteremia: resistance to antibiotics, risk factors, and patient mortality. Medicina (Kaunas). 2010;46(7):490-5.

131. Al-Hasan MN, Wilson JW, Lahr BD, Eckel-Passow JE, Baddour LM. Incidence of *Pseudomonas aeruginosa* bacteremia: a population-based study. Am J Med. 2008;121(8):702-8.

132. Chamot E, Boffi El Amari E, Rohner P, Van Delden C. Effectiveness of combination antimicrobial therapy for *Pseudomonas aeruginosa* bacteremia. Antimicrobial agents and chemotherapy. 2003;47(9):2756-64.

133. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrobial agents and chemotherapy. 2005;49(2):760-6.

134. Gransden WR, Leibovici L, Eykyn SJ, Pitlik SD, Samra Z, Konisberger H, et al. Risk factors and a clinical index for diagnosis of *Pseudomonas aeruginosa* bacteremia. Clin Microbiol Infect. 1995;1(2):119-23.

135. Joo EJ, Kang CI, Ha YE, Kim J, Kang SJ, Park SY, et al. Clinical predictors of *Pseudomonas aeruginosa* bacteremia among Gram-negative bacterial infections in non-neutropenic patients with solid tumor. J Infect. 2011;63(3):207-14.

136. Kollef MH, Sherman G, Ward S, Fraser VJ. Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest. 1999;115(2):462-74.

137. Hauser AR, Rello J. Severe Infections Caused by *Pseudomonas aeruginosa*: Springer US; 2012.

138. Cattaneo C, Antoniazzi F, Casari S, Ravizzola G, Gelmi M, Pagani C, et al. *P. aeruginosa* bloodstream infections among hematological patients: an old or new question? Ann Hematol. 2012;91(8):1299-304.

139. Trecarichi EM, Tumbarello M, Caira M, Candoni A, Cattaneo C, Pastore D, et al. Multidrug resistant *Pseudomonas aeruginosa* bloodstream infection in adult patients with hematologic malignancies. Haematologica. 2011;96(1):e1-3; author reply e4.

140. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med. 2002;137(10):791-7.

141. Chatzinikolaou I, Abi-Said D, Bodey GP, Rolston KV, Tarrand JJ, Samonis G. Recent experience with *Pseudomonas aeruginosa* bacteremia in patients with cancer: Retrospective analysis of 245 episodes. Arch Intern Med. 2000;160(4):501-9.

142. Fraser A, Paul M, Almanasreh N, Tacconelli E, Frank U, Cauda R, et al. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. Am J Med. 2006;119(11):970-6.

143. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest. 2000;118(1):146-55.

144. Retamar P, Portillo MM, Lopez-Prieto MD, Rodriguez-Lopez F, de Cueto M, Garcia MV, et al. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. Antimicrobial agents and chemotherapy. 2012;56(1):472-8.

145. Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Pitlik SD. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Intern Med. 1998;244(5):379-86.

146. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrobial agents and chemotherapy. 2005;49(4):1306-11.

147. Kang CI, Kim SH, Kim HB, Park SW, Choe YJ, Oh MD, et al. *Pseudomonas aeruginosa* bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003;37(6):745-51.

148. Zaragoza R, Artero A, Camarena JJ, Sancho S, Gonzalez R, Nogueira JM. The influence of inadequate empirical antimicrobial treatment on patients with bloodstream infections in an intensive care unit. Clin Microbiol Infect. 2003;9(5):412-8.

149. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrobial agents and chemotherapy. 2010;54(11):4851-63.

150. McGregor JC, Rich SE, Harris AD, Perencevich EN, Osih R, Lodise TP, Jr., et al. A systematic review of the methods used to assess the association between appropriate antibiotic therapy and mortality in bacteremic patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007;45(3):329-37.

151. Giamarellou H, Zissis NP, Tagari G, Bouzos J. In vitro synergistic activities of aminoglycosides and new beta-lactams against multiresistant *Pseudomonas aeruginosa*. Antimicrobial agents and chemotherapy. 1984;25(4):534-6.

152. den Hollander JG, Horrevorts AM, van Goor ML, Verbrugh HA, Mouton JW. Synergism between tobramycin and ceftazidime against a resistant *Pseudomonas aeruginosa* strain, tested in an in vitro pharmacokinetic model. Antimicrobial agents and chemotherapy. 1997;41(1):95-100.

153. El Amari EB, Chamot E, Auckenthaler R, Pechere JC, Van Delden C. Influence of previous exposure to antibiotic therapy on the susceptibility pattern of *Pseudomonas aeruginosa* bacteremic isolates. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001;33(11):1859-64.

154. Craig WA, Andes D. Aminoglycosides are useful for severe respiratory tract infections. Semin Respir Infect. 1997;12(4):271-7.

155. van Delden C. *Pseudomonas aeruginosa* bloodstream infections: how should we treat them? Int J Antimicrob Agents. 2007;30 Suppl 1:S71-5.

156. Pena C, Suarez C, Ocampo-Sosa A, Murillas J, Almirante B, Pomar V, et al. Effect of adequate single-drug vs combination antimicrobial therapy on mortality in *Pseudomonas aeruginosa* bloodstream infections: a post Hoc analysis of a prospective cohort. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;57(2):208-16.

157. Bowers DR, Liew YX, Lye DC, Kwa AL, Hsu LY, Tam VH. Outcomes of appropriate empiric combination versus monotherapy for *Pseudomonas aeruginosa* bacteremia. Antimicrobial agents and chemotherapy. 2013;57(3):1270-4.

158. Bodey GP, Jadeja L, Elting L. Pseudomonas bacteremia. Retrospective analysis of 410 episodes. Arch Intern Med. 1985;145(9):1621-9.

159. Paul M, Lador A, Grozinsky-Glasberg S, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. Cochrane Database Syst Rev. 2014(1):CD003344.

160. Mitchison DA. Prevention of drug resistance by combined drug treatment of tuberculosis. Handb Exp Pharmacol. 2012(211):87-98.

161. Paul M, Silbiger I, Grozinsky S, Soares-Weiser K, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. Cochrane Database Syst Rev. 2006(1):CD003344.

162. Bliziotis IA, Samonis G, Vardakas KZ, Chrysanthopoulou S, Falagas ME. Effect of aminoglycoside and beta-lactam combination therapy versus beta-lactam monotherapy on the emergence of antimicrobial resistance: a meta-analysis of randomized, controlled trials. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(2):149-58.

163. Vestergaard M, Paulander W, Marvig RL, Clasen J, Jochumsen N, Molin S, et al. Antibiotic combination therapy can select for broad-spectrum multidrug resistance in *Pseudomonas aeruginosa*. Int J Antimicrob Agents. 2016;47(1):48-55.

164. Paul M, Dickstein Y, Schlesinger A, Grozinsky-Glasberg S, Soares-Weiser K, Leibovici L. Beta-lactam versus beta-lactam-aminoglycoside combination therapy in cancer patients with neutropenia. Cochrane Database Syst Rev. 2013(6):CD003038.

165. Baumgart AM, Molinari MA, Silveira AC. Prevalence of carbapenem resistant *Pseudomonas aeruginosa* and Acinetobacter baumannii in high complexity hospital. Braz J Infect Dis. 2010;14(5):433-6.

166. Morrow BJ, Pillar CM, Deane J, Sahm DF, Lynch AS, Flamm RK, et al. Activities of carbapenem and comparator agents against contemporary US *Pseudomonas aeruginosa* isolates from the CAPITAL surveillance program. Diagn Microbiol Infect Dis. 2013;75(4):412-6.

167. Fuste E, Lopez-Jimenez L, Segura C, Gainza E, Vinuesa T, Vinas M. Carbapenem-resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa*. J Med Microbiol. 2013;62(Pt 9):1317-25.

168. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. Antimicrobial agents and chemotherapy. 1999;43(6):1379-82.

169. Suarez C, Pena C, Gavalda L, Tubau F, Manzur A, Dominguez MA, et al. Influence of carbapenem resistance on mortality and the dynamics of mortality in *Pseudomonas aeruginosa* bloodstream infection. Int J Infect Dis. 2010;14 Suppl 3:e73-8.

170. Giamarellos-Bourboulis EJ, Plachouras D, Tzivra A, Kousoulas V, Bolanos N, Raftogiannis M, et al. Stimulation of innate immunity by susceptible and multidrug-resistant *Pseudomonas aeruginosa*: an in vitro and in vivo study. Clin Exp Immunol. 2004;135(2):240-6.

171. Abdelraouf K, Kabbara S, Ledesma KR, Poole K, Tam VH. Effect of multidrug resistance-conferring mutations on the fitness and virulence of *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2011;66(6):1311-7.

172. Lee CH, Su TY, Ye JJ, Hsu PC, Kuo AJ, Chia JH, et al. Risk factors and clinical significance of bacteremia caused by *Pseudomonas aeruginosa* resistant only to carbapenems. J Microbiol Immunol Infect. 2017;50(5):677-83.

173. Voor In 't Holt AF, Severin JA, Lesaffre EM, Vos MC. A systematic review and meta-analyses show that carbapenem use and medical devices are the leading risk factors for carbapenem-resistant *Pseudomonas aeruginosa*. Antimicrobial agents and chemotherapy. 2014;58(5):2626-37.

174. Giske CG, Monnet DL, Cars O, Carmeli Y, ReAct-Action on Antibiotic R. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrobial agents and chemotherapy. 2008;52(3):813-21.

175. Tumbarello M, Repetto E, Trecarichi EM, Bernardini C, De Pascale G, Parisini A, et al. Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: risk factors and mortality. Epidemiol Infect. 2011;139(11):1740-9.

176. Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant Acinetobacter baumannii and *Pseudomonas aeruginosa*: a systematic review of the literature. J Hosp Infect. 2006;64(1):7-15.

8. Erklärung zum Eigenanteil

Ich erkläre hiermit, dass ich meine Promotionsschrift mit dem Titel: „Outcome of sepsis due to *Pseudomonas aeruginosa*: Impact of antibiotic resistance and therapy" selbstständig und ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche gekennzeichnet.

Die Arbeit wurde in der Abteilung Innere Medizin I der Medizinischen Klinik Tübingen unter Betreuung von Frau Prof. Dr. Evelina Tacconelli durchgeführt.

Die Konzeption der Studie erfolgte durch Frau Prof. Dr. Evelina Tacconelli.

Die Datenerhebung wurde von mir durchgeführt. Ich habe die Literaturrecherche durchgeführt, einen ersten Entwurf des Manuskripts verfasst und das Manuskript in die finale Fassung gebracht.

Die statistische Analyse wurde von Frau Dr. Deepthi Kattula durchgeführt und die statistische Auswertung erfolgte eigenständig durch mich.

Herr Dr. Michael Buhl hat die Arbeit betreut und das Manuskript überprüft. Das Manuskript wurde auch von Frau Prof. Dr. Evelina Tacconelli überprüft.

Die vorgelegte Dissertation wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt. Mit der Arbeit wurde weder ein akademischer Grad erworben, noch eine staatliche Prüfung absolviert. Den Grad des Dr. med. habe ich noch nicht erlangt.

Acknowledgement

I would like to express my sincere gratitude to Prof. Dr. Evelina Tacconelli for giving me the opportunity to write the thesis under her valuable guidance. I also thank her for always taking time out of her busy schedule whenever I needed advice and help.

I would like to thank Dr. Michael Buhl for his constructive criticism, friendly advice and support. I could not have imagined a better advisor and mentor for my thesis study.

My sincere thanks also goes to Dr. Deepthi Kattula for sharing her knowledge and supporting me whenever I was in need.

Last but not least, I thank my family for their love and support. This thesis is heartily dedicated to you, for you made my medical studies possible**.**